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Project acronym: ISAFRUIT

Project title:

Increasing fruit consumption through a trans-disciplinary approach delivering high quality produce from environmentally friendly, sustainable production methods
T5.4.1.1

Instrument: Integrated Project

Thematic Priority Food Quality and Safety

Final Publishable Activity Report 2006-2010

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ACTIVITY REPORT 2006–2010

I. PROJECT EXECUTION

This report is intended to be a major overview of the activities in ISAFRUIT during the project period from January 1st 2006 until September 30th 2010. Although September 30th 2010 was the last official day of the project, much reporting work have been done after the officeial end. Many scientific papers and other publications are still published based on resulats of the project and even some research is wrapped up based on other ressources. So one thing is the measureable results in terms of reports and publications, but much value of all the research is that the results are the basis for further work in the different research disciplines we have been dealing with. Our project has produced some briks to the house of knowledge that the future can build on.
An example of an activity after the official end of the project is that the Coordinator and pillar coordinators gave a half day symposium at INTERPOMA in Bolzano, Italy in November 2010, where the main results were presented.
We also still see that PhD students partly funded from the project are finishing their PhD degree.
The main results of the activities are the publications listed in section two of this activity report. Also the future effects of scientists working together cannot be measured, but many have got new colleagues around in Europe that they will build on for their future carreer. A majority of the Management Group of ISAFRUIS is now working together on establishing a technology platform for fruit science and industry.
This report is organized pillarwise according to the pillars of ISAFRUIT.

Pillar 1. Consumer driven and responsive supply chain

Summary

The integrative approach of Pillar 1 to enhance consumer-driven innovativeness in fruit supply chains was structured as follows.

First in Work Package (WP) 1.1, public data sources were used to give an overview of fruit consumption patterns across the EU based on a number of indicators, and to select a shortlist of seven countries that were representative of all other EU countries. More in-depth figures on fruit consumption were then collected for these seven countries, ranging from macro-level (markets and regions) to micro-level (households and individuals), in order to select four representative product/market combinations (countries and/or regions) as a starting point for the rest of the studies in Pillar 1.

Second, publicly available statistical data and expert opinions were combined in a Trend-Impact Analysis in Greece, Poland, Spain, and The Netherlands The aim was to produce scenarios for future fruit consumption based on forecasted developments in underlying trend factors, such as increasing consumer orientation towards personal health and out-of-home consumption. Expert opinions were solicited both from within and from outside the fruit industry, using the so-called Delphi method, in which experts comment upon each others statements in subsequent rounds of interviews. Scenarios for future fruit consumption were formulated, while realizing that the forecasts depended on assumptions about future developments in influential factors, of which supply chain members would have to make optimal use.

Building on these first two initial steps, three research projects were started: WP 1.2 investigated those factors underlying consumer preferences for existing fresh, prepared, or processed fruit products, and the motives and barriers to fruit consumption. WP 1.3 studied those factors that influence consumer innovativeness and the adoption of new fruit products. WP 1.4 was on supply chain management and organizational structures to enhance fruit supply chain innovativeness.

These three WP's were aligned, as much as possible, in terms of their selection of consumer characteristics and the examples of fruit product innovations and fruit supply chains. Whenever possible, selected fruit product innovations and fruit supply chains were matched to developments in other parts of the ISAFRUIT Project, to create common ground for providing guidance in research.

Both WP 1.2 on consumer fruit preferences, motives and barriers for fruit consumption, and WP 1.3 on consumer innovativeness and adoption of new fruit products, started with reviews of the relevant literature to identify a theoretical framework. These were then fine-tuned on the basis of focus-group discussions in each of the four previously selected countries (Greece, Poland, Spain, and The Netherlands).To test the theoretical frameworks, and to assess the strength of underlying factors, a consumer survey was designed in each WP and applied to samples of approx. 500 consumers in each of the four countries. Before carrying out the surveys, questionnaires were tested in a pilot study, with a sample of approx. 70 consumers per country. The consumer survey data were also used to identify cross-cultural European consumer segments. In WP1.3 an additional choice experiment was carried out to study substitution effects that occur following the introduction of novel fruit products in different consumption situations. Also, a validation study was carried out to see whether findings could actually explain the success or failure of past fruit product innovations This list of innovations has been compiled using inputs from researchers across the entire ISAFRUIT Project.

WP 1.4 focused on supply chain management and organizational structures to enhance innovativeness in the fruit supply chain, and also started with a literature review to derive a theoretical framework linking supply chain management and organizational structures to chain innovativeness, as one aspect of overall chain performance. Subsequently, an overview of the fruit industry in Greece, Poland, Spain, and The Netherlands, in both qualitative and quantitative terms was prepared.

Based on the theoretical framework, two questionnaires were developed for a number of case studies. One questionnaire focused on chain organizational structures and was administered to representatives of companies from a number of fruit supply chains in face-to-face interviews in Greece, Spain, Poland, and The Netherlands. The second questionnaire focused on supply-chain innovativeness, in relation to supply-chain performance, and underlying managerial and organizational factors.

Building on the findings from these three Projects and from the other ISAFRUIT Pillars, the final step in Pillar 1 will involve the development of a roadmap for the transition towards a European fruit industry that has consumer-driven innovativeness as one of its competitive advantages (WP 1.5). Based on the literature, strategies for such implementation of innovation and transition were developed for a number of cases covering: (1) different consumer segments; (2) different fruit industries and chains (fresh, preserved, and processed); (3) cultural and regional aspects; (4) different product/technology market combinations; and (5) communication and promotion with respect to the adoption and diffusion of innovations. These were discussed with other ISAFRUIT researchers in a Workshop, as well as in interviews with European experts in the field of strategic fruit production, distribution, and marketing. A maximum of four integrated strategies were formulated, including critical success factors. Based on the Workshop and the interviews, these strategies were adapted, after which they were discussed with representatives from the European fruit industry and other stakeholders, including public agencies, to refine them, make them more robust, and make them economically feasible.

RESULTS

Results show that fruit quality is important, but, as an added-value, this is mainly a past trend. Fruit quality is the standard which gives companies ‘a license to deliver’. It is expressed in appearance, shelf-life, taste, and safety and is associated with quality-of-life and healthy living. Product quality was identified as the most important driver in the supply chain.

Consumers’ first association with ‘fruit’ is *fresh fruit* and *healthiness*. Nevertheless, basic conditions for the consumption of existing, as well as novel fruit products are *good taste* and *nice appearance*. Sensory cues (i.e., a fresh look, ripeness, natural color, etc.) are crucial for the success of any new fruit product on the market.

Large differences in fruit consumption patterns and purchase behaviors exist across Europe. Spanish and Greek consumers eat fruit mainly at home, as a dessert, and prefer buying it from a greengrocer. Polish and Dutch consumers eat fruit as a snack, or between meals, and buy fruit wherever it is most convenient (e.g., a market, or supermarket).The main advantage of innovative processed fruit products is that they increase the availability of fruit. New purchase channels seem to be necessary to increase the adoption of fresh fruit innovations.

The safety of existing fruit products appears to be a worry in Poland and Greece, but not in Spain and The Netherlands. However, safety does appear to be crucial for novel fruit products and new fruit-production technologies. Steps in the production process such as genetic modification that violate the publics’ ideal image of “naturalness”, cast doubt on both the healthiness and safety of a fruit product which can not be remedied by any special benefit.

Consumer perceptions of convenience seem to affect their preferences and behavior, and appear as the main reason for the increasing consumption of prepared and processed fruit products as a healthy alternative to regular snacks (e.g., chips, chocolate products, etc.). Arguments such as lack of time, difficult to carry, need to peel and/or to use a knife, need to wash the fruit, need to wash hands, etc., are barriers to the consumption of various kinds of fresh fruit. It seems that everyone likes to eat fruit, as long as it is ready-prepared.

Results from the cross-cultural benefit segmentation learned that multiple benefits and different situations are important for the identification of cross cultural consumer segments. Moreover, these consumer segments can be used to formulate strategies to support promotion campaigns and product development with regard to fruit.

Results from the choice experiments learned that price information still seemed to be the most important. As a consequence one could wonder whether information on packaging will do the trick, since price is still an important driver of consumers to buy fast moving consumer goods (e.g. new fruits) in all countries. From the case study results, premium pricing strategies still seemed to be important for producers and retailers. This does not mean that price strategies are the only way in selling new fruit products, but still has be kept in mind when starting to develop new products that could be too expensive for the market at the moment. Premium pricing means that the additional value of a product has to be visible for and accepted by consumers, which is not always the case in current promotional strategies.

Furthermore, based on case studies and literature, a theoretical framework for the mechanisms underlying fruit chain performance and innovativeness was developed. This resulted in a toolkit which can be used to analyse, design and implement multiple supply chain network variants. Four ways of governing consumer driven innovative fruit supply chains were identified. Two attributes were selected as important in organising a consumer driven innovative fruit supply chain. These are the use of the chain's network (high or low usage) and the innovation strategy (formal or informal).

Finally a roadmap was developed to help key stakeholders of consumer-driven and responsive fruit supply chains implementing expert-informed strategies for innovation and transition aimed at higher fruit consumption. This roadmap was derived from all gathered information in Pillar 1 and multiple stakeholder workshops.

CONCLUSIONS

An integrated approach, studying both consumer behavior and chain management and organization, is vital to provide research guidance and recommendations for the European fruit industry. This approach can only be successful if different disciplines from the social, natural, and technical sciences interact towards product and market development. For the fruit industry, this means investing in the interactions between different areas of science to deepen consumer-driven strategies on product and process innovation. More attention to the integration of consumer-behavior research in product development and marketing and in chain relationships will be crucial to increase fruit consumption.

The paradigm of consumer-driven innovativeness implies that the results of research in other Pillars of ISAFRUIT are exploited, without loosing the opportunity to generalize our findings. Such generalizations allow for the change from a "push" towards a "pull" marketing strategy, which is necessary in order to increase fruit consumption in an already saturated and highly competitive food market.

General objectives as defined in Annex 1

The overall goal of Pillar 1 was the development of consumer-driven, efficient, responsive, and innovative supply chains for the growth of fruit consumption in Europe and for a competitive and sustainable Fruit Industry.

This overall goal was divided in several objectives which were assigned to specific work packages, WP1.2, WP1.3, WP1.4, and WP1.5.

WP1: To better understand and describe fruit consumption trends and to increase and improve interaction among researchers, producers, other supply chain actors and consumers. (EUFCON)

WP2: To understand the forces that drive consumers with respect to fruit and fruit products in order to identify consumer segments to stimulate consumption. (CONPREF)

WP3: To understand the determinants of adoption and dissemination of innovations by consumers and individual chain members. Gathered results yields insight into consumer behaviour with respect to new or modified products and identifies opportunities for fruit innovation. (INNOFRUIT)

WP4: To identify the supply chain organization and management structure that maximizes supply chain innovativeness and performance, in terms of effectiveness and efficiency, in dynamic and/or developing markets. (INNOCHAIN)

WP5: To collect and integrate relevant results from all work packages and pillars in order to develop strategies for innovation, implementation and transition in the fruit chain aimed at increasing fruit consumption and discuss these strategies with the fruit industry, governments and (fruit) researchers. (TRANSCHAIN)

To achieve planned goals each work package was divided into tasks, which were assigned to competent partners working for the project (Table 1). Some partners were engaged in the completion of multiple work packages and numerous tasks.

Table 1. List of Participants in Pillar 1 and their engagement in work packages and tasks

#	Participant name	Short name	Coun-try	WP en-ga-gement	Tasks engagement
	SCIENTIFIC PARTICIPANTS				
8	Wageningen UR -Applied Plant Research	WUR-PPO	NL	**1.1**, 1.2, 1.4, 1.5	1.1.1 - 1.1.5
10	Wageningen UR-Agricultural Economics Research Institute	WUR-LEI	NL	1.1, **1.2**, **1.3**, **1.4**, 1.5	1.1.1 - 1.1.5, 1.2.1 - 1.2.5, 1.2.7 1.3.1 - 1.3.10, 1.4.1, 1.4.2, 1.4.4 - 1.4.7
24	Universidad Politécnica de Madrid	UPM	E	1.1, **1.2**, 1.3, 1.4, 1.5	1.1.2, 1.1.3, 1.1.5, 1.2.1 - 1.2.4, 1.2.6, 1.2.7 1.3.2, 1.3.3
29	Agricultural University of Athens	AUA	GR	1.1, 1.2, 1.3, 1.4, **1.5**	1.1.2, 1.1.3, 1.1.5, 1.1.6 1.2.6, 1.3.2, 1.3.3, 1.3.5, 1.3.8
38	Warschau Agricultural University	WAU	PL	1.1, 1.2, **1.3**, 1.4, 1.5	1.1.2, 1.1.3 1.2.7, 1.3.1-1.3.10, 1.4.1 - 1.4.3
2	Agroscope Changins-Wädenswil	ACW	CH	1.2	
4	Institut de Recerca i Tecnologia Agroalimentàries	IRTA	E	1.2	1.2.2
	SME's PARTICIPANTS				
63	Inova Fruit BV	IF	NL		
64	Friesland Foods	FF	NL		
67	Sodexho Nederland bv (Sodexo)	Sodexho	NL		

Basic information, contractors, work performed, end results

Over the years Pillar 1 had many delays which lead to scheduling problems. WP 1.5 depended on results obtained from other work packages. Only WP 1.1 and WP 1.4 finished there tasks in time. Most delays were caused by misinterpretations of the work, lack of skills and cultural barriers.

Unfortunately, some partners had already used most of their budget without finishing the work planned. It was important to find realistic solutions for the problems encountered. Plans were made to secure that objectives of the project would be achieved by the end of the project. After July 15th 2009, WP 1.5 started on the synthesis of their data.

Although Pillar 1 suffered from many delays, we were able to overcome all problems and difficulties and in July 2010 all deliverables of all Pillar 1 work packages were finalized and shared with the public.

Contractors/participants

Within Pillar 1 six research institutes and/or universities from four countries participated actively. Each work package was lead by a different institute or university. In table 1 all involved institutes and universities can be found. Within WP 1.1 WUR-PPO had the leading role, in WP 1.2, UPM was work package leader, within WP 1.3 WAU was work package leader, within WP 1.4 WUR-LEI had the leading role and within WP 1.5 AUA was work package leader.

During the process of carrying out all tasks some shifts had been made in the leading roles of some institutes. LEI-WUR became work package leader of WP1.2 in June 2007 and of WP 1.3 in April 2009.With these shifts the continuation of the tasks and deliverables was ensured.

Finally Agroscope Changins-Wädenswil helped pillar 1 on a less active and formal way. Furthermore there were three SME's involved within pillar 1.

Comment on participants

To meet the expectations of interactions between Pillar 1 and other Pillars within ISAFRUIT several actions were taken. Cooperation with Pillar 2 was effectuated to develop together an instrument to measure "consumer knowledge about the health effects of fruit consumption". The consumer research (WP 3.3) in Pillar 3 was reviewed by Pillar 1 scientists.

On initiative of Pillar 4 and 5 the Vasco da Gama discussion on quality was started with Pillar 1. In a meeting with technical and social scientists, consumer- and chain characteristics and the implications for technology were presented and discussed. An attempt for further cooperation between Pillar 3 and 6 and WP 1.4 was not rewarded.

The interaction with the SME's that are involved in Pillar 1 was rather sporadically at the beginning. In 2008 and in 2009 events have been organized that targeted different stake-holders in the fruit chain. ISAFRUIT has held a roundtable at FruitLogistica 2009 in Berlin addressing consumer preferences and health-related aspects of fruit consumption. The pod-casts from this roundtable were distributed via the internet-based blog channel.

WP 1.4 cancelled their workshop due to time and financial restrictions. Instead a brochure was developed which described the results of the case study, a list of critical success factors and guidelines for innovations.

During spring 2010 final workshops were organized in Spain, The Netherlands, Greece and Poland with fruit experts and fruit supply chain stakeholders to exchange information and to evaluate the theoretical based innovation implementation strategies. These workshops were organized by WP 1.5.

Comment on organisation matters

During the first year of the ISAFRUIT project it became clear that communication strategies and plans were not sufficiently elaborated, both for ISAFRUIT as a whole and for Pillar 1. Therefore, a communication strategy was developed for Pillar 1 for the remaining years, both for communication to a professional audience and the general public (e.g. through the web-site) as well as to the scientific community (e.g. at conferences). This communication strategy resulted in news items for the website of ISAFRUIT every quarter in 2009. For different occasions but especially for the GA Angers, Pillar 1 provided a cluster of posters and special presentations about the work of Pillar one and its individual WP's.

All efforts in 2009 resulted in Pillar 1 contributions to a variety of conferences (e.g. Isafruits meets Interpoma 2010 Italy, EGEA Brussels 2010) and a special ISAFRUIT session at the IHC Lisbon conference.

Vasco da Gama

On October 1st 2009, Pillar 5, Pillar 4, Pillar 7, and Pillar 1 organized a Vasco da Gama (VdG) meeting in Amsterdam to continue the process that at the end of the ISAFRUIT-project, it should be able to document, how the technology, that was developed, contributes to increasing fruit consumption in Europe. At the GA Angers, the VdG-team presented a workshop to introduce the goal and process to the scientific audience of ISAFRUIT. Pillar 1 presented the methodological framework and the applied implications of the approach of the VdG process. Further Pillar 1 cooperated closely with Pillar 4, 5 and 7 for the ISAFRUIT-workshops at VdG-process, FruitLogistica 2009. In 2010, Pillar 1 continued and finalized the VdG-process at the IHC conference in Lisbon.

General meetings

From the beginning Pillar 1 actively participated at all ISAFRUIT General Assembles with both oral and poster presentations. Furthermore Pillar 1 organized yearly meeting with all its WP partners to interact and share and discuss each other's work, knowledge and information. These meetings were held in June 2006 (Madrid), March 2007 (Athens), October 2007 (The Hague) and March 2008 (Warsaw) .

During these meeting members from other Pillars were invited as well. In Athens Pillar 1 was visited by members of Pillar 3 and in The Hague a member of the scientific advisory board ISAFRUIT was present.

Comment on reporting and dissemination of results

Most of the 2010 activities in Pillar 1 concerned finalizing WP 1.3 and WP 1.5, both accomplished during summer 2010. In 2008, WP 1.1 was already finalized and in summer 2009 WP 1.2 and WP 1.4 were completed.

Except for one (1.2.6) all deliverables have been made public. Furthermore a variety of papers have been written and some are already published while others are still under review.

A list of all Pillar 1 deliverables, papers and posters, can be found in annex B. They are categorized per year and by importance.

Relationships between scientific, technological objectives of Pillar 3 and work packages for the period 2006 –2010

Objectives	WP	Relationship need to be adjusted
A. Increase fruit quality	1.1	WP 1.1 shows that quality is still important, but it is mainly a trend of the past. Quality is a standard which consumers expect, i.e. 'a license to deliver'. Quality is expressed in appearance, tenability, taste and safety. Quality of fruit is associated with quality of life and a healthy way of living. In D.1.1.6, suggestions are given for fruit chain members to be better prepared for expected future trends and to contribute to an increasing consumption.
	1.2	Regarding consumer preferences and behaviour, appearance and taste are considered to be the most important product characteristics of fresh fruit. Taste orientation and taste preference are items in the consumer survey and revealed that benefit segmentation are most effective to inform consumers.
	1.3	Results show that sensory characteristics (fresh look, ripeness, natural colour, etc.) are crucial for success of new fruit product on market. In general, consumers value taste, health and convenience as most important. New product development should keep these product characteristics in mind.
	1.4	Three fruit supply chains with increased quality products are being studied as cases: a Polish and a Greek fresh apple chain, as well as in a Spanish peach chain with improved taste. Success factors in chain performance and innovativeness on increasing quality were identified. Fruit or product quality were mentioned, often as a (most important) part of the mission of companies in the fruit industry; this goes in combination with aiming for a high margin in companies and chains.
B. Increase fruit safety	1.1	Health is an important trend, both in the past as well as in the future (D.1.1.5 and D.1.1.6). Health means according to some experts, also aspects like health ingredients and sustainable production and trade, lack of harmful components. WP 1.1 therefore shows the importance of increasing fruit safety.
	1.2	Regarding the focus groups participants' attitude towards food safety, their interest and concern was clearly differentiated. In some countries (Spain and The Netherlands) people did not even mention it; while in others (Poland or Greece) people seemed to be very concerned. In the consumer survey, safety orientation and safety as output of the production methods of fruit are items in the question-naire. Results revealed that it is important to take multiple situations and consumption moments into account as well as multiple benefits like health, taste and safety. The study showed that fruit campaigns aimed at health may be interesting forconsumer segments: "*Safety! and Health*" and "*Caring women*", and product development for these two segments should incorporate health as an important product benefit (for example by means of labeling or packaging).
	1.3	Fruit safety seemed to be a great consumer concern. Most people

		are not confident about the technologies and production methods applied to novel products. People would like to be ensured that the special properties of fruit products are not a result of genetic modifications. Apparently, one (additional) functional value of a fresh fruit is not the same as another (GM)
	1.4	A Polish chain on sustainable (organic) fruit has been studied as a case. Production and processing in respect of safety and chain performance and innovativeness were analyzed.
C. More convenience	1.2	Consumer perceptions about convenience do affect preferences and behaviour. Arguments such as: lack of time, difficult to carry on, need to peel and/or need of a knife, need to wash the fruit, need to wash the hands, etc. act as barriers for fruit consumption. In the consumer survey convenience orientation together with convenience as a product attribute of fruit are items in the questionnaire. Positioning fruit as a quick and tasty snack is may be more interesting for consumer segment: "*Convenience - Quick and easy satiety*" and "*Snacking pragmatics*". In this study, product development for the "*Convenience - Quick and easy satiety*" and "*Snacking pragmatics*" can focus on convenience aspects of the product (for example, ready-to-eat or quick to prepare products).
	1.3	Convenience appears as the main reason for increasing consumption of prepared and processed fruit products. Convenient properties of innovative fruit products make them healthy alternative to regular snacks (chips, chocolate products, etc.).
	1.4	Two supply chains with increased-convenience products have been studied as a case: a Dutch food service chain and a Spanish watermelon chain aimed at children. In both cases the consumer driven performance of the chain were analysed. In-Home versus Out-of-Home consumption; Because of the results of WP 1.2 and 1.3 on healthy and convenient products, the main number of cases is on products for in-home consumption.
D. Better availability	1.1	In D.1.1.6, scenario's are given and suggestions for chain members to improve availability of fruit, meeting consumer demands in future.
	1.2	With relation to buying/consumption habits, Spanish and Greek consumers eat fruit mainly at home, as a dessert, and prefer buying it at a greengrocer's; whereas Polish and Dutch consumers eat fruit as a snack or between meals, and buy fruit wherever is more convenient (market, supermarket).
	1.3	Increasing availability of fruit products seems to be the most relevant opportunity for introducing innovative processed products on the market. Development of availability by new purchase channels is fundamental for increasing fresh fruit innovations adoption.
	1.4	In all chains that were selected for case studies, the objective 'better availability' was a central issue. Not only the performance and innovativeness but also the cost efficiency (related to performance) was topic of research.
E. Raise consciousness	1.1	WP 1.1 has not had a direct effect on consumer consciousness, but hopefully is has raised the consciousness of researchers of Isafruit

		as well as other partners about trends in consumer demands.
	1.2	Consumers seem to have a positive attitude towards fruit, they associate it with health. But if it comes to eating of fruit it seems to be more complicated. Still taste and pleasure play an important role in eating fruit, but there are also some disadvantages like to lazy to prepare fruit. . Fresh fruit was more or less regarded as a synonym for health. The fresher, the less processed, and the more natural the fruit product, the healthier it is perceived to be. Especially, pleasure and convenience are conflicting with this.
	1.3	Results of qualitative study indicate the importance of communication in adoption of new fruit products. It influences mostly by decreasing uncertainty and risks related to new products. Role of communication seems to be different among countries. Participants from developed markets are more familiar with market activities; they know how to approach promoting activities. They are also aware of quality certificates and have opinion about its reliability. Furthermore, choice experiments showed that different product messages appeal to consumers in different countries and that optimal messages can be different for different products.
	1.4	In all chains that were selected for case studies, the objective to ’increase consciousness’ on involving consumer needs in innovation in new products, processes, marketing etc. were basic aspect to discuss with the European fruit industries. Not only focussed on consumers needs but also focused on chain partners and their capacity to become responsive chains.
F. Lower Fruit price	1.2	Regarding price, there is also a difference among countries. In some countries people do not mention the relevance of price of fruits (Greece and Spain), while in others, some fruit is considered to be very expensive (Poland and the Netherlands).
	1.3	Price is significant factor hampering adoption of innovative fruit products. Unknown properties invoke already uncertainty. Additional financial risk often is too much for consumers and results rejection of the innovative product. A new functional fruit needs to be perceived as healthy and better than a regular fruit to have higher willingness to be bought. The consumer segmentation study revealed that even the GM apple and a radical innovation like a new purchase channel seem to have positive buying intentions among consumers, when targeted with the right marketing strategy. Moreover, taste, health reasonably priced and safety seemed to be important consumers’ motives for both new fresh fruits and for new fruit products.
	1.4	Lower prices did not seem so much an issue in supply chains; companies want to make an interesting margin on a good turnover. As long as this goes well, they will keep the same price level. This result has been discussed in the case studies with respect to R&D investments and (fruit) innovations

Description of achievements within each work package during the course of the project

ISAFRUIT Pillar 1 consists of five work packages: WP1.1 EUFCON, WP1.2 CONPREF, WP1.3 INNOFRUIT, WP1.4 INNOCHAIN, and WP1.5 TRANSCHAIN.

WP1.1 finished its work in December 2007 according to plan.

WP 1.2 produced the last 2 deliverables based on the cross cultural consumer survey and finished its activities in June 2009.

Based on de the decision of the MC, WP 1.3 changed the work package leader and designed a new and feasible planning to finish the work package before August 2010, which they did.

The research activities of WP 1.4 also finished exactly as planned, in December 2008, although the four remaining deliverables were submitted in June 2009.

WP 1.5 started in June 2008 and was on schedule, although they had to reschedule activities due to the delay in other WPs. They finished in August 2010.

WP 1.1 Trends in European Fruit Consumption (*EUFCON*)

The objectives were:

1. To conduct a thorough analysis of European fruit consumption in order to identify and understand major trends and other dynamics regarding fruit consumption and develop scenarios for future fruit consumption.
2. To analyze and understand consumption trends, development of fruit consumption indicators, their impact on future fruit consumption, and scenario development for fruit consumption.

Conclusions and General remarks

With the deliverables of WP 1.1 an overview of fruit consumption and future trends is available. The used method Trend-Impact Analysis (TIA) appeared to be suitable and reliable enough for the purpose of forecasting fruit consumption in the future. This was illustrated by the results, which showed no divergent image. However, a lot of remarks have to be made.

Confronting the results of work package 1.1 with the original objectives learned that:

- Indicators for fruit consumption are formulated, but are, due to the restriction of data of free available sources, difficult to find. Proxies were used, if possible.
- Major consumer trends and developments over the last years were identified.
- Trends and developments for future fruit consumption were identified, but with some restrictions due to the method used and the available data. The TIA added existing expert knowledge to the forecast of fruit consumption and particularized the influence of each important trend factor.
- Scenarios for future fruit consumption were developed with respect to the fact that the trend lines already showed an increase of fruit consumption. To make optimal use of the trend factors, a bandwidth was measured about which space chain members had to use.

Confronting the results of work package 1.1 with the original Isafruit objectives learned that:

- Isafruit stated health, convenience, sustainability, quality and price as important to increase fruit consumption. Health, convenience and economic factors (like price) were considered as important trends for future fruit consumption by the interviewed experts. Those trends also showed an increasing fruit consumption. Quality was mentioned, but more as a trend of the past. Quality seemed to be a standard, i.e. ‘a license to deliver’. Sustainability was not mentioned separately, but could be considered as license to produce or as part of the health trend.

- In-Home versus Out-of-Home consumption: Out-of-Home consumption was considered to be an important trend. However, it did not appear as one of the three most important trends as seen by the experts. Besides, data about out-of-home consumption were not available in free available data sources.
- Isafruit emphasized differences between fresh-, prepared-, processed fruit (products). This was a good emphasize as it was seen as part of product variation, which is an upcoming trend in all countries.

WP 1.2 Consumer preferences (*CONPREF*)

The objectives were:

1. To analyse and understand perception, attitude, and preference formation of European consumers with regard to fruit and fruit products.
2. To identify the role of product characteristics, personal, situational, and contextual or environmental (retailer) characteristics in consumer attitude and preference formation for fruit and fruit products.
3. To identify the role of cognition and affection in consumer attitude and preference formation.
4. To develop a cross-cultural benefit segmentation for consumers.
5. To determine the influence of product information on consumer attitude and preference formation for fruit.

The results on consumer benefit segmentation are linked to WP 1.3 INNOFRUIT, WP 1.4 INNOCHAIN, Pillar 2 and Pillar 3 and WP 5.3 and WP 6.3 (objectives 4, 5).

Conclusions and General remarks

This study gives more insight in motives and barriers for European fruit consumption, as a basis to meet consumer requirements in developing new types of fruits and fruit products and to develop interventions. Therefore, focus group discussions were held in Spain, Greece, Poland, and The Netherlands. Consistent with existing literature, healthiness, (sensory) pleasure, and (lack of) convenience emerged as major drivers of fruit consumption, with appearance, habit, and price as additional drivers. Talking about fruit, participants have fresh, unprocessed fruit in mind. Fresh fruit was more or less regarded as a synonym for health. The fresher, the less processed, and the more natural the fruit product, the healthier it is perceived to be. Especially, pleasure and convenience are conflicting with this. Implications of these conflicting drivers and solutions for product development and interventions are given.

The aim of the second study was to explore the role of situations in the importance consumers attach to benefits in the context of fruit. Furthermore its aim was to formulate strategies to support promotion campaigns and product development with regard to fruit based on benefit segmentation in different situations.

A large survey was carried out in four European countries that consisted of questions regarding the importance consumers attach to food related benefits in general and for specific situations, personal orientations of the consumers, the perception of fruit products and non-fruit products, their fruit consumption behavior and personal characteristics of the consumers. A cross-cultural benefit segmentation with regard to fruit in different situations was conducted. This study showed that taking into account both benefits and situations is relevant.

Moreover, the results revealed that it is important to take multiple situations and consumption moments into account, as well as multiple benefits regarding different aspects of health, convenience, safety, sensory and personal norms. The exploration of benefit situation segmentation is a challenging and interesting route which seemed promising for application in the domain of food.

Next this study showed that based on benefit segmentation a more balanced strategy can be applied to promote fruit consumption among European consumers by means of product development and promotion campaigns. Strategies based on benefit segmentation can more effectively inform and reach consumers with fruit products that are more in line with their motives and lifestyles. While some segments can be reached with more general fruit campaigns, either because these segments already have a high fruit intake or because they attach importance to all benefits, other segments demand a more targeted approach to enhance fruit consumption. More importantly, when targeting specific segments, the segments that can be reached by a general campaign are also addressed. For example, our study showed that fruit campaigns aimed at health may be interesting for "*Safety! and Health*" and "*Caring women*", whereas positioning fruit as a quick and tasty snack is may be more interesting for "*Convenience - Quick and easy satiety*" and "*Snacking pragmatics*". In addition, either strategy can be used to effectively communicate to the members of the first segment (*Safety! and Health*). The same applies for product development. Some segments can be reached with a whole range of different fruit products, whereas other segments have specific product needs. In our study, product development for the "*Convenience - Quick and easy satiety*" and "*Snacking pragmatics*" can focus on convenience aspects of the product (for example, ready-to-eat or quick to prepare products), whereas product development for the segments "*Safety! and Health*" and "*Caring women*" should incorporate health as an important product benefit (for example by means of labeling or packaging).

WP 1.3 Innovations in the European Fruit Industry (*INNOFRUIT*)

The objectives of WP 1.3 were:

1. To develop an integrated framework for understanding the determinants of consumer decision making with respect to fruit innovations.
2. To analyse the stages in the process of consumer acceptance and choice of new products, and the extent to which adoption is based on preference.
3. To classify consumers according to their attitude towards innovations.
4. To understand consumer choice in the presence of innovative fruit products.
5. To develop research guidance for researchers and the Fruit Industry with respect to future fruit product innovations.

Conclusions and General remarks

A first lesson learned is that explaining consumers' adoption of new fresh fruits of new fruit products is dependent on a wide range of product characteristics, consumer characteristics and social environmental characteristics. In this respect, not only developing qualitative better products itself but also insight into consumer perceptions of these products will be important. Consumers in general already seem to know that consuming fruit is healthy and they are willing to adopt new fruits if they have some additional value. Sensory characteristics are certainly important, but experienced as a pre-condition. In other words, the general consumer seems to expect a tasty, good looking, reasonably priced product. One could argue that future research should not focus solely on technical product development.

From the results among the ISAFRUIT researchers (list of innovations), the results on the focus groups, the consumer survey and consumer choice experiment and the case studies among retailers, there still seems to be a gap between the different stakeholders in the chain. Producers could put more effort in gaining information on what retailers do and the other way around. For example, what are retailers' target groups and how could producers concentrate more on these target groups in their product development activities. Consumer wishes are complex and differ between countries and consumer segments. More specific knowledge on consumer segmentation at the producer level could increase the acceptance of specific new fruits and fruit products.

In short, product characteristics are still important. In general, consumers (qualitatively or quantitatively) value taste, health and convenience as most important. New product development should keep these product characteristics in mind. For example, exploring differences in the evaluation of sensory aspects of fruits between different consumer segments (e.g. taste, juiciness, aroma, sweetness, and firmness) could also improve the acceptance of innovative fruits and fruit products. Therefore, (international) consumer tests on these sensory aspects should be part of new product development for fresh fruits and fruit products, Besides product innovation, researchers and industry should keep in mind that there are three additional main types of innovations: process innovations, marketing innovations and organizational innovations.

While WP1.4 INNOCHAIN mainly focused on process and organizational innovations, WP 1.3 INNOFRUIT gives a better understanding of possible marketing innovations besides product innovations. WP 1.3 gave more insight into what kind of product innovations are most attractive and how specific consumer groups differ in the way they would like to be approach. "*Average Joe*" accepts a different message than a "*Naturally conscious consumer*" or a "*Health oriented consumer*" for the same product to be interesting to them.

Furthermore, choice experiments showed that different product messages appeal to consumers in different countries and that optimal messages can be different for different products.

A main lesson from WP1.3 INNOFRUIT is that on the one hand, one could say that it is difficult to compare apples and peaches, since consumers perceive different characteristics important for both products. On the other hand, also consumers perceive certain characteristics equally important for both products.

An example of the former is that a GM apple with non-allergic properties is not the same as a functional food like a cholesterol-lowering peach and that consumers in the Netherlands perceive these products differently than consumers in Spain. A GM apple has to be mainly perceived as natural to have positive buying intentions.

A cholesterol lowering peach needs to be perceived as healthy and better than a regular peach to have higher willingness to be bought. Furthermore in general, buying intensions for a GM apple are lower than a new functional peach. Even if both products are presented in a way to consumers that they claim to taste the same as regular products. Apparently, one (additional) functional value of a fresh fruit is not the same as another.

An example of the latter (compare apples with peaches) is that overall the most attractive products were associated with convenience (e.g. mini nectarines without stones and fruit mix salad).

The list of innovations from the technical researchers in Pillar 2-6, the focus groups as well as consumers in the quantitative studies seemed to agree with convenience products being (most) important. Both, product evaluations as well as buying intention were positive for most fresh fruits and fruit products. The consumer segmentation study revealed that even the GM apple and a radical innovation like a new purchase channel seem to have positive buying intentions among consumers, when targeted with the right marketing strategy. Moreover, taste, health reasonably priced and safety seem to be important consumers' motives for both new fresh fruits and for new fruit products.

WP 1.4 Innovative consumer-driven fruit supply chains (*INNOCHAIN*)

The objectives are:

1. To investigate the rationales underlying consumer driven, innovative, and cost efficient fruit supply chains;
2. To unveil critical success factors for chain performance.
3. To adopt an integrated perspective, including interrelationships between chain actors.
4. To embed relevant concepts, possibly chain structure and co-ordination, partnerships and other relationships, supply chain responsiveness and flexibility, and business environment.
5. Provide an overview of successful supply chain management practices and formulate recommendations and critical success factors.

WP1.4 deploys research output of WP1.1 and WP1.2 and is strongly related to WP 1.3 and WP 1.5. It will also provide valuable input to the formulation of research guidelines that will be used in other pillars (3, 4 and 5).

Conclusions and General remarks

Comparing the results of WP 1.4 INNOCHAIN with the WP 1.4 objectives showed that:

- Based on reviewed major scientific publications on supply chain performance and innovativeness WP 1.4 has developed a preliminary theoretical framework for the mechanisms underlying fruit chain performance and innovativeness. The developed framework has shown to support the ability to innovate that is required for this flexibility. A toolkit has been provided for analysis, design and implementation of multiple Supply Chain Network variants.
- WP 1.4 has formulated indicators for performance and innovation based on theoretical findings and insights from the Fruit Industry that focus on consumer orientation with respect to consumer benefits.
- The case studies gave insight in the lay out of the supply, and its organization, governance and innovativeness. From literature and the case studies Critical Success Factors (CSF) for stimulating an innovative fruit supply chain have been derived. Based on the CSF's practical guidelines were developed for stimulating supply fruit chain innovativeness and becoming consumer driven innovative chains.
- A typology has been developed to identify four ways of governing consumer driven innovative fruit supply chains. Two attributes were selected as important in organising a consumer driven innovative fruit supply chain. These are the use of the chain's network (high or low usage) and the innovation strategy (formal or informal).

Confronting the results of Work Package 1.4 INNOCHAIN and the overall ISAFruit objectives learned that:

- ISAFruit stated health, convenience, sustainability, quality and price as important to increase fruit consumption. From the perspective of WP 1.4 health, convenience, taste and price orientation were issues in the case studies on consumer driven innovativeness of fruit supply chains.

- Fruit or product quality were mentioned, often as a (most important) part of the mission of companies in the fruit industry; this goes in combination with aiming for a high margin in companies and chains.
- In-Home versus Out-of-Home consumption; Because of the results of WP 1.2 and 1.3 on healthy and convenient products, the main number of cases is on products for in-home consumption. One case is studied on the food service channel.
- ISAFruit emphasized differences between fresh-prepared-processed fruit (products). The parallel in WP 1.4 was the case study of innovative fruit supply chains and different product-market-combinations.
- WP 1.4 showed how firms in the fruit industry can collaborate to form innovative and responsive fruit supply chains that, by focussing on consumer demand, would increase fruit consumption.

WP 1.5 Transition Fruit Chains (TRANSCHAIN)

Objectives were:

1. To collect and integrate relevant results from other work packages and pillars in order to develop strategies for innovation implementation and transition in the fruit chain aimed at increasing fruit consumption and discuss these strategies with the Fruit Industry, Public partners and (fruit) researchers.
2. To formulate consistent and robust strategies based on (1) consumer segments, (2) Fruit industry and chain features (fresh, preserved and processed), (3) cultural and regional aspects, and (4) product/technology-market combinations with respect to adoption of innovations.
3. To developed a road map to implement innovation and transition which will be used in pillar 7.

Conclusions and General remarks

WP 1.5 has collected and integrated results from other ISAFRUIT WP's and pillars in order to develop strategies for innovation implementation and transition in the fruit chain aimed at increasing fruit consumption. It has summarized the various types of innovations that have succeeded or may succeed in the future to increase European fruit consumption. Next, WP1.5 reviewed both innovations proposed in the scientific literature and those identified by ISAFRUIT researchers while conducting field or laboratory research. Hundreds of fruit-related innovations were scanned and the 19 most promising were selected. After performing an exhaustive literature review on innovation and innovation strategies, a comprehensive theoretical framework was designed that linked the desired increase in fruit consumption to innovation implementation strategies. Furthermore a list of factors was made that affect crucially whether a fruit-related, consumption-boosting innovation will be accepted by consumers. Subsequently four expert meetings in Spain, Poland, Greece, and the Netherlands were organized where fruit experts and fruit supply chain stakeholders were asked to review the 19 innovations and evaluate which of the theoretically derived innovation implementation strategies were most relevant for each of the studied innovations. The following strategies were evaluated by the experts: (1) detection and correction of errors; (2) product modification/improvement; (3) gathering and disseminating market information; (4) consumer/customer education; (5) communication within organization; (6) targeting specific groups of consumers/customers; (7) learning about competition; (8) collaboration with universities and research centers; and (9) collaboration with other stakeholders (e.g., policy makers, consumer organizations, etc).

Furthermore, the experts were asked to evaluate six factors hypothesized to affect whether and by how much an innovation will be accepted by consumers as well as policy and institutional factors that might encourage higher fruit consumption in EU countries. The evaluated factors were: (1) consumers' technological awareness; (2) consumers' commitment to brand name; (3) speed to market; (4) technological competence; (5) cultural idiosyncrasies; and (6) consumers' learning ability.

The results of the four expert meetings were analyzed in order to modify or improve the list of theoretically derived innovation implementation strategies and to produce a pan-European roadmap of innovation implementation strategies.

'Market orientation' and 'continuous learning and knowledge acquisition' surfaced as the innovation implementation strategies with the highest success potential. However, the optimal mix of strategies depends on the particular innovation as well as the geographic and cultural characteristics of the targeted consumer population. Furthermore, improving technological competence through investments in R&D or collaborative agreements with specialised research centres is the single-most important factor affecting fruit innovation adoption. A drawback of this research is that the reported results are derived from four small groups of arbitrarily chosen experts and supply chain stakeholders. Nevertheless, the described results provide several insights useful to policy makers, consumers, and entrepreneurs. In designing innovations, fruit chain actors should first and foremost consider the price premium consumers will have to pay. Another implication is that for some innovations geographic or cultural characteristics become very important. Therefore, fruit companies and chains should design their innovation implementation strategies accordingly. A third consequence is that mixing or combining fruits, which are considered by consumers as healthy products, with less healthy food may result in lower, instead of higher, fruit consumption.

Pillar 2. Fruit and human health.

Project execution

Pillar 2 consists of four workpackages (WP2.1 – 2.4), each with several tasks. The objective includes raising the consciousness of consumers by generating and disseminating knowledge on the short- and long-term effects of fruit consumption on human health. The objective of RTD Pillar 2 is to create new detailed knowledge about the health and safety of fruit, in particular in such areas where this knowledge can support education along the food chain and of the consumer, i.e. in areas such as risk of overweight, cardiovascular disease, and cancer. Increased fruit safety is addressed by identifying low allergenic fruit cultivars and by monitoring safety of new production systems, including minimally processed fruits and fruit produced by improved pre- and postharvest procedures.

The contractors included the Technical University of Denmark (Dk), University of Oslo (N), Wageningen agricultural University (NL), Danish Cancer Society (Dk), University Medical Center Groningen (NL), Research Institute of Pomology and Horticulture (Pl), Technical University of Lodz (Pl), and University of Copenhagen (Dk).

The work performed in ISAFRUIT pillar 2 has been progressing to reach all objectives. In short:

WP 2.1 ***FRUITEFFECTS*** set as its objective to provide the currently lacking knowledge about functional effects of processed fruit products in comparison to whole fruit and to elucidate in more detail the mechanisms behind the health effects of fruit.

We have conducted two large human intervention studies and several mouse and rat studies on whole fruit and on many processed products, including clear and cloudy juices, pomaces, dried fruit, apple pectin, and puree. The studies are mainly with apples but cherries and black currants are also studied in one of the mouse experiments. We have confirmed a strong effect of whole fruit on cholesterol lowering in rats and in normal weight humans and a strong decreasing effect on postprandial lipaemia. While cloudy juice and especially pomaces retain some of the effect of whole fruit, clear juice seems to have a limited health potential, at least with respect to plasma cholesterol lowering. Moreover the effect could not be observed in overweight individuals. In observational studies we have substantiated that fruit intake is in fact protective against cardiovascular diseases and colorectal cancer (CRC), however fruit fibre did not affect CRC. Pomes seem to have an especially potent effect on acute coronary syndrome. We have used several novel techniques in our search for the biological mechanisms behind these effects, including profiling of metabolites, gut microbiota and the gene transcripts. The results from this research points towards several important mechanisms, including changes in bile acid synthesis, short chain fatty acid production, steroid synthesis, lipid metabolism and foreign compound metabolism as a consequence of fruit intake. Several of these mechanisms are known to affect risk markers of cardiovascular disease and potential risk markers of cancer.

WP 2.2 ***FRUITSLIM.*** The objective was to establish the efficacy of work place fruit campaigns on weight management and to investigate the effect of fruit consumption on weight maintenance.

An intervention study with free fruit offered at the workplace vs. none has been conducted in a population with a very limited initial fruit intake. The study revealed that workplace fruit campaigns tend to increase the overall intake of fruit, also at home, thereby increasing the efficacy.

The results of the main trial also showed an effect on BMI, body fat, and blood pressure. This result was corroborated by a review of other studies on fruit and obesity and by a cross-sectional study showing a significantly lower body weight among those consuming more fruit.

WP 2.3 ***FRUITSAFE*** had as its objective to investigate fruit varieties with low allergenicity and to design a GMO apple with decreased allergenic potential.

We have tested 60 apple varieties twice by skin prick tests in individuals with birch pollen allergy thereby providing a ranking system for all common apple varieties. Selected varieties have additionally been tested by oral challenge tests which have largely confirmed the results that Elise, McIntosh, Modi, and Santana are the most promising varieties for minimizing the reaction from apple allergy sufferers.

We have grown GMO apple cell cultures expressing RNAi silencing of the allergenic Mal-d1gene into fruit bearing trees. We have continuously checked that the plant materials from these trees are still genetically silenced for the allergen. We have grown 10 such accessories and tested a subset for their allergenic potential by skin and oral provocation tests with promising results.

WP 2.4 ***FRUITCLEAN.*** The objective is to document the safety of new fruit production methods.

We have developed the analytical procedures necessary for evaluation of a large number of pesticide residues and contamination with a toxic fungal metabolite. We have received samples from the field trials going on within ISAFRUIT on new spraying techniques and new postharvest treatment methods in order to evaluate their efficacy with respect to reduce contaminants. We have confirmed that the new mild post-harvest treatments decrease the risk of fungal contamination. We have also confirmed by well controlled analyses that the pesticide contamination can be reduced by more ‘intelligent’ and environmentally friendly spraying techniques.

Each of these work packages have reached their scientific objectives thereby potentially contributing to the consumer awareness of fruit and health and to increased safety for consumer and the environment as depicted in the table below. We have used several advanced technologies in order to reach these goals and we have shown some new ways that will lead to new scientific advances and pointed towards fruit products that may lead to new bioactive fruit products for European consumers.

Relationships between scientific, technological objectives and Pillar 2 WP's

Objectives	Work package	Relationship
A. Increase fruit quality	-	
B. Increase fruit safety	23	This workpackage targets the safety of those who are allergic to fruit. The objective is met by identifying low-allergenic cultivars and identifying fastors along the chain contributing to allergenic potential of fruit. Moreover, transgenic fruit with silenced genes for allergens are produced in pilot scale.
	24	This workpackage aims to monitor the new production technologies in pillar 4 and 5 to assess that they decrease pesticide load as well as fungal toxins. The objective is met by documenting actual leves of toxins in fruit from the new production systems.
C. More convenience	-	
D. Better availability	21	This workpackage aims to identify health effects of fruit and processed fruit products. By identifying the health potential of processed fruit and hitherto unused fruit fractions the potential for production of new healthy fruit-containing products is increased, thereby increasing the overall availability of fruit products.
	23	By identifying the low allergenic fruit varieties, lower-risk fruit will become identifiable and thereby more available to fruit allergics.
E. Raise consciousness	21	By identifying the mechanisms by which fruit leads to increased health, the health professionals and producers will have additional knowledge and arguments to communicate health messages about fruit to consumers and to combat the common misunderstandings, particularly about the health potential of processed fruit products.
	22	This workpackage aims to provide new knowledge on fruit and weight management. This knowledge is currently lacking and relationships between fruit intake and weight management is largely unknown to the public. Providing and disseminating such knowledge will raise conciousness about fruit and health and may help combat overweight.
	23	Providing useful knowledge to allergic consumers on fruit varieties and fruit fractions with low allergenic potential should raise the consciousness of these consumers on how to get fruit without risking allergic episodes.
F. Lower Fruit price	-	

Pillar 3. Improved appeal and nutritional value of processed fruit.

Summary

Pillar 3 has been quite complex and covered various fields of processing. It was composed of four work packages dealing with:

- Minimally processed fruits;
- Juices;
- Dried fruits;
- Dietary fibre and fruit phytocomponents.

The common idea for the first three packages was the production of high quality finished products, as close in composition as possible to the raw material used, preserving fruit healthy components to the maximum possible extent, or enriching some of them into new components such as prebiotics and probiotics and at the same time fulfilling various needs of consumers: safety, convenience, nutrients content, pleasure, wellbeing etc.

To increase fruit consumption in the processed form a few new products were developed or technology of existing products improved: probiotic apple slices, or apple slices covered with chocolate or with prebiotic inclusions. High quality crunchy fruit bars were also developed by SME in cooperation with research institutes. Flexi dried products enriched in prebiotics were developed using combined technique of osmo-dehydration and conventional drying and well chemically characterized considering changes during various stages of processing. Technology of plum juice production from fresh fruit was developed using mixture of macerating enzymes, which was tested on an industrial scale. In a few cases (for dried fruit and juices), large consumer trials were conducted to find out what are the expectations of the consumers. Raw material suitability for different products was studied based on exchange of material between partners. Variety trails were of special interest in the case of scab resistant apples (over 20 apple cultivars studied), considering quality of obtained clear and cloudy juices in reference to Code of Practice of the AIJN (European Fruit Juice Association). The results supply useful information for the producers in their endeavors to adjust to market requirements.

In work package dealing with dietary fibre and fruit phytocomponents the methods of components extraction, purification, and separation were elaborated and biologically active components derived from fruit mash/pomace of apples, sour cherries, black currants and plums were characterized. Some were used to supplement other fruit products.

In pillar 3 there was an extensive cooperation of partners working in particular work packages but also between work packages. This covered sharing material for technological trails and analysis, exchange of personnel (between INRA, France and Poland), common work on deliverables, common publications, posters etc. Pillar 3 also cooperated with other pillar in particular Pillar 2, for which fresh fruit and processed products were supplied for testing in animal and human trials. The material was well characterized in respect to chemical composition, including residues, which if present might offset correctness of results.

During the whole period of ISAFRUIT an extensive work on dissemination of information about the project and pillar 3 in particular and dissemination of results was carried out. With knowledge accumulation, this work was intensified. Knowledge was disseminated at ISAFRUIT general assemblies, various symposia, conferences and congresses. Two symposia were organized as part of Pillar 3 activity within ISAFRUIT (in France and Poland). The results planned in Annex 1 have been achieved and described in reports (deliverables) and in numerous publications. Altogether 42 publications were already published in peer-reviewed

journals, and some more are being prepared. Two partners are considering applying for a patent (one in reference to new species of bacteria blocking development of food born pathogens and another one in reference to extraction of polyphenols from fruit mash. Results obtained indicate that processing pillar succeeded achieving objectives planned in the Technical Annex (Annex 1).

General objectives of Pillar 3 as defined in Annex 1

The overall goal of the Project was increasing fruit consumption in Europe through the improvement of fresh and processed fruit quality and safety and a better understanding of consumers' needs and behaviour.

In Annex 1 (p. 10) scientific and technological objectives were established, which in the case of pillar 3 covered the following topics:

- Increase quality of fruit products meeting the consumer preferences, improving quality of selected processed fruit products;
- Increase fruit safety by enhancing safety of minimally processed fruit;
- Augment the convenience of fruit products by improving the quality and stability of minimally processed fruit and by developing novel convenient processed products with snacks characteristics;
- Improving availability of quality fruit by means of developing minimally processed fruit and novel products;

To achieve planned goals each work package was divided into tasks, which were assigned to competent partners working for the project (Table 1). Some partners were engaged in completion of two or even three work packages and numerous tasks. The tasks are listed in Annex 2 and results reported in deliverables listed in Annex 3.

Basic information about Pillar 3

As mentioned in the Summary, Pillar 3 has been quite complex and covered various fields of fruit processing. However, two ideas were common for all work packages. First one – introduction of innovations, and the second one – desire to increase competitiveness of small and medium European business. Considering innovations in two most prospective fruit processing branches (minimally processed fruit and juices) and in a promising area of dehydration industry the objectives planned were directed toward improving technologies and new products development to fulfil expectations of European consumers in respect to product values (health components, convenience, pleasure).

Consumers are concerned about food safety risks, particularly due to foodborne pathogens, residue levels and GMO-containing food. Consumers are also concerned about food additives and preservatives, even it they do not create a serious health hazard. They undoubtedly would accept "natural" products more readily than those in highly processed form, and products for which health claims[1] may be used. They also accept new pasteurization and packaging technologies such as high-pressure post-packaging pasteurization that minimize negative temperature effects, and are more closely associated with freshness or health. In some countries "natural" is at the top hierarchy of consumers expectations, and 'better taste' has been ranked higher than "saving money". Interest in naturally functional foods is growing and it is foreseen that it will remain one of the strongest health trends for the next 10 years.

[1] EFSA recently prepared a list of accepted health claims for different constituents (vitamins, minerals etc.) and products (e.g. some fruit juices).

Specialists also pointed out that combining foods naturally high in antioxidants and nutrients is a good strategy. Most of the above-mentioned premises concerning consumers' attitude toward food (in particular processed fruit) are reflected in planned work packages.

The competiveness of the European SMEs' could be strengthened by popularizing production and consumption of direct juices at the site of fruit production (Annex 1, part B 3. point 5). Highly concentrated clear fruit juices are microbiologically stable at room temperatures and are imported from other continents strongly competing with European products. Import of cloudy half-concentrates is of course possible but much more expensive and Europeans would gain economic advantage producing direct, cloudy juices locally. Substantial health argument has been found in Pillar 2 in favour of this kind of juices consumption.

It was unticipated that pomace, a waste product in juices production contains nutritionally more valuable compounds than the clear juice. However, so far the only rational method of its utilistion was using it as a feedstuff. Dealing with juices it was reasonable to look deeper into properties of pomace, its composition and give at least some proposals of its utilistion. Determinatioon of detailed composition of pomace and proposed methods of extraction of valuable compounds may evoke interests of specialist inside and outside of food processing branch, especially if one considers findings of our partners in Pillar 2 on health effects of pomace.

Contractors/participants

In Pillar 3 participated 6 research institutions (institutes and universities), representing 5 EU countries. However, in the case of Institut National de la Recherche Agronomique (INRA, France) we had in fact 3 independent partners (Table 2), who separately reported their research contributions and moreover 2 of them chaired 2 work packages (INRA Genial WP 3.3 and INRA Avignon WP 3.4).

In the pillar 3 there were also 5 SMEs, 4 industrial partners, and an agricultural university (École Supérieure D'Agriculture D'Angers). Beside that in Pillar 3 participated representative of Pillar 2, who cooperated in execution of tasks being of common interest.

Comment on participants. Scientists involved in research carried out within Pillar 3 represented different specialties, there were food technologists, food chemists, food scientists, sensory specialists, marketing specialists, biochemists and medical scientists (in cooperation with Pillar 2 (Partner No 3, and later Partner No 21). Thus the team was a true inter- and trans disciplinary. Moreover, in each work package there was a small or medium enterprise specializing in particular commodity of food products, the work package was interested in. One of the SME (Partner 65 – ESA) was specializing in sensory research and was responsible for coordination of consumer tests within the pillar. Moreover specialists of pillar 3 were also engaged in specific areas of research carried out in other pillars and in particular pillar 6 (helping to conduct consumer test carried out on peaches and apples) and vice versa, received when necessary, extensive help from others outside their work-packages or even pillar. Engagement of individual partners in realization of tasks (table 1 and Annex 2) and in particular deliverables has been shown in Gantt charts prepared for each planned periods of research.

Although within Pillar 3 there were 4 work packages, two of them (WP 3.2 and WP 3.4) were closely cooperating during the whole course of the project to achieve aims described in Annex 1.

The pillar coordinator opinion on partners involved has been very positive, irrespectively of some delays in supplying deliverables. The reasons for delays were commented in periodically prepared reports. Some partners were excellent in basic research, others in practical approach but all did their best according to the principle of due diligence and all fulfilled their obligations.

The world crisis in recent years negatively affected each SME partner involved into the project realization. For example, the financial situation of Val de Vire Bioactive (Partner 48) has deteriorated and the company stopped its activity (see Annex No 4). Luckily, this happened in the last year of the project and most planned experiments had been carried out. Other SMEs' also experienced difficulties. Partner 55 (Celiko) had to abandon their plans to invest into packaging machine and other equipment necessary for production of developed crunchy fruit bars. Partner 54 (Alpex) invested large amount of money into pomace dryer, however producer of the dryer bankrupted and did not carry out its task till the end (the dryer caught fire during the early stages of testing). Market situation for minimally processed fruit also deteriorated and Partner 56 (NBL) temporarily stopped production of ready-to-eat fruit. In the time of recession, the producers preferred to rely on products, which had an established market share and guaranteed profitability, fulfilling the basic needs of the consumers.

Table 2. List of Participants in Pillar 3 and their engagement in work packages and tasks

#	Participant name	Short name	Country	WP engagement	Tasks engagement
SCIENTIFIC PARTICIPANTS					
4	Institut de Recerca i Tecnologia Agroalimentŕries	IRTA	E	3.1	3.1.1÷3.1.8
5	Institut National de la Recherche Agronomique	INRA	F		
	INRA URC, Rennes			3.2 & 3.4	3.2.1÷3.2.5 3.4.1÷3.4.4
	INRA AgroParisTech/Genial, Massy			3.3	3.3.1÷3.3.4
	INRA Avignon			3.2 & 3.4	3.2.1, 3.2.2, 3.4.1÷3.4.4
10	Wageningen UR-Agricultural Economics Research Institute	WUR-LEI	NL	3.3	3.3.4
11	Research Institute of Pomology and Floriculture	RIPF	PL	3.2, 3,3 & 3,4	3.2.1÷3.2.3, 3.2.5, 3.2.6 3.3.1÷3.3.4, 3.4.1÷3.4.3, 3.4.5
23	Universitat de Lleida	UdL	E	3.1	3.1.1÷3.1.5, 3.1.7, 3.1.8
34	Teagasc, The National Food Center	NFC/AFRC	IRL	3.1	3.1.1, 3.1.2, 3.1.4, 3.1.5, 3.1.7÷3.1.9
37	Technical University of Lodz	TU-LODZ	PL	3.2, 3,3 & 3,4	3.2.1÷3.2.5, 3.3.1÷3.3.3, 3.4.1÷3.4.3, 3.4.5,
SME's PARTICIPANTS					
48	Val-de-Viere	VDV	F	3.4	3.4.1÷3.4.3, 3.4.5
54	Alpex	ALPEX	PL	3.2 & 3.4	3.2.1, 3.2.2, 3.4.1,
55	Celiko	CELIKO	PL	3.3	3.3.1, 3.3.3
56	Nature's Best Ltd.	NBL	IRL	3.1	3.1.1, 3.1.2, 3.1.4÷3.1.9
65	Association Groupe ESA	ESA	F	3.2 & 3.3	3.2.2, 3.2.3, 3.2.6, 3.3.4,
PARTICIPANTS FROM PILLAR 2					
3*	Danish Institute for Food and Veterinary Research	DFVF	DK	3,4	3.4.3
21*	Royal Vet. and Agricultural University	KVL	DK	3.2	3.2.1

Comment on organisation matters. Pillar 3 did reasonably well during the whole period of the project, what was positively commented by the reviewers in their periodic reviews. This might be due to responsibility of partners involved who actively participated in organized yearly pillar meetings aimed at mutual acquaintance of partners and their research abilities, exchange of ideas, reporting currant progress in their experiments (as a rule power presentations by each participant), working out plans for subsequent stages of the project etc. The pillar meetings were organized in Poland (2006), France (2007) and Ireland (2008). In pillar meetings, also participated representatives of the other pillars (mainly Pillar 2 leader) interested in cooperation. Beside that if it was absolutely necessary there were occasional meetings of participants within work packages. Participants of pillar 3 were active during ISAFRUIT General Assemblies, presenting numerous posters and if required oral presentations, and new products developed within ISAFRUIT (in Bologna 2007 and Angers 2009). Pillar 3 organized national symposia (in 2008 in France and in 2009 in Poland). This activity was well documented in annual reports for the EU Commission.

Comment on reporting and dissemination of results. Most deliverables produced were not available publicly considering that their authors in the table "dissemination level" on the front page of each deliverable indicated "restricted to other programme participants". The reason for this approach was justified by the fact that deliverables were in most cases not consummate "masterpieces" to be communicated to the public, but just consecutive steps in achievement of planned aims. At the end of the project, upon suggestion of reviewers more and more reports were prepared in ready, or almost ready, to be submitted for publishing form. Dissemination of knowledge/project results over the years of the project duration was taking place by oral and poster reports during numerous conferences, symposia, congresses etc. and through scientific publications. The drawback of this approach was such that in most cases it was targeted to scientists. On the contrary articles published in journals destined for industry professionals and lectures delivered at conferences organized for them or organized by producer associations definitely contributed to extension of practical knowledge with probability of its application.

Some deliverables were marked as confidential – this was in the case when partner considered patenting of the results or in the case of SME's wanted to keep results secret for its own use. In fact, SMEs' were not interested in patenting considering that competitors instead of buying a patent might try to evade it.

Results obtained by pillar 3 teams probably did not bring direct effect in increased consumption of processed fruit during the period of recession[2]. However, in a long run will have a positive effect by changing attitudes of industrial professionals and consumers toward fresh and processed fruit products, considering continuation of efforts on implementation of results also by scientists who obtained their PhDs' within ISAFRUIT. PhDs' are an additional effect of the Project of great significance, considering that among others they prove high rank of results obtained, whereas each of theses was reviewed by at least 2 reviewers and presented before scientific committees of universities and research institutes. In each of the thesis listed in Annex 5 there is a reference to ISAFRUIT project within which the theses were initiated and completed. Granting a chance to work on PhD thesis within ISAFRUIT stimulated hard work of the employed young scientists, and this was advantageous for the project. Results obtained within ISAFRUIT may definitely shift consumption of one commodity to another one. This may be the case of a shift from clear juices toward cloudy juices, which according to results obtained in Pillar 2 are definitely healthier. This was expected at the beginning when theses of pillar 2 and 3 were formulated (in Annex 1 it was stated "*the evaluation of health effects of processed fruit products in particular patient groups is yet another field where fruit processing technology may create new markets and competitiveness*"). The shift

[2] In Poland there was a significant increase (by 18,8%) of juice consumption in 2009 versus 2008, which may primarily be attributed to the '5-a-Day' campaign, supported by EU and the Polish government (AIJN European Fruit Juice Association market report 2010, Liquid fruit).

from clear to cloudy must however proceed reasonably slowly not to impart negative effects for the industry. Consequently, the change will strengthen EU industry (Annex 1: ***Technology-Competitiveness:*** *Improvement of the technology to produce direct juices and similar products, manufactured at the site of fruit production will stimulate development of local industry and will increase its competitiveness on the global market*).

3. Relationships between scientific, technological objectives of Pillar 3 and work packages for the period 2006 –2010

Objectives	Work package	Relationship
A. Increase fruit quality	3.1	It was proved that quality of minimally processed apples may be enhanced through selection of apple cultivars with low browning tendency and proper sensory parameters, texture was considered especially important. SME partner has been interested in use of particular cultivars. Tests with browning inhibitors and firming agents also showed potential usefulness. Implementation of results into practice is feasible. Apple desserts with nutraceuticals (BeneoHSI or with BeneoHSI + Beneo-Orafti) received acceptability scores equal to or better than the control; the inclusion of pomace on its own reduced acceptability. With peach, BeneoHSI improved sensory acceptability compared to the control. Trials conducted on the application of nutraceuticals to apple puree desserts processed by cook-chill, freeze-chill and *sous vide* proved that *sous vide* was the most satisfactory of the three processes in terms of logistics and product quality. For this reason, these trials were extended to plums and peaches. In all deserts there was excellent oligosaccharide retention (at least 94%) and this was attributed to the mild *sous vide* process.
	3.2	Knowledge on raw material composition and processing value (fruit species and cultivars) has been enlarged. Data collected on chemical composition of raw material and juices are the bases for control of finished products on the marked, to prevent their adulteration. Experiments to improve processing technology to increase biological value of processed products were successful. It was proved that cloudy juices and nectars made of black currant and plums have higher antioxidative activity due to higher content of ascorbic acid, polyphenolic compounds (including antocyanins and procyanidins) then clear products. Recommendations for the industry on ripeness degree of raw material, use of specific enzymes, doses of ascorbic acid in plum juice production and product storage have been prepared. Information on currant achievements have been transferred to processing industry.
	3.3	It was proved that technology of osmotic dehydration may be used as an initial pre-treatment of sour cherries and black currant, increasing sensory properties of final products. Use of invert sugar enhances sensory properties and texture of the products obtained largely than sucrose. Texture of product improved with extension of dehydration time. New product – sour cherries dehydrated in a mixture of concentrated apple juice and invert sugar were in consumer tests more appreciated than available on the market rasins. Conditions of fruit dehydration were optimised to improve sensory quality and dietary value. Products were enriched with functional ingredients. Processing industry showed interest in developed semi-finished products (produced experimentally by SME) to be included into their final products. Crunchy fruit bars developed by SME are considered as an interesting product due to their nutritious and excellent sensory properties.
	3.4	Components extracted from pomaces increased the content of health pro-

		moting compounds, however did not improve organoleptic quality of enriched juices. Other ways of their utilisation have been looked for, e.g. addition to desserts produced within WP 3.1. Basic research carried out within this package opens new way for better extraction of biologically important components from pomace/raw material to final products.
B. Increase fruit safety	3.1	Vulnerability of raw material to support growth or survival of foodborn pathoges (FBP) on a few species of fruit was established (pineapple the most resistant). Out of about 200 strains of microorganisms tested two proved to be very effective in reducing *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* in fresh-cut apples and peaches.- for one of them with potential commercial application patent document has been prepared. It was also proved that LGG is able to reduce *L. monocytogenes* population on fresh-cut apples by 1-log unit but it has no effect against *Salmonella*. Several GRAS substances were tested on fresh-cut apples against *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes*. Peroxyacetic acid and hydrogen peroxide showed the best results and were safe to use breaking down to safe and environmentally friendly residues. Peroxyacetic acid was selected to be combined with High Pressure Treatment (HPP) to obtain a safer novel product. Optimal conditions of microwave pasteurization and HPP treatment were established. HPP significantly reduced the microbial load and could be an alternative to heat treatments, especially in apple purée and smoothies. However, storage temperature for these products should be 5 ºC or even lower, as some of the studied microorganisms were able to grow at 10 ºC.
C. More convenience	3.1	Extension of shelf life of minimally processed fruit through use of microwave and high pressure processing has been investigated, and looks promising. The shelf life of processed apple products, and specifically a convenient apple puree desert with nutraceuticals, had high quality shelf life of at least 12 days at 2÷4 ºC. A few different kinds of ready-to eat minimally processed desserts were developed. High pressure processing is a suitable technology for processing smoothies and the product had good physicochemical, sensory and microbiological properties/scores during storage for 30 days at 2÷4 ºC.
	3.3	Consumer tests showed that market would accept dried fruit with functional properties. This allows designing new brand of breakfast products (freeze-dried, conventionally dried - flexi version, dried fruit enriched in prebiotics) and dried fruit in the form of a bar, considering their quality and convenience. SME plans to start production before end of the project and put the product on the market through wholesaler ‘Vita Nature’ (Bogucin, PL).
D. Better availability	3.1	Work on minimally processed fruit deserts was successful. SME producer has been cooperating with WP 3.1 partners labs. SME intends to introduce new products on the market to meet demands of consumers. Among the new products are fruit salads with nutraceutical and other bioactive compounds. Probiotic apple wedges containing *Lactobacillus rhamnosus* are an alternative for people allergic or intolerant to dairy products and are highly desirable in the market place. Commercialisation of the outcomes is being progressed by Nature’s Best Ltd and Valio, Finland (who make a powdered-based version of the bacterium) in cooperation with AFRC.
	3.2	Positive results at laboratory scale were obtained with production of clear and cloudy plum juices and cloudy black currant juices. These were apparently not available on EU market yet (at least not in Poland), despite abundance of raw material in Europe. Industrial trials were made on plum juice production. For production of cloudy juices from coloured fruit to

		obtain high stability of turbidity a mixture of macerating enzymes must be used, with low activity of pectinmethylesterase and high activity of pectin lyase. Widely scheduled trials of scab resistant cloudy apple juices may facilitate the industry introduction of cloudy juices on the European market giving the consumer better quality juices.
	3.3	A database on consumers' expectations toward dried fruit was collected creating the starting point for new product development. Work on new products has been carried out by SME in cooperation with research institutes and university and ended with success. Two types of new products were prepared: flexi breakfast fruit and crunchy fruit snacks in the form of a bar. In the later case two kinds of products are to be launched in 2010: Crispy Fruit" - Apple Cubes and "Crispy Fruit" - Cherry Cubes. First batches of crispy fruit were delivered to Polish specialty shops to be sold through 'healthy food shelf'.
	3.4	Precise knowledge was gained on composition of pomaces from various fruit (apples, black currant, sour cherries, and plums). Possibilities of better utilisation of different fractions of pomaces and extracts have been investigated. Methods of extraction of biologically active substances (including polyphenols) have been worked out.
E. Raise consciousness	3.2	Lectures for the fruit growers in Poland and contribution to ISAFRUIT leaflet concerning quality of cloudy juices raise consciousness of the consumers. In promotion of a product, information on fruit health properties becomes extremely important. Consumer tests conducted in supermarkets, besides acquiring the data on consumers expectations also proimoted fruit products.
	3.3	Dried black currants served to consumers with information on nutrients content were significantly higher appreciated than those served without such information. This was not the case for dried sour cherries, which were appreciated primarily for their taste not nutrients content.
F. Lower Fruit price	Not planned within Pillar 3, however, data on producer, wholesaler and market prices of fruit in Poland were supplied by Pillar 3 to collaborator in Switzerland for analysis and comparison with other countries.	

Description of achievements within each work package during the course of the project

In Annex 1 the specific goals to be achieved within Pillar 3 were formulated for each work package and their fulfilment are described:

Work Package (WP) 3.1 Stability and quality of minimally processed fruits and novel products *(MPFRUIT)*. Leader: 34, NFC (Dr. Ronan Gormley - after his retirement the leadership was taken over by Dr. Nigel Brunton); other partners: 4, IRTA; 23 UdL; 56, NBL. Work period 0÷57 mo.

1. To develop a new generation of fruit products with added value, i.e. extended shelf-life, high technical quality, good consumer acceptance, nutritive value and safety, by using minimal processing (tasks[3]: 3.1.2; 3.1.3; 3.1.4; 3.1.5; 3.1.9).
2. To elucidate the effect of interactions between raw materials (including added ingredients) and the minimal processing techniques on product quality attributes as a route to the selection of raw materials based on the most suitable varieties and husbandry

[3] Tasks are listed in Annex 2

practices (tasks: 3.1.1; 3.1.2; 3.1.3).

3. To develop and validate a fast and safe new industrial technology for producing novel fruit products, including the safety of packaging materials used in micro waving, and to compare product quality and shelf life of new developed products versus traditional ones (tasks:3.1.2; 3.1.3; 3.1.5; 3.1.6; 3.1.7).
4. To develop a comprehensive marketing plan for project outcomes based on strong dissemination and technology transfer procedures that will ensure effective dissemination of results to all potential end-users, and especially to SMEs (task 3.1.8).

Ad 1. Fruit salads with nutraceutical and other bioactive agents were developed

Minimally processed fresh-cut **apple wedges containing nutraceuticals,** namely Beneo™ P95, which is a prebiotic, dietary fibre and sweetener were developed. Apple circles were produced containing Beneo™ P95 and Aquamin (highly available algal calcium) using laboratory scale equipment. The task here was to apply the prebiotic to apple wedges using pilot scale vacuum infusion equipment, i.e. Inject Star vacuum tumbler. Prebiotics act as a feedstock and encourage proliferation of the beneficial bacteria in the human gut thereby 'protecting' gut health. The infusion trial gave apple wedges with a modest content of Beneo™ P95 (and hence a modest prebiotic effect) in the absence of AS1; the content of infused Beneo™ P95 was lower in the presence of AS1 (browning inhibitor). The level of Beneo™ P95 infusion in the wedges was less than that found previously for Braeburn circles (thinner cross section than wedges). The trial with edible coatings applied to apple wedges gave mixed results. The alginate used had good adherence to the apple wedges but did not totally overcome the translucency problem found in vacuum-infused apple wedges.

Probiotic apple wedges (D 3.1.10, D 3.1.43) containing the microorganism *Lactobacillus rhamnosus* (Lbs-r) were developed. Such a product is desirable in the market place as many consumers are allergic or intolerant to dairy products and are precluded from eating probiotic yoghurts. Probiotic apple slices are thus an alternative for people allergic to dairy products. Physicochemical and sensory evaluation indicated that dipping apple slices in a probiotic solution resulted in slices of acceptable quality with sufficient numbers of Lbs-r adsorbed on the surface for a probiotic effect. Moreover, *Lact. rhamnosus* reduced growth of *L. monocytogenes* on apple slices stored at 5 and 10ºC.

Another product developed in this workpackage was so called **synbiotic fresh-cut apple wedges** (D 3.1.42) containing Lbs-r and prebiotics in the form of oligofructose and inulin. Synbiotic fresh-cut apple wedges with a shelf life of at least 14d/2÷4 ºC were successfully produced in these trials. Fructan analysis showed that the prebiotic inclusions in the apple wedges remained relatively stable over 14 days at 2÷4 ºC. All samples sets contained ca. 10^8 cfu/g of probiotic bacteria over the test period, which is sufficient for a probiotic effect, and is comparable to counts of probiotic bacteria in commercially available dairy products. Browning index, firmness, acidity and dry matter remained stable throughout the 14 days, and applying prebiotic coatings resulted in an increase in soluble solids in the wedges. Sensory assessment indicated that synbiotic apple wedges had high levels of acceptability. HPLC analysis showed that levels of polyphenolic compounds in the apple wedges decreased during storage. No difference was found between O_2 and CO_2 headspace concentration and volatile production of synbiotic samples and samples only treated with probiotics.

Freeze-chill and sous vide processes were tested as an alternative technique of preservation of ready desserts. Products prepared in the form of an apple puree (with nutraceuticals in the form of inulin and apple pomace) were processed (by chilling, freezing, *sous vide*) and tested (D 3.1.2, D 3.1.23). The products had good sensory acceptability, especially when it is considered that the samples were unsweetened (except for samples with BeneoTM as this inclusion has sweetening properties). The levels of inclusion of BeneoTM HSI (8%) and AIS (10%) were significant in functional food terms resulting in a increase in the dietary fibre and prebiotic content of the samples respectively. The shelf life of *sous-vide* and freeze chilled apple purees with added nutraceuticals was also assessed over a 30 day period (D 3.1.44). Apple puree desserts (with nutraceuticals) processed by *sous vide* had a high quality shelf life of at least 30 days at 2÷4 ºC based on the physicochemical tests used. The retention of Beneo HSI™ during storage was >90% (D 3.1.45) indicating minimal hydrolysis in the apple puree. For apple purees with added pomace results show that pomace inclusions give a darker colour in *sous vide* processed apple purees from ‘Bramley’ apples compared with a control. However, the effect was much smaller with pomace from ‘Shampion’ apples, and suggests that this pomace has potential as a functional ingredient (i.e. as a provider of dietary fibre and antioxidants) in fruit based ready-desserts. The samples with ‘Shampion’ pomace also received good sensory scores but some tasters detected graininess in product texture due, presumably, to pomace inclusion. A second generation of apple purees/wedgees were also developed (D 3.1.13). These had accompaniments (cream, aerosol cream or custard) and processed by chilling, freeze-chilling or sous vide methods. Ready-desserts that do feature on the market are usually ’heavy’ in dairy products, e.g. yoghurt-like products with a small amount of fruit. The focus here was the opposite, i.e. to produce ready-desserts high in fruit content with smaller amounts of other inclusions, i.e., cream, soy-cream or custard. These desserts were assessed in terms of their appearance, flavour, texture and consistency. The wedge samples looked better than the purees. Of the accompaniments in-pack, samples with cream looked good but the aerosol cream lost its structure, probably due to the vacuum treatment of the bags. All samples, with the exception of aerosol cream, tasted too sour due to the high acidity and astringency of the ‘Bramley’ apples. The samples with aerosol cream were sweeter as the aerosol cream contained sugar. The *sous-vide* samples had the best texture (firmest) and the freeze-chilled ones were too soft. This was reflected in the instrumental texture tests. Presumably, the over-soft texture of the freeze-chill samples was due to tissue damage during freezing. The apple purees with custard had a low consistency, which was not affected by freeze-chilling. The chilled samples with cream and aerosol cream had the highest consistencies, but values were lowered by freeze-chilling or *sous vide* processing, especially in the case of samples with aerosol cream. A third generation product was also developed which consisted of ready-dessert apple products (purees) with accompaniments (cream, soy-cream or custard) and with nutraceuticals (D 3.1.23). The nutraceuticals included in the 3rd generation products were a prebiotic (BeneoTM HSI) and an antioxidant dietary fibre (apple pomace from ’Shampion’ apples). Physicochemical and sensory assessment indicated that these third generation products had good physicochemical and sensory properties and are now ready for commercialisation. In addition, investigation if the added prebiotic was stable during storage of the apple puree based desserts was carried out. While both thermal and HPP processing did result in hydrolysis of the prebiotic inclusions, storage of the purees did not result in any further increase in hydrolysis. These products are potential new entrants to the ready-dessert range currantly available in retail outlets.

The survival of foodborne pathogens on fresh-cut fruit packaged under different conditions was also tested. This was to understand the behaviour of pathogenic bacteria on low-pH food matrices, which have been considered 'safe' from microbiological point of view.

Growth of *Salmonella*, *L. monocytogenes* (*L. innocua*) and *E. coli* O157:H7 was determined in fresh-cut apples and peaches, as planned in the technical annex. Challenge tests included: effect of variety, storage temperature, packaging atmosphere and the use of an antioxidant treatment (D 3.1.6 and D 3.1.32). In these experiments, films were provided by Nature's Best Ltd. (Partner 56). Fruits were provided by IRTA-Lleida (Partner 4). Experiments with fresh-cut melon and pineapple were also carried out as they are often used in fruit salads. Results (D 3.1.26) confirmed that the three studied pathogens were able to survive and even grow at refrigeration temperatures and were not influenced by packaging atmosphere and antioxidant treatment. Risk of growth was higher in melon, as its pH was not as low as for apple, whilst no growth and low survival was observed in pineapple. The three pathogens grew on apple and peach at ambient temperature. At refrigeration temperature, only *L. innocua* was able to grow but the other pathogens survived during the storage period.

Concurrently with work on new products, experiments were carried out on the feasibility of using **alternative disinfectants** to replace chemical preservatives such as sodium hypochlorite for the production of minimally processed fruits. These alternatives should be more environmentally friendly and safer for consumers.

In particular the use of biopreservation (biocontrol agents or biopreservative cultures and bacteriocins) and GRAS substances (organic acids, essential oils, ...) on reducing FBPs population and preventing FBPs growth on fresh-cut apples and peaches was investigated. With regard to **biocontrol agents or biopreservative cultures**, about 200 strains of microorganisms isolated from the surface of apples, peaches, nectarines and fresh-cut fruit samples were tested against a strain of *E. coli* O157:H7 on fresh-cut apples and peaches at *in vivo* conditions. The best microorganisms were also tested against *Salmonella* and *L. monocytogenes* at room and refrigeration temperature. Two microorganisms proved to be very effective in reducing *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* in fresh-cut apples and peaches under laboratory conditions (D 3.1.7). Experiments to determine optimal concentrations, mode of action and determination of pathogenicity on tobacco plants were carried out and the strains were identified. One of these was identified as a new species and the other belongs to *Pseudomonas* spp.. Their effect on fruit quality was also determined (D 3.1.28). One of the biocontrol agents was tested against *Salmonella* and *L. monocytogenes* on fresh-cut apples at conditions simulating commercial application (with antioxidant and packaged in passive modified atmosphere packaging and stored at refrigeration conditions). Although the reduction of food borne pathogens tested was significant it is recommended to use the biocontrol agents in combination with a previous disinfection treatment. Considering the results obtained and the opinion of a patent's agency the patent document is being written and it is expected to be submitted this year. For this reason there are no publications on biopreservative cultures. Additionally, in collaboration with AFRC, the antimicrobial effect of the probiotic strain LGG against *L. monocytogenes* and *Salmonella* on fresh-cut apples was tested. Results demonstrated at semi-commercial conditions that LGG is able to reduce *L. monocytogenes* population on fresh-cut apples by 1-log unit but it has no effect against *Salmonella*.

Another biopreservation method studied by IRTA team was the use of bacteriocins (antimicrobial peptides mainly produced by lactic acid bacteria). More than 100 lactic acid bacteria were isolated from fruits and vegetables and their ability to produce bacteriocins was tested at *in vitro* conditions. None of the tested strains produced bacteriocins (D 3.1.16). Afterwards, the efficacy of a commercial nisin preparation to reduce *L. monocytogenes* on fresh-cut apple and melon (for their different pH) was evaluated. Results indicated that the efficacy of nisin was equivalent to a standard of sodium hypochlorite treatment (D 3.1.29).

The effect of some **GRAS substances** (organic acids, vanillin, carvacrol, Citrox, hydrogen peroxide, peroxyacetic acid and many others) has been also tested on fresh-cut apples against *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes*. Peroxyacetic acid and hydrogen peroxide showed the best results (D 3.1.30). Reductions observed were not higher than 2.2-log units. The advantage of peroxyacetic acid and hydrogen peroxide is that they decompose to safe and environmentally friendly residues. Peroxyacetic acid was selected to be combined with High Pressure Treatment (HPP) to obtain a safer novel product - HPP treated apple slices. The experiment was carried out in collaboration with IRTA-Monells and UdL.

Spanish partners also collaborated in experiments to determine the antimicrobial effect - of polyphenol fruit extracts provided by TU-Lodz (2 batches of samples) and VdV. Anti-microbial efficacy has been tested at *in vitro* conditions and against 5 bacteria (4 human pathogens and 1 spoilage, D 3.1.30).

Conclusions

1. A number of new mostly apple based products have been developed and are ready for commercialisation. Of these probiotic apple slices and *sous vide* desserts may have the greatest chance to be implemented into practice.
2. Recommendations regarding the effective use of biocontrol and GRAS agents to extend the shelf life of minimally processed fruit have been made.

Ad 2. The effect of interactions between raw materials (including added ingredients) and the minimal processing techniques were elucidated

The effect of interactions was studied on an ongoing basis to find out the best raw material for particular process and products. Evaluation of suitable cultivars is an essential requirement for production of minimally processed apples. Fresh-cut fruit salads with a shelf life of up to 10 days at 4 °C are an increasing feature on supermarket shelves in a number of countries. Consumers purchase them for convenience and for health reasons (the five-a-day message) and they represent considerable added value over and above the constituent fruits. The composition of the salads varies, but apple slices are usually a significant component. A low browning tendency (colour retention post-processing), crisp texture and good sensory acceptability are essential quality criteria for apple wedges used in these salads. A good high quality shelf life is also a key feature as it aids distribution and retailing, and allows short-term refrigeration in the home before consumption. Considering that development of products requires testing raw material for process applications and for use in ready-desserts one of the aims was to evaluate the suitability of apple cultivars and other components obtained from ISAFRUIT partners.

Apples, peaches and related items (e.g. pomaces, other fruit fractions, etc.) were sourced from the project partners (based on their recommendations on high-performing cultivars from different husbandry regimes) and locally (to take account of special local cultivars, e.g. ‘Bramley’s Seedling’ apples) for use in minimal processing trials. This work continued throughout the project and facilitated exchange of ideas and information amongst partners in ISAFRUIT. For example apple cultivars were sourced from RIPF in Poland (partner 11) for three years running and suitability of these cultivars for use in ready desserts and fresh cut fruit salads was assessed by determining their physicochemical (colour, acidity, total soluble solids, sensory acceptability). Apple pomace samples were also obtained from RIPF, Poland throughout the course of the project and these were used as a functional ingredient in ready made desserts. NBL has provided NatureSeal and packaging films and TU-Lodz provided polyphenol samples. In addition, samples of the probiotic *Lactobacillus rhamnosus* LGG were exchanged between AFRC and IRTA (partner 4). This probiotic was used as an added functional ingredient for minimally processed apple wedges at AFRC and it's efficacy against pathogenic bacteria was assessed at IRTA Lleida. In addition, UDL have shared bacteria strains and protocols with IRTA-Lleida. In addition, exchange of methods between partners took place. For example high pressure protocols developed at AFRC on a laboratory scale were replicated at pilot scale by IRTA Monells.

Twelve apple cultivars were tested in trials carried out by AFRC, over two years. Results indicated that the cultivars 'Gloster', 'Cortland', 'Alwa', 'Bramley' and 'Granny' were closely grouped in terms of their suitability for processed desserts based on these tests (D 3.1.1, D 3.1.21, D 3.1.23, D 3.1.41). The remaining seven cultivars are less suitable with 'Jonagold' and 'Rajka' as the lowest performers overall. The next objective was to develop protocols for the application of freeze-chill and sous-vide processes to a range of fruit products (phase 1) with emphasis on apples at this stage in the project. This embraced a number of trials using these processes to deliver minimally and fully processed products, with added nutraceuticals, for use as ready-desserts in both puree and apple wedge formats. For apple wedges the results show a large difference between cultivars and between processes for a number of the quality parameters. 'Bramley' was the only 'cooking' cultivar of the four tested and its lack of sweetness and its high acidity was evident in the sensory tests. However, this cultivar performed well in respect of slice retention, the lowest colour change pre- to post-processing, the lowest gravity drip, and the second firmest wedges (after 'Granny') post-processing.

There were large differences between the physicochemical properties and antioxidant status of the different cultivars with 'Topaz', 'Gloster' and 'Idared' exhibiting the best potential for fresh-cut fruit salad applications. 'Shampion' cv. which was characterized by a low browning tendency (due to low polyphenoloxidase activity) seemed potentially an interesting cultivar for fruit salads. However, due to too low firmness, its texture was not acceptable irrespectively if treated by MCP or not. Dipping in browning inhibitor AS1 had a significant beneficial effect on colour retention during storage at 2÷4 °C for 5 days and caused slice firming.

In microwave processing of apple puree it was found that reduction in levels of gram-negative bacteria (*E. coli* O157:H7 NCTT12900) and gram-positive bacteria (*Listeria innocua* CECT-917) was slightly lower in 'Shampion' cv. than e.g. in 'Granny Smith', which indicates that the apple cultivars are an important factor to be taken into account (D.3.1.39).

Not all fruit wedges can be processed by HPP as some of them suffer from textural and sensorial modification following processing. Experiments showed that apple wedges tend to be more affected by treatment than peaches. In addition, deliverable D 1.36 has proven that for the some fruits, especially apples, cultivar has an important impact on the acceptability of the fruit product processed by HPP. Of the seventeen cultivars tested, only seven were not rejected. In the case of peaches, the cultivars selected had similar ºBrix and hardness and were equally affected by HPP.

Conclusions

1. Selection of an appropriate cultivar is essential for good quality products. Recommendations were made as to which cultivars should be used and which should definitely be rejected.
2. Enzymatic browning problems can be avoided by choosing cultivars with low browning tendencies or the use of antibrowning agents.

Ad. 3. To develop and validate a fast and safe new industrial technology

Microwave processing. In development of new products by using innovative techniques a series of experiments were conducted to assess the key factors involved in a mild (below 1 minute) microwave processing. This technology gives also the opportunity to save energy leading to a reduction of production cost andmaking it more environment friendly.

To be successful fruit products should have a shelf life of at least 15 days, should be devoid of the cooking flavour occurring above 85 ºC and should allow inactivation of enzymes present in the product. Microwave treatment of food products implies involvement of knowledge of several parameters, chemical composition, dielectric properties, geometry and process of the products. This technology was selected considering that it could be treated as a minimal process due to small heat load compared to traditional pasteurisation. Three different apples cultivars have been studied; 'Granny Smith' and 'Braeburn' were supplied by a local cooperative growers and 'Shampion' was supplied by partner 11. Most of the studies were conducted on 'Granny Smith' apples that are easily obtainable in Spain. First experiments using a laboratory Microwave system were carried out in order to design apple products and to establish a minimal microwave treatment (D 3.1.4). Further tests were made using a pilot microwave tunnel. The tunnel had a maximum power of 24 kW, delivered by 12 adjustable magnetrons. Most of the work, using the pilot scale microwave tunnel, was made to establish:

a) Appropriate microwave process (speed, power, product weight, etc.) in accordance with temperature parameters defined in laboratory experiments,
b) Optimal packaging tray size and shape,
c) Estimation of a the shelf life with the new packaging,
d) Sensory and nutritional analysis of apple product processed by microwave technique.

Apple pure was a commodity of special interest. A few packaging films and trays were tested to obtain maximum product temperature between 70 ºC and 90 ºC and a heating rate of 2.0 ºC/s. In the first experiments (using a conveyor speed of 200 mm/min and a power of 6.0 kW to 9.0 kW) the heating rate could not be achieved (it was less than 1.0 ºC/s) and parameters of the tunnel had to be adjusted. Experiment with new films and packages ended with success (D 3.1.15) and shelf life of produced apple puree was monitored. To maintain the integrity of the packaging a maximum power used was 18 kW, a belt speed 200 cm/min and a time in the chamber 55 s. Under these conditions the average temperature profile gives a heating rate of 1.5 ºC/s with a maximum temperature of 72.6 ºC.

Final products were analysed for sensory properties such as colour, viscosity and acidity as well as vitamin C and total polyphenols levels. Not all packaging may be used in microwave processing, the most resistant ones are trays made of Polypropylene (PP) and Crystallized Polyethylene Terephthalate (CPET). Another point is the lidding material or films. For a treatment at 70-80 ºC, there are films on the market which are flexible enough to absorb the vapour generated during processing, with good barrier to oxygen and anti-fog. For temperatures above 80 ºC the vapour generated during processing has to escape from the packaging through a hole made in the film or using a valve. In the first solution the hole will be sealed by a food compatible label (D 3.1.27).

The effect of different microwave treatments on the viability of the best novel products selected by IRTA-Monells on the viability was determined on different foodborne pathogens (artificially inoculated samples) and on indigenous microbiota (total aerobic plate count) was determined. Studied products were apple slices and a formulated apple ('Granny Smith' and 'Shampion') purée (D 3.1.5). The effect of the selected microwave treatment for 'Granny Smith' apple purée on artificially and inoculated samples and stability of the product stored at 5 and 10 ºC for 21 days was also determined (D 3.1.18). *L. innocua* population remained below the detection limit during the whole experiment and microbial counts of uninoculated (control) samples remained very low (around 100 cfu/g). The effect of microwave treatment on standard and nutritional quality of purée and compared it with a pasteurization process was also investigated. The results demonstrated that microwaving at 900 W for 35 s could be a minimal process to reduce microbial contamination of apple purée, in particular *L. innocua*. However, it does not completely eliminate *E. coli* O157:H7 and indigenous microflora, which means this novel product must be stored at refrigeration temperatures. The combination of low pH and storage temperature represents a hurdle that some microorganisms could not overcome (D 3.1.18).

It was concluded that apple cultivar, sample geometry and packaging design play an important role in the success or failure of obtaining a product with fresh appearance and a shelf life of 15 days. A modification of any of these parameters, especially packaging and geometry will require a modification of the parameters of microwave processing. Based on the mild pasteurization of apple purée, an estimation of the productivity and cost was done, giving a price of 0.12 cents per kilogram of fruit product (D 3.1.27).

High Pressure Processing (HPP). High pressure processing (HPP) has been proposed as an alternative method to decontaminate fruit products without heat in order to create a fresh like fruit product with an appreciable microbiological stability and a good retention of nutritional quality attributes. In recent times, HPP technology has proven to be an interesting technology to pasteurize foodstuff without heat. Several studies (Fernández García et al., 2001a, 2001b; Lavinas et al., 2008; Nienaber & Shellhammer, 2001a, 2002b, Sánchez-Moreno et al., 2003; Guerrero-Beltran et al. 2004) have evaluated the impact of the technology on the micro-biological stability and on the retention of nutritional quality attributes of fruit and vegetable products. At industrial level the main application is the processing of fruit juices, mainly of the ‘smoothies’ type.

As part of ISAFRUIT the suitability and evaluation of other fruit products have been the subject of investigation. Most work was conducted at IRTA using pilot scale equipment and some was replicated at AFRC using laboratory scale equipment. IRTA focused its research on different types of fruit preparations treated by High Pressure Processing (HPP) and compared with the traditional process. Three types of products were studied: Granny Smith apple purée, different apple cultivars peeled and cut in wedges and yellow-fleshed peaches cut in pieces. All this products were submitted to different HP processes at different pressures (D 3.1.36). Normally samples were processed in a vacuum packaging with a low permeability to oxygen. In the case of apple purée, metallic bags were used to reduce oxidation. Depending on the final application appropriate packaging was selected.

None of the HPP treatments used in the experiment were fully successful in preventing undesirable changes during shelf life of **apple puree**. Nevertheless based on the results some positive aspects can be outlined. The 400 MPa pressurisation did not affect substantially the initial nutritional quality of the Granny Smith purée. T|he time required for loss of 50% of total vitamin C was reached 10 days. Based on colour parameters $L*$, $a*$ and $b*$, high-pressure treatment did not fully suppress oxidative browning during cold storage. Quality constraints could be overcome by using apple cultivar with lower browning tendency or by incorporating more powerful antibrowning agents (D 3.1.36).

The effectiveness of high hydrostatic pressure (HPP) treatment at 600 MPa using an pilot scale equipment (Hyperbaric Wave 6500/120) with an ultra high pressure process at 900 MPa on a prototype equipment on the shelf-life of ‘Granny Smith’ (GS) apple purée was also studied (D 3.1.17). A mild Conventional pasteurisation process at 75 ºC served as control process. The sensorial shelf-life based on colour measurement was monitored over the storage period of 21 days at refrigeration conditions (4 ºC ± 1 ºC). In another trial GS apple pieces were submitted to 900 MPa pressurisation and the colour deterioration analysed.

The results obtained suggest, that the 900 MPa cycle could partially inactivate the polyphenol oxidase enzyme, which is responsible for enzymatic browning reactions in fruits and vegetables (a* values of the 600 MPa treated samples were affected adversely during cold storage, whereas those for 900 MPa samples showed good stability).

Results of experiments on **apple wedges** produced of 'Granny Smith and later of 'Fuji' and 'Pink Lady' apples were not satisfactory from a sensory point of view - colour, texture, taste were strongly modified by the treatment and were very different from the control samples. The alteration of the colour of 'Granny Smith' apple pieces during HHP process at 900 MPa indicates that there is not much prospect for future use of this product. Contrary to the apple purée in apple pieces the polyphenol oxidase enzyme was probably not inactivated by the process. Therefore it was concluded that HPP it is not useful for preservation of fresh cut apple products, unless other ingredients are incorporated that impair changes in sensory parameters. More promising results were obtained with **peach pieces**. Considering sensorial qualities, after an initial modification, colour remained unchanged during the storage period. HPP processing affected the hardness and cohesiveness of peach pieces, but during the storage time initial hardness (post treatment) of peach samples was retained.

Following experiments conducted at IRTA and AFRC some general conclusions may be drawn with respect to design of a HPP treatment for fruits products. Results have shown the importance of the composition of raw materials. Not all apple varieties and peach varieties are suitable for such process. Another important aspect, fruits processed by HPP should be fruit products or desserts based on fruits and not fresh cut fruits (D 3.1.37). Depending on the products (fruits wedges or juices), shelf life is in the range of 21÷30 days. Because most of the undesirable effects occur during the processing (during storage investigated in these studies physical and chemical parameters remained mostly unchanged, except ascorbic acid), the first step is to conduct sensorial and nutritional studies comparing samples before and after treatment. In a second step, the shelf life can be determined for the products followed by a validation of the stability of the sensorial and nutritional qualities. Finally, following the cost calculated for the processing an optimisation of the process can be undertaken.

HPP treatment can be used to obtain a fresh like fruit product/dessert with a reasonable processing cost. The average cost for such processing is around 0.21 €/kg of product; to reduce this cost the solution is to reduce the holding time, if the microbiology is unchanged, to improve operation duration by day and to change the equipment if the market is mature.

A series of analysis were conducted at UDL and IRTA to check effects of HPP on the microbiological stability of obtained products. Studies were conducted on the survival of indigenous microbiota after pressurization and during shelf-life. Products studied were peach ('Ryan Sun') and apple ('Fuji') slices and a mixture of apple ('Granny Smith') puree csyrup and acerola. Three processes were selected for microbiological studies: 'Granny Smith' apple purée treated at 400 and 600 MPa for 5 min at 20 ºC; 'Fuji' apple slices treated at 350 MPA for 3 min at 12 ºC and 'Ryan Sun' peach slices treated at 300 and 400 MPa for 5 min at 20 ºC. The effect of these treatments on indigenous microbiota (natural contamination) and microbial stability during at least 21 days of storage at 2 temperatures (5 and 10 ºC) were determined (D 3.1.31). In some cases, standard quality (colour, acidity, soluble solids and texture) were determined in parallel with IRTA. From the point of view of microbial quality, it was concluded that HPP significantly reduced the microbial load and could be an alternative to heat treatments, especially in apple purée. However, its efficacy against foodborne pathogens could not be investigated as the use of inoculated samples was not permitted in the pilot plant.

A storage temperature for these products of 5 ºC or lower was recoomended for these products, as some of the studied microorganisms were able to grow at 10 ºC. Finally, the effect of combining HPP with antimicrobial treatments on the indigenous microbiota of apple slices and its stability during shelf life (D 3.1.46) was determined.

Conclusions

1. Out of the two novel technologies tested (microwave and high pressure processing) microwave seems to have a greater chance to be implemented for minimally processed fruit, due to its higher adaptability to processing different products.
2. High pressure processing maybe recommended for processing liquid or semi-liquid products such as smoothies and purees, but not for fruit pieces, due to problems with browning and loss of texture and aroma.

Ad 4. To develop a comprehensive marketing plan

A blueprint concerning the effective dissemination of selected information from WP 3.1 to scientists and nutritionists, but especially the industry and embraces food and other SMEs, fruit packers, distributors, retailers, and also consumers. This blueprint charts a number of routes for the dissemination of outcomes from the ISAFRUIT project, and specifically from WP 3.1 and Pillar 3 to end-users. There are many outcomes from WP 3.1 some of which are confidential. However, most of the information is likely to be of a public good nature. End-users are important and this embraces all those in the production, processing, marketing, retailing and consumption (i.e. consumers) of fruit and fruit products and those in parallel fields such as dieticians and nutritionists. Members of ISAFRUIT must be aware of their research outcomes and of potential end-users to ensure potential patents are not compromised. This requires close liaison with the patents officer in ISAFRUIT. Based on this, a selection can be made between sensitive information and general information which is suitable for widespread diffusion. Research outcomes can be regarded as scientific or technological ‘currency’ that has currant and latent potential. R & D outcomes have considerable latent value.

Targeting the information for end-users is of critical importance and a ‘horses-for-courses’ approach should be adopted. Information for non-scientists must be in user-friendly form that is succinct, short and easily understood without complex terms, and with recommendations to the fore. It should show a potential profit motive, which will encourage greater utilisation and uptake by end-users.

Potential end-users include fruit producers, packers, food processors, marketing specialists/ organisations, distributors, retailers, consumers and health professionals. Consumer groups and consumers are by far the most difficult end-user group to reach, link-with and engage in terms of dissemination. Small-to-medium-sized enterprises (SMEs) are more easily reached and are very important in employment and output terms in Europe. In transferring technical information to food SMEs a number of factors should be considered; **1)** biulding trust; networking is the key to success in many cases **2)** sell solutions or information relevant to the company **3)** suppliers (of ingredients and equipment) can be used to reach small and very small companies. **4)** timing is important; you cannot communicate R&D when today's business is the currant SME priority **5)** ready-to-use material in the 'right language' is critical **6)** concentrate on companies that have the capability of being successful in R&D uptake.

Selecting an appropriate dissemination route is very important. A number of possible routes can be selected and appropriate consideration for each must be taken into acount.

1) Patents: The dilemma 'to publish or to patent' is ever-present for scientists. It has been calculated that a patent application takes 10 times as much time as a peer reviewed scientific publication and hence many scientists are reluctant to go the patent route. Some organisations/institutions have a number of patent officers who prepare the patent submissions.

2. Journals: Scientists should be proactive in publishing their data in peer reviewed journals and in technical and trade journals. A wide spectrum of journals should also be used in order to reach as many types end-user types as possible.

3. Presentations at conferences and workshops (+the plug-in concept): Scientists should engage with every opportunity to present ISAFRUIT findings at conferences, workshops and other fora. Some of these may be by invitation but others may have to be sought.

4. Workshops, RE*tu*ER workshops, conferences: Events could be industry workshops, or of a more general nature (farm to fork) embracing all actors in the fruit chain both fresh and processed, and also those in associated disciplines such nutritionists, food safety personnel, and the media.

5. Media: Media power is immense, but the media should be engaged-with on a selective basis. Every effort should be made to see a draft of the article to be published to ensure that it has not been shortened, or if it has, that it still conveys the correct message. However, the producer often edits the interview and the question of balance and delivery of the right message becomes a concern.

6 Personal contacts/visits/exchanges: This is a powerful dissemination route as it usually involves 1:1 or small group contacts and is verbal. It offers the opportunity for dialogue, clarification, re-clarification, synergy and feedback. It may also result in follow-on or phase 2 contacts which lead to even better dissemination and/or technology transfer and/or new business or other opportunities.

7 Websites: ISAFRUIT personnel should make full use of the project website both as users and contributors. Websites have the potential to be excellent dissemination routes but much depends on the design and the information included.

Conclusions

1. Be acutely aware of your research outcomes and their potential as patentable items
2. Maintain frequent contact with ISAFRUIT and institutional patent officers
3. Be proactive in harnessing the latent value of your research
4. Target information for end-users; i.e. user friendly language; demonstrate a potential profit motive
5. Focus particularly on dissemination to SMEs (see success factors above); small firms are the real giants of the European economy
6. Be proactive in publishing/presenting your research results in/at journals/workshops and conferences; use the 'plug-in' potential to include your information in conferences being organised by others; be aware of 'journal power'

7. Host national workshops on ISAFRUIT outcomes and invite all the actors in the fruit chain, i.e. a farm to fork approach
8. Liaise with the media - but on a selective basis!!
9. Use personal contacts/visits/exchanges when practical for dissemination; these usually involve 1:1 or small group contacts and are verbal

WP 3.2 Attractiveness and quality of direct juices, nectars and concentrates *(JUICETHIRSTY).* Leader: 11, RIPF (Prof. Dr. Witold Plocharski); other partners: 3, DFVF; 5, INRA-URC& INRA Av.; 21, KVL; 37, TU Lodz; 54, Alpex; 65, ESA. Work period 0÷57 mo.

1. Improve quality of direct juices/nectars and concentrates from red fruit by technology optimisation (e.g. using new enzymes) and selection of raw material (tasks: 3.2.2; 3.2.4).
2. Determine quality of single cultivar juices according to the Code of Practice of the European Fruit Juice Association (Association of the Industry of Juices and Nectars from Fruits and Vegetables) (task 3.2.5).
3. Selection of high acidity scab resistant apples suitable for sustainable orchard management and for juice/concentrate production, considering juice yield, and quality (tasks: 3.2.3; 3.2.4; 3.2.5).
4. To supply analytical data and products for juices/nectars to pillar 2 (tasks: 3.2.1; 3.2.5).
5. Elucidation of consumer perception of single cultivar juices and nectars (tasks: 3.2.3; 3.2.6).

Ad. 1. Improve quality of direct juices/nectars and concentrates from red fruit

General remarks

Red fruit are very rich dietary source of phenolic compounds, which characterize with healthy properties and antioxidative potential. Among red fruit blackcurrant and plum play an important role. Black currants are mostly processed into nectars, whereas plums are mostly dried. Due to lack of suitable technology plum juices are seldom produced on industrial scale (the exception is an extraction method used in prune juice production). Therefore, considering availability of raw material, work on plum juices was justified. Anticipating higher nutritional and health value of cloudy juices (being closer in composition to the starting material) experiments were directed toward production of cloudy blackcurrant and plum juices and nectars (D 3.2.5).

Technology optimization/development and cultivars testing.

Black currant juices and nectars. Experiments conducted aimed at improving technology of cloudy black currant juice or cloudy nectar production, selection of most appropriate cultivar and determination of quality of produced cloudy products in comparison to clear juice/nectar. The experiments were carried out in two stages: labaoratory and pilot scale. In the first stage technology of production of an acceptable cloudy product was investigated. To achieve this aim, four commercial enzymatic preparations were tested, which had different enzymatic activity. Pectinex BE Colour, Pectinex BE XXL, Rohapect PTE and Rohament PL. To optimize mash treatment different doses of enzymes and different treatment times and temperature were used (D 3.2.5). Conditions of enzymation of the mash had a pronounced effect on juice yield and stability of cloudiness. Use of macerating mixture composed of Rohapect PTE and Rohament PL (2:1) at a dose of 200 g/t allowed, at optimal conditions (50 °C, 1 hour treatment), to obtain cloudy juice having acceptable cloud stability and color. Low temperature treatment was adventageos for the sake of energy saving. Rohapect PTE characterizes with high pectic lyase (LP) activity and low activity of pectinmethylesterase (PME), whereas Rohament PL has high polygalacturonase activity (PG). Enzymatic preparations having both high PME and PG activity and low LP activity (Pectinex BE Colour and Pectinex BE XXL) allowed to obtain high juice yield, however, were not suitable for cloudy juice production. In the second stage of experiment, carried out at a pilot scale in two seasons, clear and cloudy nectars were produced out of 3 black currant cultivars ‘Tiben’, ‘Tisel’ and ‘Ben Lomond’, using technology developed in the first stage. Nectars were stored for 12 months in the dark at 20 ^{o}C to determine changes occurring in product quality during storage. Analysis were made directly after production and after 6 and 12 months. Large differences among cultivars and seasons in juice yield and quality parameters were found. During nectars’ storage, there are large losses of ascorbic acid, anthocyanins and procyanidins. The losses were at quite the same level in clear and cloudy nectars. Considering the above, storage time should be shortened to 6 months or products should be stored at low temperature conditions.

It was found that technology of juice production has strong influence on nutritional value of nectars made of black currants. Cloudy nectars had higher antioxidative activity than the clear nectars due to higher content of ascorbic acid and phenolic compounds, including anthocyanins and highly polymerized procyanidins (αDP 21÷42; D 3.2.12). The differences in some cases were large and e.g. cloudy nectars stored for 12 months contained more procyanidins than clear nectars directly after production. The overall sensory quality of cloudy nectars were very much the same as for clear nectars, about 7 points in 10 point scale. The best cultivar for production of cloudy juices out of the three cultivars investigated was ‘Ben Lomond’. Results obtained justify introduction on the market cloudy black currant nectars, which might be an attractive product due to high antioxidative values. Both clear and cloudy nectars fulfil requirement of EU legislation and of the Code of Practice of the European Fruit Juice Association.

Plum juice and nectar. In production of cloudy plum juice there are two major technological problems: colour retention of the juice and cloudiness intensity and stability. To solve the problems very much the same experiments were conducted as described for black currants. Experiments were carried out on 3 scales: laboratory, pilot plant and industrial. Laboratory scale experiments were carried out to establish conditions of enzyme treatment, time, temperature and kind of enzyme used. Pilot scale experiments were used to produce enough material for expert panel and consumer tests. Industrial scale experiment was designed to prove on a large scale efficiency of the developed method. It was carried out at SME Alpex (Partner No 54). To optimise technology different commercial enzymes were used in laboratory experiments: polygalacturonase, pectin lyase, polygalacturonase macerating, arabanase and different technological parameters were investigated: initial heating (90 ^{o}C followed by cooling to 50 ^{o}C, or heating only to 50 ^{o}C), enzyme doses, enzymation times, etc. (D 3.2.5). Based on the results obtained the optimal conditions of processing stoned plums were established as follows: disintegration of fruit with ascorbic acid addition, heating to 50 ^{o}C, enzymation with Rohapect PTE + Rohament PL (2+1) in the dose of 100 g/t for 1 h at 50 ^{o}C (D 3.2.16). After enzymatic treatment mash was pressed, obtained raw juice was centrifuged and pasteurized at 94÷96 ^{o}C for 30 s using plate heat exchanger, hot filled into bottles, capped and cooled. Irrespectively of the scale pressing yield was very high, usually between 94÷96%. Stable cloudiness of the juice was at least 531 NTU. It was found that increase of enzyme dose leads to lower cloudiness. Content of anthocyanins in juices with addition of ascorbic acid was by 60÷120% higher compared to juices without addition of ascorbic acid. However, addition of ascorbic acid has to be minimised as much as possible due to its negative effects on colour stability during storage. Out of 3 investigated cultivars the most suitable one was ‘Najbolia’.

As concerns the clear plum juice, it can be produced using Pectinex BE for mash treatment (which significantly increased polyphenolics extraction and yield of juice). The major problem in production of clear plum juice was clarification, which required special enzyme treatments. Through the series of laboratory trials, it was prooved that an enzyme cocktail containing Pectinex BE XXL 100 g/t; Rohapect B1L 50 g/t; Rohament PL 50 g/t for 2 h at 50 ^{o}C was the most effective in reduction of stable turbidity (D 3.2.16).

Plum juices were analysed for chemical components (at INRA Avignon, RIPF and TUL), including soluble and insoluble fibre, polyphenolics, antioxidative activity and were subjected to sensory tests. Juices contained high amount of bioactive substances thus might be valuable for human nutrition. Cloudy juices had higher content of phenolics and higher antioxidative capacity than the clear plum juices. It was also found that the polyphenolics, in particular hydroxycinnamic acids and anthocyans, underwent degradation and polymerization processes (almost 50% loss) caused probably by the conditions of the process, the kind of raw material and plum cultivar (D 3.2.18).

Regarding to chemical composition very large differences were found in sorbitol content - “Dąbrowicka” contained only 7 g/L while ‘Promis’ 57 g/L (D 3.2.16). This suggests specific suitability of plum juices for human nutrition as sorbitol has laxative properties.

Sour cherry juice was produced on a laboratory scale with or without enzyme application (D 3.2.16). Treatment of mash with enzyme increased yield by 4÷7% and slightly increased anthocyanins extraction into the juice. Irrespectively of treatments, the turbidity of juices obtained was too low to produce cloudy sour cherry nectars. No improvement of technology could then be proposed.

Analytical methods. Concurrantly with technological experiments the teams were analyzing chemical composition of raw material and products produced. In a few cases this required adoption or even development of suitable analytical methods, particularly needed for analysis of phenolics. Sophisticated analytical methods had to used within the ISAFRUIT project to separate and quantify phenolic compounds in raw material and products obtained. They were needed for control of extraction of health beneficial components from fruit mash into the juice. The challenge was to elaborate an HPLC method in which both procyanidins and anthocyanins could be measured in the same analysis. Major problems in analysis of raw material and processed products for procyanidins were solved in INRA URC with contribution of the Polish Ph.D. student during two study visits (D 3.2.3, D 3.2.4, D.3.2.12). Comparison between thiolysis and phloroglucinolysis methods used for the characterisation and quantification of procyanidins in plum samples containing anthocyanins showed that both are giving comparable results (no significant differences between the two). It was proved that plums and black currants are very rich sources of highly polymerized procyanidins. DP*n* for plums is in the range 6÷12.5 and DP*n* of procyanidins in blackcurrants is 21÷42.

Data were collected on polyphenolics composition in red fruits, which showed large variation particularly between cultivars - for example among plum cultivars tested ‘Węgierka Dąbrowicka’ is by about 5-times less abundant in phenolics than ‘Najbolia’ and ‘Promis’.

Transfer of phenolics from fruit/mash into the juice. Considering that a lot of phenolics are contained in the skin and after mash pressing are retained in pomace another challenge was an increase of transfer of phenolics into the juice. Therefore, the experiments on improved transfer of procyanidins (D.3.2.7) and other polyphenols from red fruits into juice were made. In D 3.2.25 quantification of the transfer of various polyphenol classes from plum fruits to different types of juices is reported. It was found that the transfer was lowest for procyanidins (circa 10%). This is in accordance with the high affinity for cell walls (pomace) as already reported for apple. Very high transfer rates were found for the polyphenols present in the plum skin: this might be due to the process itself, which systematically included a freezing step. To increase the transfer, the proportion of polyphenols which were extracted from the plum to the plum juice were investigated, taking into consideration the specific composition of the skin (anthocyans, flavonols) and flesh of this fruit, and the type of juice produced (clear or cloudy) and the oxidation level (D 3.2.25). Contents of procyanindins in the cloudy juice were higher than in clear juice due to omitting clarification, during which polyphenolics are precipitated after reaction with the clarifying agents.

Conclusions

1. The technology of cloudy plum juice was developped, which may easily be adapted to production of juicies from other coloured fruit.
2. Analytical methods for separation and quantification of phenolic compounds, including procyanidins were improved. Due to this achievement unique set of data on composition, extraction and stability of phenolics in produced juices have been collected.

Ad. 2. Determine quality of single cultivar juices according to the Code of Practice

In the European Union quality control of juice market has been carried out in reference to Code of Practice developed by the European Fruit Juice Association (AIJN, http://www.aijn.org/). This document sets absolute quality requirements (section A) and criteria for the evaluation of identity and authenticity of juices (section B) produced from 19 different fruit juices.

There are no reference values for plum juice yet. The Code of Practice (CoP) was accepted and brought in use by all members of AIJN and by some national food inspection organizations. Moreover fulfilling requirements of the CoP is mandatory for participants of the European Quality Control System (EQCS) and members of the International Raw Material Assurance Organization (SGF/IRMA). Reference to Code of Practice will appear in the amended Council Directive 2001/112/EC (already accepted by all parties involved).

Considering involvement of ISAFRUIT in investigation of new cultivars, particularly scab resistant, destined for industrial orchars[4], and technologies modifying juice composition it was necessary to check products compliance with CoP requirements.

The material for the experiments were scab resistant apple cultivars (24 to 28 depending on the season), blackcurrants (3 cvs.) and plums (3 cvs.). Apples were processed into clear or cloudy juices on laboratory scale. As a standard was used 'Shampion' cv., which is unique in certain characteristics, and was used in medical trials. Selected, most interesting cultivars were also processed on a pilot scale to compere quality of obtained juices with French products, made of 4 cultivars. Juices produced on a pilot scale were also destined for expert panel test carried out in ESA (Parner 65) and a few selected ones for consumer tests carried out in France and Poland.

Black currants and plums were processed into juices on laboratory and a pilot scale. In one season plums were processed into cloudy juices on industrial scale. Technologies of juice production were described in reports D 3.2.5; D 3.2.6 and D 3.2.9.

[4] There is a growing interest of establishing orchards fulfilling needs of the processing iundustry

Apple juice. The data on apple juices were described in detail in Deliverables D 3.2.08 (first season), D 3.2.19 (second season) and in D 3.2.29 the third season plus summary for all three seasons. In juices determined were most important criteria according to CoP: specific density and Brix value, individual sugars, individual acids, mineral components, formol number, sugar free extract and proportions of some constituents. No deviation concerning absolute quality requirement parameters, it is relative density and soluble solids, for single strength juices and year of the apple processing was found. Also content of organic acids, sorbitol and formol number in scab resistant apple juices was generally comparable to those found in typical apple juices. However, in several cases saccharose in juices was above Code of Practice limit (up to 45 g/L), irrespectively of the processing season. Out of CoP range was also content of glucose and fructose (less than required), ratio of glucose/fructose and % of saccharose in sugars (D 3.2.19, D 3.2.29, D 3.2.37). Saccharose content to a high degree depends on maturity stage. The problem might not appear if apples were processed after storage, but this is usually not the case. According to CoP sorbitol content should be within the range of 2.5÷7 g/L. There was no sample with sorbitol content above the maximum CoP limit; however, in rare cases lower sorbitol content was found (D 3.2.19).

It may be concluded that Code of Practice data require some correction particularly in reference to saccharose content in direct juices produced from several apple cultivars. If this is not the case producers may be accused of adulterating their products. In production of juices right after harvest of apples it is advisable to avoid production of single cultivar juices. However, on some markets these kinds of juices are becoming popular, particularly among SMEs. Considering the above the Polish Voluntery Control System (subordinate to Polish Association of Juices Producers) has been notified of the possible problems with discrepancies toward Code of Practice.

Comparison was also made of juices produced from 4 French cultivars versus 6 Polish cultivars, using the same technology (D 3.2.8). French juices in most cases did not fulfil the requirement for calcium, potassium and magnesium content, but on the contrary to the Polish ones, had saccharose content within CoP limits. Taking this into consideration it can be said that French and Polish apple juices are quite different.

Blackcurrant juices. Technology of juices production was described in deliverable D 3.2.16. Data presented cover two years of investigations, three blackcurrant cultivars, two juice types and two technological replications. Single strength blackcurarnt juices made of the three most popular in Poland cultivars (‘Tiben’, ‘Tisel’ and ‘Ben Lomond’) had most parameters within the Code of Practice reference values. Much higher than the minimum CoP values was relative density and soluble solids content. Small deviations in single components e.g. phosphorus (D 3.2.37) cannot be regarded by experts as a real problem. High content of soluble solids may be of significance due to dilution practice. Concentrated juices diluted to 11.6 Bx (minimum Brix in the reconstituted juice according to the Code of Practice) may not fulfil requirements for most parameters including ascorbic acid content, for which minimal content has been defined in the CoP (D 3.2.37). Therefore changes in the CoP reference values may be needed or producers may be required to increase input of juices into nectars produced.

Plum juices. Technology of plum juice production was developed and described in deliverable D 3.2.16. As no reference data for plum juices do exist, results obtained will be an initial valuable source of information for estimation of quality and authenticity of industrially produced plum juices or nectars. High differentiation of total soluble solids, glucose, fructose, sorbitol and mineral compounds contents in reference to cultivar and season was found

(D 3.2.37). Results showed that the minimum density of the Polish plum juices could be fixed at 1.037 while corresponding Brix cannot be lower than 9.1%. In southern European countries this values may be considerably higher. Depending on the season (or climate), degree of ripenes (especially if mechanical picking is used) or cultivar this values may be substantially higher. Plum juices are abundant in glucose which was dominating over fructose at least 1.2 times Sugar free extract was highly differentiated and was always higher than 42 g/L. Very important parameter is sorbitol content, which strongly depends on cultivar used and may be found in the range from 6.7 up to 59.1 g/L (D 3.2.37). The titratable acidity of plum juices was from 4.2 up to 20.4 g/L with malic acid dominating. Citric acid was found only in some samples in concentrations not higher than 0.23 g/L. These data can be used as authenticity parameters as other red fruit juices usually have high concentration of citric acid. Plum juices were characterized by potassium content between 1900÷3600 mg/L, which is comparable to blackcurrant juices.

Conclusions

1. In single seasons, discrepancies were found between Code of Practice data and the data obtained for investigated juices, particularly in the content of sugars and their proportions.
2. Some corrections in the CoP may be needed. If not, production of single cultivar juices right after harvest should rather be avoided.
3. Data for plum juices are unique, considering that the Code of Practice does not contain reference values for this kind of product, and may partially fill the gap in the AIJN and SGF/IRMA database.

Ad. 3. Selection of high acidity scab resistant apples

Work on scab resistant apples suitability for juice production started in the first year of the project and continued for 3 years (D 3.2.6, D 3.2.13 and D 3.2.24). For screening of large number of cultivars, laboratory method of juice pressing was used (Instron texture press with special attachment). Number of cultivars tested in laboratory scale was between 24÷27, depending on raw product availability. As a standard ‘Shampion’ cultivar was used throughout the tests. Based on results of laboratory scale 6 cultivars were selected to be processed on pilot scale to obtain large enough amount material for extended chemical analysis and consumer tests. Considering possibility of both clear and cloudy juice production juices were pressed with and without mash enzymation according to the procedure described in D 3.2.6 and were investigated for important technological parameters such as: yield, acidity, soluble solids, sensory characteristics, using standard methods.

In general processing of apple mash into cloudy juices resulted in pressing yield of 77.0% whereas for clear juices it was 83.7%. However, large variations due to technology, cultivar and season were observed. Some cultivars showed high variability of juice yield from year to year if pressed without enzymation (Angold’, ‘Sawa’ and ‘Shampion’). On the contrary, other cultivars (‘Ariwa’, ‘Gerlinde’, ‘Rebella’, ‘Florina’ and ‘Topaz’) were pressed with high juice yield irrespectively of the investigated season. In case of cloudy juices, the pressing yield is not the crucial parameter. The press cake after cloudy juice production may once again be pressed and the juice obtained used for concentrated juice or drinks production. The most important in cloudy juice production is the quality of the final product – it must be a premium product, to compete with cheaper clear juice produced from concentrate.

Summarizing three years of laboratory scale experiments (D 3.2.24) it may be said that some cultivars are valuable due to high acidity or soluble solids content or both these parameters.

High acidity cvs. were: ‘Reglindis’, ‘Antonovka’, ‘Rewena’ and ‘Renora’. Very high soluble solids content was found in ‘Regina” and ‘Topaz’ (above 14%). Several cultivars had more than 13,5% soluble solids (‘Enterprise’, ‘Shampion’, ‘Rubinola’, ‘Gold Milenium’, ‘Renora’ and ‘Rebella’). Those rich in soluble solids had high juice density - usually above 1.055 (correlation coefficient above 0.90). There was no correlation between soluble solids and acidity content and no correlation between acidity and juice density. ‘Regina’, ‘Topaz’, ‘Enterprise’, ‘Shampion’, ‘Renora’ and ‘Rebella’ were the 6 cultivars most suitable for concentrated juice production considering content of soluble solids and/or acidity.

It may also be concluded that some cultivars are very interesting due to sensory characteristics and balanced soluble solids to acidity ratio. These were ‘Shampion’, ‘Gold Milenium’, ‘Rajka’, ‘Melfree’ and ‘Ariwa’. Very interesting is the position of ‘Shampion’, in all three season there was a distinct difference between this cultivar and other investigated. It does not contain enough acids to satisfy concentrated juice producers but it is highly suitable for direct, cloudy juice production - light colour juice (and pomace) is obtained due to low browning tendency of this cultivar, although it is very rich in phenolic compounds. Therefore, less ascorbic acid may be used in production of cloudy juice from this cultivar.

In the package dealing with juices it was also planned to investigate scab-resistant apple cultivars (French:‘Chanteline’, ‘Judaine’, ‘Judeline’and Polish grown: Gold Milenium’, ‘Melfree’ and ‘Rajka’) in respect to their suitability for juice production including specialty, high quality products using traditional technologies (D 3.2.35). Juices were produced using Polish and French protocols. In clear juices production similar to industrial technology was used while cloudy ones were processed without pectinolysis of the mash and juice filtration, using sedimentation (French technology) or centrifugation (Polish technology). Production of juices was carried out in both countries on the same raw material for the best evaluation of the technology and cultivar impact on juice quality.

Results showed that technology does not affect soluble solids, most sugars, acids and most mineral compounds content of the juice. However, the antioxidant activity measured by $ABTS^{\bullet +}$ method, monomeric phenolics, procyanidins and pectic substances content strongly depend on juice technology and cultivar (D 3.2.8). The cultivar has a dominating effect on the juice phenolics. It was shown that production of cloudy juices under limited oxidation conditions and proper cultivar selection may be the key for substantial increasing the content of healthy compounds in the apple juice.

Cloudy juices are then definitely closer in composition to the raw material used and may deserve a health claim.

Conclusions

1. Among scab resistant cultivars there are some highly suitable either for cloudy or clear juice/concentrate production.
2. Cloudy juices were more abundant in phenolic compounds and in general were closer in chemical composition to fresh fruit due to omitting enzymation stage of the mash and clarification and limited oxidation during production.
3. Taking into account the advantage of processing cultivars with low browning tendency (also for other products) it would be desirable if breeders considered working on fruit with low polyphenoloxidase activity.

Ad. 4. Supply analytical data and products for juices/nectars to pillar 2

Pillar 3 and Pillar 2 had very close cooperation being oriented toward health aspects of processed products. One of the general questions asked in Pillar 2 **“*Are processed fruit just as***

***healthy as fresh fruit*"** could not be answered without having processed products obtained from the same raw material, all well characterized considering chemical composition.

Fresh apples of 'Shampion' cultivar as well as processed products such as: clear and cloudy juice, puree and pomace (with or without seeds) were supplied by the Research Institute of Pomology and Floriculture in Poland to Partner 3, Danish Institute for Food and Veterinary Research (DFVF) in required quantities and according to agreed supply scheme (D 3.2.1; D 3.2.9, D 3.2.21, D 3.2.28). Technology of processing was the same as used in the industry, including ultrafiltration of juices if required. Apples and processed products were used in DFVF in experiments planned within Task 2.1.1 dealing with short-term animal studies on fruit nutrigenomics and later human studies.

Considering necessity to characterize chemical composition of the material supplied to DFVF sub-samples of fresh apples or pomace were either lyophilised or dried and together with processed products sent to Partner 5, INRA-URC (France) and Partner 37, TU-Lodz (Poland) for physical and chemical analysis. Some analyses were also made at RIPF. Result of analyses of fresh apples and all processed products are reported in deliverables: D 3.2.2, D 3.2.10, 3.2.21. Analysis covered basic components, soluble and insoluble fibre, as well as polyphenols content and antioxidant properties. The most important observations were as follows. Processed apple products characterized by significant differences in health-beneficial properties e.g. polyphenols contents. Cloudy juice was richer in polyphenols, particularly procyanidins, than clear juice. The results of analysis prove that apple pomace is a valuable source of phenolics; practically all quercetin glycosides and nearly half of procyanidins present in whole fruits are present in lyophilized pomace, while air dried pomace contained slightly less quercetin glycosides and three times less procyanidins. Dried pomace is a rich source of fibre.

In 2008 (D 3.2.21) beside apple and apple products also sour cherry fruits of 'Schattenmorelle' cultivar (frozen) and blackcurrant of 'Ben Lomond' cultivar (frozen); as well as the products obtained from them i.e. lyophilized fruits, juices and pomaces after juice pressing, as well as commercial blackcurrant pomaces prepared for medical studies were sent to Denmark and subjected to physical and chemical analysis in France and Poland. Moreover, blackcurrant pomaces obtained on industrial scale in ALPEX company (Partner No 54) as a real waste product was used in the investigations.

Considering that the objectives of WP 2.1 of Pillar 2 concern explanation of health effects of processed fruit and fruit components apple fibre for biomedical studies was produced and analyzed in TUL (Partner 37, D 3.2.28). Attention was paid to obtain product with low granulation to be easy mixed with diet components and create tasteful dish without 'sand' effect. Considering that during drying and granulation high temperature is increasing the level of sugar degradation products such as hydroxymethylofurfural, mild conditions in both of these processes were used during production of the dietary fibre preparation. The obtained product was thoughtfully analyzed and compared with available commercial products. It contained significant amount of dietary fibre 61%. Well micronized product was a good source of polyphenols, which had high antioxidant activity. Microbiological analyzes proved that product was acceptable for diet supplementation.

Conclusions

1. Results obtained in Pillar 2 give an answer to a question "*Are processed fruit just as healthy as fresh fruit* ?" and are extremely important for promotion of processed fruit products which contribute to improvement of human health. Moreover, they support the prediction set during formulation of objectives of pillar 3 that processed fruit should be as close to the raw material used as possible.

2. Close cooperation between the two pillars has been manifested in several common publications.

Ad. 5. Elucidation of consumer perception of single cultivar fruit juices and nectars

Apple juices. Sensory attractiveness of clear and cloudy apple juices produced of single cultivars were evaluated using screening procedure and a consensus method in RIPF laboratory (D 3.2.6). Among investigated 27 scab-resistant cultivars six most promising ones were selected for larger scale production. Clear juices were produced according to the standard production protocol, whereas cloudy juices were produced with addition of of ascorbic acid to prevent oxidation.

Juices were sent for expert panel test carried out in ESA, France (partner No 54) to describe their sensory properties in comparison to French juices. Altogether 20 clear and cloudy apple juices from France (8) and Poland (12) were tested to select the most contrasted products for consumer tests (D 3.2.11). It was found that the Polish juices could be distinguish from the French ones. Juices were subjected to a number of chemical analysis to find the reasons in differences of sensory properties. Analysis made for particular components at the same laboratory covered not only basic composition but also phenolic compounds (D 3.2.15). Juices showed a wide range of variation in investigated parameters. The differences in basic chemical parameters were due to the combined effects of apple cultivar and juice origin. In case of phenolics content an effect of cultivar and processing technology was observed. Extremely high differences in phenolics content were found (e.g. in clear juices from ‘Novamac’ - 58.5 mg/L and in ‘Chanteline’ 579.7 mg/L).

Selectioon of products, single cultivar juices and nectars for consumer analysis, took place in the third year of the project and was based on opinion of ESA expert panel used before (D 3.2.11). Results of trained expert panel were necessary to have full knowledge on quality of limited number of samples served to the consumers. Consumer tests were carried out in France and Poland, in each country in two towns observing gender proportion (50/50%) and a good distribution of different ages (25% per category). Only consumers who drink fruit juices including apple juices participated in the test, altogether 313 (103 consumers in France, 210 consumers in Poland).

The results showed that perception of juices in both countries was quite similar. There were some differences between clusters of consumers, however, in both countries ‘Melfree’ and ‘Chanteline’ were most appreciated. Both cvs. had high content of phenolic compounds (over 500 mg/L). In the case ‘Melfree’ high soluble solids content and a proper balance of sugars to acids might play a decisive role. The obtained results proved that the scab resistant cultivars could be useful material for production of excellent quality juices.

Results of consumers interview how they perceive healthiness of different kind of apple juices, nectars and drinks were quite interesting. Packaging in glass bottles was associated with product healthiness. Surprisingly there was no difference between pure cloudy and pure clear juices – both were ranked very high. Consumers indicated that a chilled apple juice is healthier than apple juice at ambient temperature. They seem to be more confident in the “enriched in vitamins” claim than in “naturally rich in polyphenols”. Surprisingly apple juice sold in cardboard packaging and those sold at ambient temperature were classified statistically in the same group as apple nectar. Drinks were ranked the lowest.

Plum juices. Developing new products such as cloudy juices it is required to test and describe their sensory properties. In the first sensory trials carried out at ESA plum juices were considered too sour. Therefore, the proposal was to study the impact of the sweet/acid ratio on the consumer appreciation (see 3.2.33). It was concluded that the cultivar effect was most pronounced. No difference between clear and cloudy plum juices were observed.

Black currant nectars. Black currant is one of the most important berry fruit in Europe with a production close to 200 000 T. The fresh market is marginal and juice industry represents two-third of the utilisations of European blackcurrants (Duponcel, 2007). Black currants are too sour to be consumed in the fresh state and in the form of juices and these must be diluted and sweetened to obtain an acceptable product (Brennan, Hunter et al., 2003). In order to improve the attractiveness and quality of black currant nectars most often adjustment of proportion of sugars to acids is made to obtain optimal balance between these components.

To obtain reliable information on optimal sugar/acid balance samples prepared from the same concentrate at RIPF (Partner 11) were tested by trained panel at ESA (partner 65). Using, dilution and sucrose addition, sweetness and sourness proportion of nectars were modified (D 3.2.33). At the same time bitterness, astringency and aroma are modified. The sourness and bitterness were strongly correlated ($R^2 = 0.97$). Perception of astringency decreases with increased sweetness.

Results of expert panel were then validated in consumer tests carried out in France (105 respondents) and Poland (106 respondents) observing proper proportion of gender and ages, and allowed to measure the consumer appreciation of blackcurrant nectars. For all consumers there was a high correlation between appreciation and sweetness perception of nectars. With hierarchical cluster analysis, four clusters of consumers with clear different behaviour could be separated (1. did not like sour; 2. did not like the sweetest and preferred a proper balance of sugars to acids; 3. preferred the sweetest product; 4. small group (7%) did not like the product at all). The conclusion is that the producers should adjust parameters of nectars observing the balance between sugars and acids and preferences of individual groups of consumers. the group which liked all products were mostly Polish people who are acquainted with the product and its high nutritional value. Considering that majority of French and Polish consumers welcomed nectars there is a possibility to increase their consumption also by adjustment of their composition and by supplying information on product nutritional value.

Conclusions

1. Juice made of ‘Melfree’ cultivar may be considered a good example of highly acceptable single cultivar product.
2. The results of survey on consumer perception of juice healthiness indicated that several opinions should be corrected. The most important is correction of perception of clear and cloudy apple juice taking into consideration results obtained in ISAFRUIT.
3. Considering that, there was diverse appreciation of black currant nectars in France and Poland slightly different strategy of promotion of these products might be used. In France, nutritional value might be a good argument, whereas in Poland more attention to proper balance of sugars and acids should be paid.
4. Plum juices made of Polish fruit were generally too sour to be appreciated sensorially. The product for nectar production, particularly cloudy.

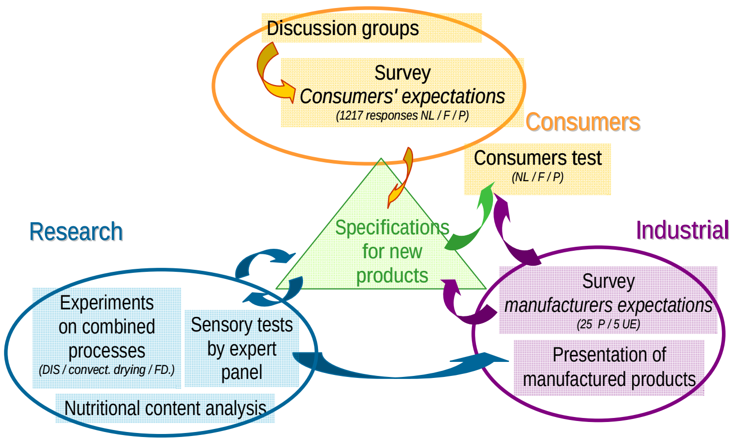
WP 3.3 Dried fruit *(DRYFRUIT)*. Leader: 5, INRA-Genial (Dr. Catherine Bonazzi); other partners: 11, RIPF; 37, TU-Lodz; 10, WUR-LEI; 55, Celiko; 65 ESA. Work period 0÷57 mo.

The objectives were:

1. Development of a fruit based dried product, characterised by a high content of biologically active compounds, which may be offered as ready-to-eat dry snacks or used as food ingredients (tasks: 3.3.1; 3.3.2; 3.3.3; 3.3.4).
2. Production of tasty dried fruits of high nutritional quality by optimized combined processes (tasks: 3.3.1; 3.3.2; 3.3.3), taking into account the compromise between sensorial and nutritional quality of the final product.
3. Optimisation of quality of osmo-dehydrated dried fruit products (tasks: 3.3.1; 3.3.2; 3.3.3; 3.3.4).
4. Increased knowledge on the interaction between mass transfer phenomena and reactions leading to the formation of compounds involved in the definition of the nutritional, toxic and/or organoleptic quality of the products (tasks: 3.3.1; 3.3.2; 3.3.3).

General remark

In WP 3.3 the multidimensional approach was taken including laboratory and pilot scale experiments, analysis of consumers' expectation and perception, and the conception of new products from the industrial point of view. The structuring of this iterative cooperation between industry, food process scientists and social scientists is described in the following figure.



WP 3.3 (in RIPF) also produced and sent for testing in Pillar 2 dried apple samples which were ground into powder. The powder has been subjected to detailed analyses including polyphenolic compounds contents (D 3.3.6).

Ad.1. Development of a fruit based dried product, characterised by a high content of biologically active compounds, which may be offered as ready-to-eat dry snacks or used as food ingredients

Specification for new dried products have been formulated on the basis of information gathered within a consumer survey (D 3.3.8), and the feedback of 25 enterprises specialized in products containing dried fruits (based on their opinion concerning samples of experimental batches of dried fruit). The opinions indicated that consumers might accept dried fruit products as carriers of functional ingredients especially if they are offered in products consumed within regular meals, like breakfast with muesli. Analysis of declared intentional behavior of consumers also indicated that there is a gap on the market, that allows introducing such novel dried fruit based product. A proposal has been prepared for new products with functional properties, offered in crunchy and flexi versions, which could be proposed in separate packaging for mixing with breakfast cereals or dairy beverages, or for direct nibbling. However, as launching a new brand on the market is expensive and risky, another type of product containing dried fruit with functional properties has been proposed in a form of bars of ready-to-eat fruit snacks for health conscious consumers (D 3.3.10). Such products should substitute widespread highly energetic snacks (with hydrogenated fats) which are generally unhealthy foods. Different recipes were tested in order to obtain sticks of freeze-dried fruit with a stable crispy structure during a few hours in normal atsmospheric conditions (D 3.3.21). End users preference analysis confirmed that bars were the most preferred products, and the osmotic dehydration conditions were modified according to their evaluations for reducing hygroscopy (using trehalose) and for obtaining more tasty bars containing whole sour cherries, whole blackcurrants, and apple cubes with higher fruit flavor and lower sweetness (D 3.3.32). Fruit bars with sour cherries and apples' cubes have finally been produced at the semi-industrial scale (D 3.3.37).

The proposed fruit bars are a good source of monosaccharides, mainly fructose, with low glycaemic index and high content of dietary fibre. Sour cherry bars have also a high content of polyphenols. One retail package of fruit bar (50 g) is equal to 150 g of raw sour cherries or apples. It corresponds to about 10% of daily energy supply. It can be a good supplement for breakfast or proposed as an afternoon snack.

The calculated price was too high for shelf-type snacks/crisps dedicated for young people, and SME 'Celiko' is exploring an introduction through health food shelf.

Results obtained have also been used for designing a new processing line for osmotic-dehydration (25 kg/batch) to be used prior to convective or freeze- drying (D 3.3.15), and for developing products (attractive from the sensory point of view, easy to use, as well as having positive effects on human health thanks to significant amounts of antioxidants and fibre). The performed researches lead to the creation of a portfolio of 14 prototypes of products (D 3.3.17). Operating conditions for pilot production of osmo-convective dried fruit were determined (D 3.3.18).

Evaluation of consumers' response to novel product promoted as healthy snacks

In the process of new product development, it was important to have insight into consumers' perception of dried fruit. An exploratory study designed as a group of discussion (using group discussion association - as one of the projective technique, Kelly repertory grids, and discussions on fruit and dried fruit perception and consumption) was first held in the Netherlands and in Poland, in order to understand how consumers perceive dried fruits, and to elaborate a questionnaire (D 3.3.7). From that, it appeared that Polish consumers perceived dried fruits as convenient and easy-to-store products while Dutch consumers considered them mainly as old-fashioned. About products with dried fruits, Polish consumers seemed to appreciate the presence of fruit as a source of better taste and diversity. Both Polish and Dutch consumers declared to pay attention to taste and to the nutritional value of the products, but they were not totally convinced of the fact that dried fruits were as healthy a fresh ones. A quantitative survey of 1092 respondents (age above 18) *via* Internet allowed then to gather a lot of original data on consumption and perception of fresh and dried fruits or products with dried fruits in the Netherlands, in Poland and in France (D 3.3.8). The survey's results were then used by SME for product design and promotion of novel products on the market. Data were first analysed in terms of preferences of Polish, Dutch and French consumers, focused on the frequency of consumption of dried fruits and dried fruit based products in general, on the preferences for different dried fruits and dried fruit based products, on the context of consumption of dried fruits and dried fruit based products, and on the locations and activities influencing dried fruits and products with dried fruit consumption (D 3.3.20). The consumers' perception was then analysed in terms of health (perception of healthiness of dried fruits and dried fruit based products), convenience and feelings connected with dried fruits and products with dried fruit.

The sensory perception and acceptability of impregnated dried fruit was then tested. A large combination of osmo-dried or osmo-freeze-dried apples, blackcurrants and sour cherries was tested by an expert panel, samples were sent to Polish enterprises with a questionnaire, or proposed to consumers in different places (Privat Label Foods / Amsterdam, Isafruit GA). Based on all collected results, a sensory test was then conducted involving 289 consumers in Poland, in the Netherlands and in France. Red fruits, first osmo-dehydrated and then convectively dried were choosen as the most promising and the most suitable for such a consumers' test. Two samples of sour cherries (of 'English Morelo' cultivar) and two samples of blackcurrants (of 'Tiben' cultivar), with sweet (impregnated with sucrose) or sweetly acid (impregnated with invert sugar + FOS or invert sugar + fruit juice, respectively) taste profiles, were selected (D 3.3.24). The main aims of this survey were to check whether consumers could accept the product, and to select the mostly appreciated dried fruit. The respondents evaluated each product twice: the first time, only the hedonic aspect, for testing the acceptability of fruit products, and the second time with nutritional values of products and relevant health claims, in order to appreciate the influence of nutritional and health information. Irrespectively of the country, respondents significantly more appreciated the dried sour cherries than blackcurrant products. Sour cherries of sweet-sour taste profile were higher ranked than the sweet ones, on the contrary to blackcurrants. Apart from well-balanced taste, the high evaluation of sour cherries with sweetly acid taste profile was explained by a tender texture and an appealing glossiness of samples' surface. Although the level of appreciation of products varied between countries and age groups, the unequivocal highest scores for sweetly acid dried sour cherries indicated that this kind of fruit snack might be successful on the European market (D 3.3.29). The collected data also indicated the usefulness of information about nutritional and health value on consumers' acceptability, especially for blackcurrants, with higher contents in health beneficial nutrients (D 3.3.38). The technology of osmo-convective drying offers possibilities for increasing the sensory appreciation of blackcurrant product by elongation of the processing time, but the effect depends on the osmotic agent. The best sensory appreciation was obtained for soaking in sucrose for 150 minutes, thanks to good texture harmonization and a high sweet taste. The chemical analyses showed no significant loss in antioxidants properties. The only dietetic disadvantage is enrichment with sugar during osmotic dehydration. Further experiment would be advisable with other kinds of carbohydrates (D 3.3.33).

A complementary experiment was done in order to check whether osmo-convectively dried sour cherries produced within the ISAFRUIT project can compete with raisins, and to verify whether muesli with higher fruit content can be accepted and appreciated by consumers. It was performed on 58 respondents in Poland, who had first to complete a questionnaire about their consumption preferences, and then to evaluate osmo-convectively dried sour cherries and raisins, individually and added to cereals. The actual high perception of osmo-convectively dried sour cherries indicated that they could compete with dried fruit present on the market and have a chance to be adopted as muesli additives (D 3.3.30).

Conclusions

1. SME partner ‘Celiko’ has decided to enter the retail market with two types of fruit snack bars, under the name of "Crunchy fruit": Apple Cubes & Cherry Cubes.
2. The fruit bars, rich in antioxidants and dietary fibre and highly appreciated by consumers, can be a good supplement for breakfast or proposed as an afternoon snack.
3. Perception and consumption of dried fruit differed between countries (Polish respondents eat more dried fruit and consider them as healthy, when Dutch respondents are more familiar with products containing dried fruit), most respondents appreciated the naturalness of dried fruit, and antioxidants were indicated as the most interesting functional ingredients.
4. Health information (if allowed) can be useful for dried fruit promotion.
5. Semi-dried sour cherries of sweetly sour taste profile were highly appreciated, and could be proposed as new products for nibbling or for muesli supplementation. Health conscious consumers looking for snacking fruit products could accept such products.
6. Semi-dried blackcurrants with high antioxidant contents could be proposed, but with relatively high sugar contents.

Ad 2. Production of tasty dried fruits of high nutritional quality by optimized combined processes taking into account the compromise between sensorial and nutritional quality of the final product

Raw material from crops 2006, 2007 (D 3.3.11), and 2008 (D 3.3.26) were systematically characterized. Carbohydrates, sorbitol, total polyphenols, total anthocyanins, ascorbic acid, total proteins, total dietary fibres, and acidity were determined for apples (Idared, Marie Menard, Guillevic, Belchard, Gala, Reinette, Avrolles), sour cherries (English Morello, Botermo), and blackcurrants (Tiben, Tisel) used in osmotic dewatering performed in TUL (Poland), RIPF (Poland) and INRA-Genial (France). Out of all tested materials blackcurrants turned out to be the richest source of bioactive and health-promoting compounds like polyphenols, ascorbic acid, and dietary fiber.

The 48 osmo-dried or freeze-dried products obtained with the 8 soaking solutions were evaluated by an experts' panel. At the same time, the nutritional value was estimated by measuring sucrose, glucose, fructose, sorbitol, maltitol, polyphenols, anthocyanins, vitamin C, FOS, protein, dietary fiber contents and acidity on all raw materials and dehydrated fruits (D 3.3.3). The stability of FOS in dehydrated fruits was specifically checked, and FOS was found to be very stable in all investigated species. FOS can therefore be used for supplementation of products containing dried fruits and submitted to long storage (D 3.3.13).

Sorption isoterms were measured at 20 °C for selected products: First, in 2006, on convectively dried and freeze-dried sour cherries and blackcurrants osmodehydrated in FOS preparation (51.7% w/w in dry substance), and on convectively dried and freeze-dried apples osmodehydrated in concentrated apple juice (D 3.3.4). Secondly, on final products potentially interesting for futher product development by ‘Celiko’, in 2008, freeze-dried apples dehydrated in apple juice, freeze-dried apples dehydrated in apple juice and trehalose, freeze-dried sour cherries dehydrated in the fructooligosaccharide concentrate, freeze-dried sour cherries dehydrated in the fructooligosaccharide concentrate and trehalose, convectively dried sour cherries dehydrated in saccharose, convectively dried sour cherries dehydrated in mixture of concentrated apple juice and inverted sugar, convectively dried black currants dehydrated in saccharose, convectively dried black currants dehydrated in the fructo-oligosaccharide concentrate with invert sugar. Sorption isotherms were then fitted using GAB, Peleg, Henderson, BET and Lewicki models which all well fitted data for high-sugar and dried food products. The Peleg model turned out to be the best one. The optimal water activities for storage were determined (D 3.3.27). The influence of sugar impregnation on sorption properties was also measured for different fruits species (apples, pears and apricots) (D 3.3.14).

The content of some bioactive substances and organoleptic properties of osmo-dried fruit (final products) were determined during 12-month storage. Performed tests proved good stability of polyphenols and vitamin C in freeze-dried sour cherry, blackcurrant and apple products stored at 18 °C, but poor anthocyanins retention in convectively dried red fruits. Sensory tests proved that producers of freeze-dried fruit products would have to pay special attention to moisture tightness of packaging materials for minimizing the texture softening during storage. Moreover, a mixture of invert sugar and concentrated apple juice seemed to be suitable for sour cherries osmotic treatment, giving osmo-convectively dried sour cherries stable in colour, glossiness, texture, and flavour (D 3.3.31).

Conclusions

1. A lot of data on composition of raw and dehydrated fruit materials were collected.
2. Information on stability of compounds during long term storage was obtained.

Ad. 3. Optimization of processing of fruits by osmotic dehydration combined with drying or freeze-drying

Apples, sour cherries and blackcurrants are valuable source of bioactive compounds, and an increase of their regular consumption through snacking products could be beneficial. Sour cherries and cider apple cultivars are rich in polyphenols, but rarely consumed in fresh form because of their sour and astringent taste, respectively. The osmotic dehydration is a method that due to carbohydrates infusion can not only increase the fruit stability but also enhance their sensory properties. For obtaining stable product at low water activity, the osmotic step has to be combined with convective drying (CD) or freeze-drying (FD).

The impact of eight different soaking solutions (sucrose, invert sugar, fructooligosaccharide preparation, oligofructose, concentrated apple juice, apple juice after acid removing, sorbitol, galactosylosorbitol) on the sensorial acceptability and nutritional values of osmo -dried or -freeze-dried sour cherries, apples and blackcurrants was systematically tested.

In most cases the use of sucrose (S) as osmotic agent increased the flavor and the overall appreciation of quality (D 3.3.12). Products with fructooligosaccharide (FOS) were characterized by a good sensory evaluation, and due to its prebiotic properties it was further investigated as osmotic agent, except for convectively dried sour cherries, because in that case, FOS hydrolyzed to glucose and fructose, and lost its prebiotic properties. Oligofructose (OF) seemed to have a positive effect on the retention of biological compounds and, for freeze dried products; it tended to tenderize the texture. OF was promising but needed much more experiments to check its impact on texture properties and to increase sweet taste sensation in the final products. Concentrated apple juice (FJ) significantly increased perception of characteristic aroma of raw materials. In spite of a positive sensory evaluation and a good nutritional value of products obtained by using deacidified apple juice (DeAFJ), it was abandoned because of its expensiveness. Both sorbitol (SOR) and galactosylosorbitol (GalSor) gave undesired sensorial effects (off-taste sensation and sticky surface and GalSor additionally brought tough texture). Invert sugar (IS) significantly raised sweet taste and improved sugar/acid ratio. It also increased the pleasant sensation of fruit aroma and seemed to positively influence the biological compounds retention (D 3.3.12).

When soaking in concentrated solutions containing FOS (51.7% of dry substance), the most promising results were obtained for blackcurrants. The effect of osmotic dehydration conditions on FOS intake was specifically checked on 'Idared' apples, 'English Morello' sour cherries and 'Tiben' blackcurrants (D 3.3.2). The effect of temperature (30÷70 °C), fruit/solution ratio (1/2 to 1/5, at 40 °C) and syrup concentration (50÷65 Brix) on the FOS and dry substance content of apples was determined. The highest content of FOS was obtained after 75 min at 70 °C (14.7%), and FOS impregnation was proportional to solid gain, whatever the temperature and the apple-to-solution ratio. Experiments on blackcurrants indicated that the skin had to be abraded.

Based on gathered data concerning nutritional value, sensory qualities and potential consumer attractiveness, promising combinations of raw materials, osmotic agents and drying methods were selected (D 3.3.12). In the case of apples, longer DIS times using FOS as osmotic agent did not result in any improvement of the sensory properties neither for DIS-CD nor DIS-FD products. Similar results were obtained for freeze-dried sour cherries and blackcurrants. The only product which positively responded towards higher FOS uptake was the DIS-CD blackcurrant, with combined effects of higher flavor and texture appreciation. For OF impregnation before freeze-drying, the increase in dry matter was correlated with an increase of hardness sensation and a lower texture rating. For apples and sour cherries, dehydration in OF decreased drastically the flavour and the overall quality. Despite creating crispness sensation, the OF could not be further recommended as promising agent. Within investigated cultivars of red fruits, 'Tisel' blackcurrant represented a promising alternative cultivar for DIS-CD fruits production. Despite of good sensory properties of dried products, the sour cherry of 'Debreceni Botermo' cultivar had to be abandoned because of DIS and CD longer times in comparison with the 'English Morello' cultivar. Based on these results detailed specification for producing DIS-CD products using sour cherries and black currants was worked out. Applying the sweet (S) and sweetly-acid (IS+FJ or IS+FOS) osmotic agents according to optimised DIS parameters allowed to produce for sensory testing two kinds of products for nibbling with appreciated texture properties and a good flavour, which differed in taste profile. A repetition was made for testing the impact of the season (D 3.3.16). The obtained products were subjected to sensory and chemical analyses. Sensorial results confirmed that DIS-CD products were much higher appreciated in taste, while DIS-FD were much more valuable from a dietetic point of view, based on the absolute amounts of anthocyanins in the final red-fruit products. The data confirmed correctness of decision undertaken during novel product designing (D 3.3.10), *i.e.* that DIS-CD can be chosen from making ready to eat snacks, while DIS-FD products are more valuable as components for products containing dried fruits, in which they could play a meaningful role of bioactive compounds carrier.

Production of experimental batches was performed (by SME Partner 'Celiko' using freeze drying technology, and in RIPF by convective drying). The products were presented to selected industry consumers, and also in the Private Labels Foods - the International Fair (Amsterdam, May 2007) and on the 2nd Isafruit General Assembly (in Bologna, June 2007) (D 3.3.5).

Ad 4. Increased knowledge on the interaction between mass transfer phenomena and reactions leading to the formation of compounds involved in the definition of the nutritional, toxic and/or organoleptic quality of the products

Further investigation on novel product development was focused on maximizing anthocyanins and ascorbic acid retention. Several experiments were set up to determine the impact of syrup management and drying parameters on antioxidant stability in osmo-convectively dried sour cherries and blackcurrants. It showed that FOS syrups can be successfully re-used for fruit dehydration and that 10 successive batches of dried fruit were similar as regards to the contents in carbohydrates and polyphenols, and acidity (D 3.3.28). The dried fruits were also comparable in terms of polyphenol and carbohydrate profile. Some technological recommendation were formulated in the aim of limitation of bioactive compound losses (D 3.3.19), like re-use of syrup during soaking stage for limiting anthocyanin leakage, or convective drying at variable temperatures (from 40 to 60 °C) instead of constant temperature (60 °C). But whatever the technology implementations, selecting raw materials with high initial anthocyanin and ascorbic acid contents remains the best way for obtaining high bio-active compound content in dried fruits.

Apple is naturally rich in polyphenols but studies on the impact of processing on their behaviour are scarce. Several cultivars of dessert apples (Belchard ®, Royal Gala and Reinette grise of Canada) and cider apples (Avrolles, Guillevic and Marie Ménard) were selected, through three consecutive years (2006÷2008). For each process, dehydration and degradation kinetics of ascorbic acid and different classes of polyphenols were followed, anti-oxidants being measured by HPLC-UV-DA; two temperatures (45 and 60 °C) were tested in DIS. Changes in sugars concentrations during DIS (fructose, glucose and sucrose) were also measured by HPLC-RI.

During DIS, a significant impact of the crop year was observed on mass transfer, larger than the cultivar's one. Temperature accelerated mass transfers and had a negative impact on the retention of antioxidants. However, the results revealed significant differences between polyphenols and ascorbic acid behaviours. The loss of ascorbic acid was very rapid, mainly due to oxidation and leaching with water. Concerning polyphenols, the retention differed with their chemical structure. Procyanidins underwent little loss, due certainly to the fact that they are polymerized compounds that can bind with cell walls polysaccharides. On the opposite, hydroxycinnamic acids, preferential substrate of the polyphenol oxidase with a low molecular weight, recorded higher loss (D 3.3.1, D 3.3.23 & D 3.3.35).

By comparison, convective drying better preserved ascorbic acid and phenolic compounds, without any difference due to their chemical characteristics. In addition, apple cultivar showed little impact on mass transfer (D 3.3.34).

When combined to convective drying, and even if it only represented 10% of the total processing time, DIS lead to about half of the final losses in polyphenols. Differences observed between compounds were similar to those already described for DIS. The impregnation of sucrose may nevertheless be interesting to mask the astringency of procyanidins and improve the taste of dried apples.

The impact of thermal treatment and PPO activity on levels of ascorbic and chlorogenic acids was also investigated during heating in a buffer solution at 45 and 60 °C. Concentrations in O_2 in the apple cubes and diffusion values were estimated during DIS and CD in order to corroborate the proposed mechanisms for explaining the losses in polyphenols (D 3.3.36).

Finally, the feasibility of SHS drying was tested on apple cubes at 110 and 150 °C. It allowed a very rapid dehydration of fruit pieces, with a good preservation of shape and colour, but with appearance of a 'cooked apple' aroma (D 3.3.9 & D 3.3.22).

Conclusions

1. Dehydration in invert sugar significantly raised sweet taste and improved sugar/acid ratio, increasing the pleasant sensation of fruit aroma.
2. Blackcurrant products dehydrated in fructooligosaccharide solutions were characterized by a good sensory evaluation and possible prebiotic properties.

3. Convective drying better preserves ascorbic acid and phenolic compounds than osmotic dehydration. This latter must be considered as the limitating step for producing dried fruits with high contents in natural nutrients.

WP 3.4 Dietary fibre and fruit phytocomponents concentrates for enrichment of processed fruit and food products ***(COMPONENTS)*** Leader: 5, INRA-URC/INRA Avignon (Dr. Catherine Renard); other partners: 3 DFVF; 11, RIPF; 37, TU Lodz; 48, VdV. Work period 3÷57 mo.

1. The improvement of extraction, purification, separation and characterization of biologically active components derived from fruit mash/pomace of apples, sour cherries, black currants and plums (tasks: 3.4.1; 3.4.2; 3.4.3; 3.4.4);
2. Preparation of adequate amount of dietary fibre and phytocomponents for studies in Pillar 2 and WP 3.2 (tasks: 3.4.2; 3.4.5).

General remarks

The issues for WP 3.4 were to promote a total use of the fruit biomass by proposing improved methods for “cracking” of pomace, allowing conversion of this by-product into value-added products. This leads to a better valorisation of the fruit resource and thus potentially higher revenue throughout the fruit chain, and to the proposition of health-beneficial compounds for inclusion in other foods, and primarily other fruit products.

WP 3.4 was mostly concerned with the following issues of ISAFRUIT:

- increasing the quality of fruit products;
- increasing the availability of fruit products.

This work was carried out in close cooperation with WP 3.2 ‘Juicethirsty’, as major provider of pomace.

Ad. 1. The improvement of extraction, purification, separation and characterization of biologically active components derived from fruit mash/pomace of apples, sour cherries, black currants and plums

Assessment of pomace as source of phytocomponents

Industrial wet apple, sour cherry and black currant pomaces obtained from modern juice production lines are diversified material and unstable microbiologically. They differ by seeds, flesh and peel share, and by polyphenol and dietary fibre content. It was proved that sieving wet pomace is less efficient and less selective than separation of dried pomaces, but makes it possible to obtain at least two material streams, which can be dried or extracted more efficiently than not separated material. Significant limitations for implementation of the idea of two material streams using wet separation method is necessity to construct proper installation, considering that wet pomace weights three times as much as dried pomace, and sieving capacity is twice smaller than for dried pomace. The lines for further treatment of obtained two material streams including dryer, seed separator, extractor etc. are necessary supplement. Physical separation of pomace components before or after drying is anyhow a necessary step for its optimal valorisation.

The simplest method, allowing partial utilization of wet apple, sour cherry or black currant pomace, would be hot water extraction. It allows at proper conditions obtaining polyphenol-sugar extracts containing respectively 0.2, 0.6 and 2.0% of polyphenol sum, and characterized by desired nutrition properties including properly high share of soluble dietary fibre fraction (D 3.4.36).

Initially very little was known on pomaces from blak currant, sour cherry and plum, while no systematic investigation of apple pomace variability had been carried out. A huge database of pomace composition has been build by partner 37 TU Lodz, including impact of variety, year for apple and black currant, mode of preparation of juice (at laboratory, pilot or industrial scale). The data can be found in D 3.4.1, D 3.4.8, D 3.4.13, D 3.4.24, D. 3.4.30 and D 3.4.35 and has been partially published.

Based on results obtained it may be stated that selection of high potential cultivar (taking into account juice quality), pressing with limited oxidation and mild drying can be recommended for a pomace with high antioxidant extract potential. The practical use of pomace as source of phytocomponents might be justfied if health claims for antioxidants, including phenolics, and other pomace components (e.g. soluble fiber) are accepted.

Characterisation of fibers and their polysaccharides. The dietary fiber contents were measured in apple, black currant, sour cherry and plum pomaces as a function of variety and processing. Fractional extractions were carried out on black currant pomaces, sour cherry fruit and pomace produced with or without enzyming, and on plum fruit (differentiating skin and pulp) and pomaces.

The main polysaccharide components in all pomaces were pectins and cellulose. The proportion of galactose was highest in plum and of arabinose in sour cherry. Alcohol insoluble solid mode of preparation resulted in a marked co-precipitation of proteins. Sour cherries are high in pectins, with low degrees of methylation, and held by calcium bonds. The main hemicellulose in sour cherry and in plums was a xyloglucan.The fibres in plum fruit and pomace are very rich in pectins, with a high proportion of galactose; most of these pectins were not readily available, in contrast to sour cherry. Enzyming acted with different intensities depending on the precise enzyme used, but generally resulted in a decrease in degree of methylation. Pectins in the pomaces have lower degree of methylation, molecular weight and more neutral sugars than those from fruit.

Apple pomaces are the solid by-products resulting from juice and cider manufacturing. They generally account for 15-30% of the raw material. Apple pomaces have been prepared in 2006, 2007 and 2008. Variability of the pomace composition due to processing season, cultivar and mode of processing was estblished. Cultivar was a source of variation both for polyphenols and for dietary fiber (content and relative composition). Pomaces contain large amounts of phenolic compounds, which are either located in the seeds and in the peel or associated to the cell-wall material (fibers). Because of their high sensitivity to enzymatic oxidation, not only the nature and amount of polyphenols in the pomace but also their extractability can be very variable depending on the conditions used during crushing and pressing operations. Pulp oxidation has an impact on polyphenols but not cell wall polysaccharide composition. Enzyming affects dietary fiber composition and hydration properties.

Apple pomace extracts purified by semi-preparative HPLC were analysed by ESI-MS and HPLC/ESI-MS. The study confirmed the presence of isorhamnetin glycosides (pentosides and hexosides), the substances recently detected in apples (previously believed to be characteristic for pears and not present in apples). New component, initially identified as 3-O-rhamnoside of methylated mirycetin was also detected (D 3.4.9).

Black currant pomaces. Industrial pomaces (from SME Alpex) were subjected to a complete fractionation, separating seeds from seedless fraction. The seedless fraction was further separated according to particule size. The concentration of phytocomponents is 3-4 times higher in seedless fraction comparing to fraction containing seeds; the content of dietary fibre is higher in seedless fraction as well. The fraction containing seeds is richer in fat, protein and saccharose. Black currant seeds were very rich in a mannan-type hemicellulose. Statistically significant differences were found in the content of fat, ash and total dietary fibre in the granulometric fractions. The fraction of under 0.8 mm is characterized by the lowest content of fat, saccharides and polyphenols, which results from their better transfer to juice comparing to larger size fractions. The seedless fraction of the particle size of 2÷5 mm is characterized by the greatest content of phyto-compounds, including anthocyanins and quercetin and myricetin glycosides and thus can be taken into consideration as a valuable raw material for obtaining food colorants and antioxidants.

Industrial seedless black currant pomace is a good source of dietary fibre and protein. Seedless black currant pomace has high antioxidative activity due to large amount of polyphenols present. High variability of the components has to be taken into account when industrial pomace is to be used. The composition of the pomace depends on a growing season and technology of juice extraction.

The extracts of blackcurrant pomace purified on polymer absorption bed were also subjected to HPLC/MS analysis. Three purified extracts were selected for this study i.e. anthocyanin-rich extract, myricetin, quercetin glycosides-containing extract, and aglycon-containing extract. The research confirmed that anthocyanin-rich extract is composed of the following anthocyanins: delphinidin-3-glucoside, delphinidin-3-rutinoside, cyanidin-3-glucoside and cyanidin-3-rutinoside. The same compounds are present in fruits as well. Aglycon extract contained four aglycons, where myricetin and quercetin were predominant, kaempferol and isorhamnetin were present in lower amounts. Glycosides-containing extract was the most interesting, since it contained myricetin, quercetin, kaempferol and isorhamnetin glycosides. Myricetin and quercetin galactosides were detected in black currant extract for the first time. Moreover, the presence of nitrile-containing compounds, two acylated anthocyanins and one auron type compound were detected (D 3.4.18, D 3.4.32).

Plum pomace. Pomaces were prepared from fruits of season 2007 and 2008 at the pilot plant scale from 3 cultivars ((Dabrowicka, Promis and Najbolja), pressed using pectolytic enzymes and air or freeze-dried. Cell walls, which are the source of dietary fibers in fruits, have been isolated and analysed from 5 French plum cultivars, differentiating epiderm and flesh, and 3 Polish cvs. Significant differences between the cultivars were detected. Polysaccharide extractions (pectins, hemicelluloses) have been carried out and the extracts have been analysed for neutral and acidic sugars. Results indicate very high pectin contents.

Experimental industrial plum pomace was produced in 2009 from mixed cultivars, and submitted to various drying treatments. The original dried industrial pomace contained 35% of total dietary fiber, which could be increased to 67% after water extraction, subjected to air (<70 °C) or freeze-drying, with or without ascorbic acid addtion. Freeze-dried pomaces contained on average 1.5 times more polyphenpols than air-dried. There was a significant but lower effect of ascorbic acid addition.

Conclusions

1. The highest polyphenol potentials were obtained for scab-resistant apples of cv Renora while cvs Melfree, Gold Millenium, Topaz, Novamac, Rajka and Ariwa gave the best compromise with juice production (average on three years)
2. Basic kowledge was acquired on the composition of pomace from black currant, plum and sour cherry.

3. Enzymes preparations used in production of juices are safe for further extraction of polyphenols: they produced only slight hydrolysis of phenolic glycosides, except for enzymes used in plum juice production.
4. Enzyming interacts with the composition and hydration properties of the fibers from pomace: less soluble dietary fibers were present and the hydration capacities were lower.
5. Mash oxidation leads to a decrease in amount and extractability of polyphenols in the pomaces (D 3.4.5). Formation of quinones by action of fruit endogeneous polyphenoloxidase can lead to covalent bonds with the fiber polysaccharides (D.4.19).
6. More native phenolics can be extracted from freeze-dried apple pomaces. After air-drying similar amounts of total polyphenols are recovered but their structure has changed.
7. Black currant pomaces need to be separated for an optimal extraction: denser seeds can be collected separately for oil extraction; seedless pomace is best used for extraction of anthocyans and preparation of fibers.

Extraction and purification of polyphenols and dietary fibre from pomace. Extractions were carried out at the laboratory scale for pomaces after juice pressing of apples, black currants, sour cherries and plums and scaled-up to pilot and industrial scale for apple (D 3.4.3) and black currant (D 3.4.10; D 3.4.21).

Choice of extraction conditions. The extractability of valuable components from black currant pomace in various conditions of solvent, temperature, time and mode of extraction were investigated on the seedless fraction of black currant pomace (D 3.4.2). Two steps extraction: static water extraction, followed by continuous ethanol extraction is the rational way of using black currant pomace, resulting in production of valuable polyphenols and dietary fibre preparations. By using 0 and 20% ethanol concentration anthocyanins and flavonols were extracted in low proportions. On the other hand, the highest extractability of anthocyanins was found at 40% of ethanol, while flavonols are best extractable at 60 to 80% of ethanol. The yields of stable soluble pomace components varied with temperature and reached 90% at 90 °C. However, extraction should not be carried at above 70 °C in order to avoid hydrolysis of myricetin glycosides and probable anthocyanins condensation. With the use of laboratory scale static pure water extraction at 70 °C with pomaces from two harvest seasons the A-F anthocyanin-sugar concentrates and sugarless dietary fibre preparation were obtained with yields of about 11% and 88%, respectively. With the use of laboratory scale successive static water extraction at 70 °C and continuous alcohol extraction at 45 °C of pomaces, the concentrates were obtained with comparable yields and composition from two harvest seasons. Anthocyanin-flavonol concentrates were obtained with yields of 2.5% and post extraction fibre preparation with the yields almost 85%.

Purification on polymer beds. Two different resins were used for purification of polyphenols (D 3.4.14): RP-C18 was used with black currant hot water extract, and Amberlite XAD-7HP for plum and sour cherry hot water extracts. In both cases, a fractional elution with increasing methanol concentration allowed to collected fractions with differentiated polyphenol composition. The 45% ethanol extract from black currant could be separated in an anthocyanin rich-fraction, a myricetin and quercetin glycoside fraction and an aglycon fraction. All three fractions from plum and two fractions from sour cherry had as main phenolic components hydroxycinnamic acids. They were the only polyphenols present, albeit at low concentration, in the first eluate. The second eluate was relatively enriched in anthocyanins, and the last in flavonol glycosides. For sour cherry, though hydroxycinnamic acids were always a significant component, anthocyanins were the main phenolics in the second extract (D 3.4. 26).

Industrial scale. Val-de-Vire has developed a process for production of apple fibre and apple polyphenol fractions enriched in each of the main classes from dried pomace of cider apples (D 3.4.3). The method for production of a hydroxycinnamic acid-enriched fraction has been set-up. This fraction as well as a flavanol-enriched fraction and apple soluble fiber of high viscosity have been made available to partners.

VdV procured 2 T of black currant pomace from a “crème de cassis” producer (Cassis Boudiers), at 65% dry matter and 15% ethanol; 1.95 T of black currant pomace were processed, leading to 2 kg of polyphenolic extract and 22 kg of soluble dietary fiber (D 3.4.12). Due to residual bitterness, another extraction was carried out in 2008 using pomace from Alpex and a protocole derived from that optimized at the laboratory scale by TU Lodz (D 3.4.21), allowing production of an anthocyanin-rich fraction with high colorant potential and of soluble and insoluble fibers. The anthocyan-rich concentrate obtained after water-ethanol extraction from Alpex black currant extract coud be incoroporated in red-fruit nectars, giving an organoleptically aceptable product and enhanced cloud stability.

Experiments carried out at Alpex showed the feasability of separating seeds and seedless pomace as an in-stream process from wet pomace (D 3.4.24; D 3.4.36).

Characteristics of dietary fiber. Hydration capacities were measured for pomaces and post-extraction dietary fiber i.e. the dietary fiber recovered after polyphenol extraction (D 3.4.1; D 3.4.11; D 3.4.25). These hydration capacities were generally high, with swelling between 15 and 20 mL/g for black currant, 7÷9 mL/g for apple, 9÷25 mL/g for sour cherry, with an impact of both variety and enzyme used, and circa 12 mL/g for plums. Low water binding capacities were found for black currant pomaces (< 4 g/g), slightly higher for apple (2÷5 g/g) while quite high WRC were found for pomace from sour cherry and plum (betwen 5 and 10 g/g). However, air-dried industrial plum pomace had lower hydration capacities.

Generally, dietary fibers recovered from the pomaces had a lower proportion of soluble fibers and readily soluble pectins than those of the corresponding fruit. Higher concentrations of dietary fibers were obtained after the polyphenol extraction sequences, including a decrease in metabolisable sugars. Polyphenol extraction generally has a positive impact on the fibers, specifically an increase in hydration capacities. Preparation of soluble dietary fiber was possible at the indutrial scale.

Improved schemes for extraction and purification of phenolics. Fractional extraction schemes were adapted for black currant; an optimum temperature was noted at 70 °C, while different ethanol concentrations were required for anthocyans and flavonols. A purification procedure using C18 resins and fractional methanol extraction was devised - it allows recoveries of a highly concentrated anthocyans fraction, a quercetin-enriched fraction and an aglycone-enriched fraction from black currant. Water and ethanol:water were compared for extraction of pomaces from plum and cherry; similar yields of polyphenols were obtained but water more efficiently depleted the final fiber of metabolisable sugars. Fractionation procedure was also devised from water-extract of sour cherry and plum on Amberlite XAD; however, hydroxycinnamic acids precluded obtention of fractions as specific as with black currant.

Extraction of phenolics, and notably ethanol-water extractions, are beneficial for the fiber properties: proportion of fibers is increased, colour is generally decreased while hydration capacities increase (D 3.4.1, D 3.4.11).

Innovative pretreatments. Apple skin is very rich in polyphenols which are hardly extracted to juice. Technological enzymes added to the pomace or to isolated skins to attempt a solventless extraction, yielded very poor results.

Ultrasound-assisted extraction (D 3.4.31) was carried out on dried apple pomace, both with water and with 48% ethanol (which gave the highest total polyphenol yields) as a solvent; optimal extraction conditions were sought using an experimental design with three parameters: ultrasound power (0.058 – 0.142 W/g), temperature (9.9 – 40.1 °C), extraction duration (5-55 min) and their interactions. The most significant variables were temperature and extraction duration. For total polyphenols an optimum was found at 40.1 °C, 45 min and 0.142 W/g with 48% ethanol, 40 °C, 40 min and 0.120 W/g with water, but this was close to the upper limits of the parameters. Individual polyphenols have been measured in the extracts, and a highly significant effect of ultrasound power was only found for flavonols, which might be related to their specific localisation in the epidermis. Ultrasound appeared as a useful pretreatment for enhanced extraction of polyphenols from dried pomace.

The influence of the application of pulsed electric field (PEF) and High-Voltage Electrical Discharges (HVED) treatment to apple skin to improve the extraction of the polyphenolic compounds by diffusion in distilled water at 25 °C was also investigated. The application of PEF led to a significant increase of phenolic compounds but the most efficient treatment was HVED (D 3.2.31[5]). Thus, it is evident that electrical treatments are useful technologies for extracting phenolic compounds from apple skin. These results open a new perspective for selective extraction of functional compounds with health benefits. Patent application isunder consideration.

Conclusions

1. Improved polyphenol extraction and purification schemes were established, allowing preparation of polyphenol-rich extracts up to the industrial scale.
2. Some of the purified extracts, rich in anthocyans, had high coloring power, while procyanidin-rich extracts were bitter.
3. The extracts from black currant, plum and cherry were all high in anthocyanins and flavonols; cherry and plum extracts also contained noticable amounts of phenolic acids, primarily neochlorogenic acids. Type B procyanidins were identified in plum and cherry.
4. The dietary fibres recovered after polyphenol extraction often had increased hydration capacities and decreased colour.
5. Ultrasound could enhance extraction yields from dry pomaces.
6. Application of electrical treatments (PEF and HVED) to apple pomaces gave positive results in extraction of phenolic compounds from apple skin.

Characterisation of polyphenol extracts and dietary fibre derived from pomace of apple and red fruits

Under this subject studied were effects of enzymes used in industrial processing and methods of pomace drying on phenolic compounds and dietary fibre. It was unticipated that these two processes might have negative effect on biologically important constituants of pomace, and if so, alternative methods of processing should be looked for. Effects of enzymes were of great interest considering production of clear juices and concentrated fruit juices on a large scale.

[5] The work was done within WP 3.2, however, from meritum point of view it is being reported under WP 3.4

Hydrolysis of phenolic glycosides by technological enzymes

Work was carried out on assessing the impact of enzyme preparations used in technological process in juice production (and therefore in pomace quality) on the phenolic compounds (polyphenolic glycosides) which are to be extracted from pomaces (D 3.4.7; D 3.4.22; D 3.4.33) and on dietary fibre to be utilized as functional additives in later experiments (D 3.4.11; D 3.4.25). Partner 37 (TU Lodz) has been looking for effect of various commercial enzyme preparations on enzymatic hydrolysis of quercetin glycosides at 20 and 40 ^{o}C. Experiments carried out with a mixture containing quercetin-3-O-galactoside, quercetin-3-O-xyloside, quercetin-3-O-arabinoside and quercetin-3-O-rhamnoside as substrate showed that rhamnoside was the most resistant glycoside to all enzymes (14% of hydrolysis at highest), xyloside was relatively resistant, while galactoside and arabinoside showed high degree of hydrolysis (arabinoside was completely hydrolysed by Panzyme XXL after not more then 12 hours). Among enzymes, Panzym XXL showed the greatest capacity of hydrolysis, while Rohapect MA Plus was practically "safe" to all glycosides.

The results of initial tests can be summarized as follows: resistance of quercetin glycosides to hydrolysis (decreasing) - rhamnoside, xyloside, galactoside, arabinoside; the hydrolytic capacity of enzymes (increasing) - Panzym SMASH, Rohapect MA Plus, Amylase XXL, Novoferm 43, Panzym XXL. For enzymes currantly used in juice processing technology at recommended doses and process temperature, even the hydrolysis of most sensitive rhamnoside and xyloside was limited, so the enzymes could be considered as not significantly influencing apple quercetin glycosides content.

Tests performed for blackcurrant with the huge doses of enzymes used in blackcurrant processing technology (Pectinex BE Colour, Pectinex BE XXL, mixture of Rohapect PTE and Rohament PL) showed, that the use of enzymes does not result in the hydrolysis of polyphenol glycosides (delphinidin glucoside and rutoside, cyanidin glucoside and rutoside, mirycetin glucoside and quercetin glucoside) abundant in black currant. The only observed effect of enzymes was the increase of polyphenol glycosides level extracted from blackcurrant, resulting from the increased liberation of those substances from fruit, presumably due to cell wall degradation. Phenolics of black currant: anthocyanins, quercetin glycosides and mirycetin glycosides are not hydrolysed by the enzymes used in blackcurrant juice technology.

In plum study, enzyme preparation used both in recommended and huge doses increases the recoveries of hydroxycinnamic acids, flavonol glycosides and anthocyanins from fruits, with no polyphenol hydrolysis observed.

Two of examined enzymes used in sour cherry juice technology applied in huge doses decrease the contents of cyanidin-3-soforoside and cyanidin-3-glucosyl-rutoside, with the increase of cyanidin-3-glucoside, but used in recommended doses can be considered as safe for valuable phenolic components.

Drying conditions. It was noticed early in the program that air-drying and freeze-drying resulted in different analytical results for polyphenols.

Apple pomaces were produced using two cider apple cultivars (Kermerrien and Avrolles) that differed in their polyphenol composition and in particular by the degree of polymerisation of procyanidins (D 3.4.20). Apples were pressed with limited or enhanced oxidation, and apple pomaces were air-dried at 70 °C to different residual water contents. Samples with differential moisture contents ranging from 72% (Kermerrien) and 68% (Avrolles) down to 34, 12, 4, 2 and 0% were prepared. Polyphenols were extracted first with water then with 48% ethanol using an accelerated solvent extractor, and concentrations of total polyphenols were analysed spectrophotometrically.

Overall, apple pomace drying does not seem to affect the extractability of total PPs. Only when water content is halved, the polyphenols' extractability is reduced. Further drying down to 12% of water content is interesting for industrial applications as this drying point can be easily and rapidly attained, without loosing pomace PPs.

Different drying options were tested for apple pomaces in an experimental industrial drier (D 3.4.36). The IR radiator module of the tested dryer appeared to be effective in first drying stage on the upper belt only, when humidity decreases from c.a. 75 to 25%. Nethertheless local burns were observed. The efficiency of dryer's convection part was unsatisfactory. Fire inside dryer made it impossible to carry on with the experiments.

Dietary fibers. Fibers are a major component in pomaces from apple, black currants and plums; however, they were almost absent from cherry precipitate, which comprised mostly proteins and mineral substances from processing aids.

The focus has been on identification of the impact of enzymes used in processing to juice in the polysaccharide composition. This was carried out on industrial pomace of apple (Polish and French), on "Ben Lomond" black currant pomace, on 'Schattenmorelle' cherry pomace and on plum pomaces. Very generally, use of enzymes resulted in decreased pectin contents, lower degrees of methylation of this pectin and a shift of the pectin fraction from water-soluble to alkali-soluble.

Enzyme treatments also affected the hydration capacities, increasing water retention and swelling for cherry. The ethanol and acetone treatments used for polyphenol extractions increased the AIS contents in the pomaces and slightly increased its glucose content. Overall, the material resulting from polyphenol extraction of press cakes thus appeared very well suited for commercialization as dietary fiber.

Conclusions

1. Most enzyme preparations used for juice production are relatively "safe" for the flavonol and anthocyan glycosides, i.e. only lead to limited hydrolysis of the bond between the sugar and its aglycone.
2. Air-drying of pomace leads to loss of native phenolics but not of antioxidant properties in the extracts.
3. Dietary fibers present in the press cakes were affected by the enzyming used for juice extraction, primarily by a decrease in readily-soluble dietary fibers and of pectin's degree of metylation.

Interaction between polyphenols and cell walls - understanding formation of the pomace. Two approaches were used to better understand how polyphenols become insoluble in pomace, so as to better avoid these conditions. In the first one effects of pomace oxidation were studied and in the second one chemical bonds between the cell wall and phenolic compounds were of interest.

Formation of apple pomace in different oxidation conditions. Pomaces were obtained from two cider apple varieties showing highly contrasted phenolic profiles. In addition, four different conditions of contact with air during crushing and pressing were compared to evaluate the impact of oxidation (D 3.4.5). They were subjected to enzymic (D 3.4.19) and solvents extractions (D 3.4.27) and the different polyphenol classes were analysed.

Simple phenolics including flavonols, dihydrochalcones and hydroxycinnamic acids are mainly extracted by methanol and their concentration in the extracts was reduced for oxidized pomaces probably because a part was converted to oxidation products. Procyanidins, which corresponded to the major class of polyphenol in the pomaces, were only partially extracted by solvents and the extraction yield was much lower for pomaces containing highly polymerised tannins. A clear decrease of the procyanidin extractability was observed for oxidized pomaces containing oligomers. Enzymatic actions using polysaccharidase allowed extraction of an additional tannin fraction. A chemical marker of the effect of oxidation was clearly detected on LC-MS chromatograms of the liquid extracts resulting from pectinolytic degradation of pomace residues after solvent extraction.

Phenolic compounds in the apple pomaces, were only partially extracted by solvents and the extraction yield was much lower for pomaces containing highly polymerised tannins. A clear decrease of the procyanidin extractability was observed for oxidized pomaces containing oligomers. Enzymatic actions using polysaccharidase allowed extraction of an additional tannin fraction but not all. Therefore we hypothesized that covalent bonds were formed between the insoluble cell wall and phenolics. They could not be extracted by solvents and these associations limited the enzymatic hydrolysis of polysaccharides.

Preparation of artificial pomaces. The aim was to reproduce covalent structures formed during the processing of fruits and vegetables, which to our knowledge had never been demonstrated. In this work fibers were isolated from apple (these fibers were devoid of polyphenols) and then put in contact with activated polyphenols, i.e. quinones of cafeoylquinic acid and (-)-epicatechin produced by chemical or enzymic means (D 3.4.27).

While in the absence of quinone in the control samples, phenolic compounds added and adsorbed to cell wall were removed during washing with solvents, various compounds were associated with cell wall in the samples that have undergone oxidation. This implies the existence of strong bonds. When the quinone of cafeoylquinic was put in contact with the cell walls, quinic acid could be extracted by saponification, indicative of formation of strong bonds with the cell wall polymers, either polysaccharides or proteins. The data obtained are consistent with the presence of bonds between the cell wall and phenolic compounds through their quinone formed during the enzymatic oxidation. If covalent bonds exist between walls and polyphenols they could be points of adsorption of other polyphenols by exchange of p-electrons. This stacking may explain the retention of some polyphenols on the pomace.

Conclusions

1. Oxidation of the mash during juice extraction leads to formation of strong bonds between the polyphenols and the cell wall matrix, bonds, which render part of the polyphenols unextractable.
2. Indirect evidence points to formation of covalent bonds by reaction of the chlorogenic acid quinone with cell wall polymers.

Stabilisation of red fruit cloudy juices by admixture of apple phytocomponents. Various extracts were used in an attempt to stabilise colour and cloud in cloudy red fruit nectars. Considering the problem of stability of coloured fruit juices/nectars, which are susceptible to degradation, mainly browning, we have been testing the hypothesis, which claims that in presence of phenolic acids formation of more stable addition products occurs.

Extracts tested included: apple flavonoid-rich and quercetin-rich extracts, and two black currant extracts from Val-de-Vire, apple, sour cherry and black currant from TU Lodz. They were tested in black currant nectars. Addition of phenolic extracts preparations to fruit nectars does not really improve their sensory properties and/or colour stability. Enriching nectars or possibly other types of beverages in phenolic preparations does not influence the changes of native phenolics during storage, which undergo typical nonenzymic browning rules. Some preparations did allow an increase in turbidity.

Major difficulties were the negative organoleptic impact (bitterness, astringency and off-colour) caused by many of the tested extracts. Apple extracts from Val-de-Vire, though able to stabilise the colour of anthocyans in synthetic solutions, gave too much bitterness and brown colour (D 3.4.6; D 3.4.17). The most satisfactory results were obtained with water-ethanol extracts from apple while black currant extracts could increase the anthocyan concentration but not colour stability (D 3.4.34).

Use of pomace in apple desserts was also attempted in WP 3.1, but amounts, which could be incorporated, were limited by perception of colour and texture changes.

Conclusions

1. Additions of pomace extracts/soluble preparations to either drinks or other food products may only be justified if health claims are established for particular phenolic components or groups of components.
2. Organoleptic side effects of the pomace preparations MUST be taken into account.

Ad 2. Preparation of adequate amount of dietary fibre and phytocomponents for studies in Pillar 2 and WP 3.2 (tasks: 3.4.2; 3.4.5).

Partners: No 5 INRA, No 37 – TU Lodz, No 48 Val-de-Vire and No 54 Alpex prepared different kind of materials for studies in other work packages of pillar 3 and also for pillar 2 for medical studies.

Precise information on products prepared (date, material kind, quantity, sender and receiver) are summarized in D 3.4.12, covering period from June 2006 until April 2008. This early exchange of materials allowed to test them in the subsequent months and report in deliverables in due time. Some exchange of materials was also continued in the following months. For example Partner No 37 – TU Lodz prepared samples of polyphenolic extracts and concentrates for testing their inhibitory effect on food-born pathogens, to be done at Partner No 4 – IRTA laboratories. Partner 37 TU Lodz (Poland) sent about 300 kg of dried deseeded black currant pomace to Partner No 48 Val-de-Vire (France) to test optimised procedure of phenolics extraction (D 3.4.14). Val-de-Vire successfully extracted phytocomponents from black currant pomace at an industrial scale (D 3.4.21).

Partners involved in sample exchange were: 3 – DFVF Danish Institute for Veterinary Research – receiver of samples for medical studies; 5 – INRA Institute National de la Recherche Agronomique – receiver and resender of products derived from pomaces, 11- RIPF Research Institute of Pomology and Floriculture – producer of pomace, sender and receiver of samples, 34 - AFRC Ashtown Food Research Centre, receiver of ground pomaces for their studies, 37 – TU-Lodz Technical University of Lodz, receiver of samples, producer of post-extraction pomaces and ground pomaces, 48 – VdV Val de Vire Bioactives – producer of pomaces and pomace-derived products, 54 – ALPEX – producer of pomace.

More then twenty samples were exchanged. About half of the samples were apple pomaces and products derived of them. They were prepared as required by partners who were planning to use them. Some were deprived of seeds if needed, dried, ground to proper mesh size, analyzed and sent to partners to be used in various studies: feeding animals or humans or for preparation of functional foods. Other parts of samples were blackcurrant pomaces and their derivatives. Pomaces of these two fruit species were the subject of researches in medical and interventional studies. The actual exchange of pomace samples also covered plum and sour cherry pomaces, used mostly for detailed chemical analysis.

Pillar 4. Quality, safety and sustainability – Improved postharvest chain management.

Summary

Pillar 4 covered several fields of postharvest chain management. The pillar was divided in 2 workpackages dealing with:

- Sustainable treatments against postharvest rot
- Fruit quality from harvest to point of sale

For both workpackages the research mainly concentrated on apples and peaches, as defined in the technical annex.

Both fruits are very important for the EU consumers, so results from this research will have a great impact for the whole fruit chain, including consumers. Apple and peach represent different groups of fruits and therefore different chains in the fruit industry and last but not least different groups of consumers. As a consequence innovations will significantly contribute to the general ISA Fruit project objectives.

The outcome of the research in Pillar 4 includes different levels, which are generally valid for the whole ISA fruit project.

1 Results which are immediately available for application in the fruit chain

2 Information and/or prototypes which are ready to be taken up by the private industry.

3 Basic knowledge for further research on practical application.

All these levels were represented in the research of Pillar 4. The workpackage 4.1 on sustainable treatments against postharvest rot contributed very well to the technological objectives in ISA Fruit, more specific to increase fruit safety and more general to increase sustainability.

From a social- and environmental point of view the results on alternatives for chemical rot prevention are important. During the projectperiod, retail organisations came up with extra restrictions on chemical residues on the skin on top of the current criteria from the EU and the countries. This may lead to total banning of chemical protection in the future. Especially in stored fruit detected residues are often related to chemicals against post harvest rot.

A complete replacement of all the chemicals for rot protection in peaches and apples was not the aim of the project, as resources were not sufficient for this goal. It was not possible to get alternative treatments for each variety, climatical zone, type of fungi etc. Nevertheless a lot of progress has been made in the reduction of storage losses of peaches by an effective Hot Water Treatment. This method can easily be used in practice. Also other treatments were successful, like GRAS treatments (General Regarded as Safe Substances), like allyl thiocyanate and BCA (Biological Control Agents)(antagonists), which showed a good potential to control Brownrot in Peaches. So for peaches several alternatives for chemicals to control post harvest rot were found.

The hot water treatment does not need registration. However, GRAS and BCA treatments have to be registered within the EU and require the same time-consuming and costly procedure as chemical treatment.

In apple effects of hot water, GRAS and BCA treatments were variable, depending on variety, climate and fungi present. Moreover, the huge biological variation of the fruits contributed to the erratic results. For apples the results of this research are partly directly applicable and should be further developed by the industry. The hot water treatment is a direct applicable system but needs more specific investigation on site. It is impossible to use a general protocol

for every variety, growing season/area, picking time etc. So the potential user of the hot water treatment should first test a sample of fruit before treating a whole batch.

The workpackage “Fruitquality from harvest to point of sale” concentrated on the development of a Decision Support System (DSS) for quality control of apples and peaches in the whole fruitchain. It was aimed to develop a system for different varieties, and areas in Europe. Attributes related to taste and ripening of the fruit were used as a reference, as requested by chain players. It was also aimed to include non-destructive measurements of the fruits in the DSS.

Building the DSS was a huge and difficult operation. Models had to be established and model parameters’ sets to be estimated. In order to estimate model parameters’ sets for the DSS huge experiments were done with five apple cultivars and with three peach/nectarine cultivars. This was done in different countries (orchards) and in different years. In these experiments effects of harvest date, storage temperature and storage duration on destructive quality attributes, like fruit firmness, ground colour, brix-value (total soluble solids) and acidity were determined. Besides, non-destructive measurements were done with a new Italian device: the DA-meter. In order to get reliable model parameters experiments were set up according to previously determined well-defined protocols. These experiments were necessary to quantify effects of cultivar, harvest year and orchard on model parameters. At the end of the project it can be stated that the DSS, named “Peaple” has been developed, is available for all users as a software package and works well. The DSS contains 127 sets of model parameters for apple and 16 sets for peach/nectarine.

The collection of such huge amounts of data on effects of harvest date, storage duration and storage conditions on quality attributes is very unique in food research, as well as the development of such a model.

In 2008 and 2009 huge experiments were done on the usability of non-destructive (ND) data for the DSS. The new DA-meter was used for this. This study was done for two reasons: 1. to study the potential of ND data to replace destructive traditional methods. 2. to study whether ND methods can give overall information on fruit status i.e. not only actual firmness level but also the potential for further storage.

It was demonstrated that the DA-index is not a good replacement for traditional measurements. Moreover, it does not seem to be a useful tool for prediction of the potential range of quality changes during postharvest supply chains. The DA-index has thus not been implemented into the DSS.

The DSS was tested and validated. It was generally concluded that the final ‘ISAFRUIT’ version looks very nice and professional and is easy-to-use. It can also be concluded that the DSS generally works reasonable to good for the known cultivar/orchard/season combinations. However, when non-analysed ’new’ datasets were tested differences were observed between measured and predicted values in several datasets. These differences might be attributed to differences in model parameters between analysed datasets and ‘new’ datasets, as it was demonstrated that huge differences might exist between model parameters of one cultivar from different locations or different seasons. As a consequence over- and underestimations are possible every time simulations are made on a new batch of fruits, using sets of parameters estimated for other fruits. Effects of harvest year and orchard were much bigger than ever expected and this was demonstrated and quantified for the first time on a large scale. To help a user in the selection of model parameters’ set(s) some simple experiments were defined. A user can read the protocol via a window.

Two special websites have been created in relation to the DSS. A user of Peaple“ DSS may search for help in fruit storage knowledge at the special website located at the address http://wiki.peaple-dss.pl/. Via www.peaple-dss.eu and www.peaple-dss.pl every postharvest chain player can download the“Peaple“ software, and find basic information about the way it works.

A DSS user can simulate and compare different supply chains and can then decide to adjust chain steps, e.g. the duration and temperature of different supply chain steps in order to meet the consumer demands for high quality fruit. Identification of bottlenecks in the supply chain may help in adjustments of step parameters to improve the quality of fresh fruits offered to consumers. By playing with different initial firmness levels (at harvest) and using sets of models' parameters estimated for different growth locations and seasons the DSS user may increase his/her knowledge on fruit quality.

When fruits are stored for too long, quality drops and fruits cannot be sold anymore. The optimization of supply chains will reduce fruit losses and consequently will contribute to reduction of fruit prizes.

For ISA Fruit as an integrated project the relationship between technological and consumer-science is an essential demand. Therefore Pillar 4 was involved in the so called “House of Quality“ (HoQ) approach. This HoQ has been developed as a scientific tool linking technical fruit quality attributes to consumer demand related attributes. Moreover, knowledge gaps were identified with suggestions for future research, aiming to increase fruit consumption.

General objectives as defined in Annex 1

The overall goal of the Project is increasing fruit consumption in Europe. Pillar 4 concentrated on better quality, safety and sustainability by improved post-harvest management.

In Annex 1 scientific and technological objectives are described. Pillar 4 covered the following topics:

- To provide sustainable non-chemical alternatives for pesticide use to control fruit losses by rot. As a result, fruit becomes more healthy and safe and can be consumed with the skin. Moreover, environmental losses of pesticides will be eliminated.
- To develop and test a prototype of a decision support system (DSS) and a prototype of a portable, non-destructive multi-sensor device for fruit quality assessment. With these tools, actors in the fruit chain can monitor and manage fruit quality towards consumer's demands.

Basic information, contractors, work performed, end results

Contractors/Participants

In Pillar 4 21 participants were involved, 17 research institutes and 4 SME's from 7 European member states. One participant was from the University of Davis, USA and had an advisory role.

In WP 4.1 (non chemical alternatives for post harvest rot) five research institutes were involved: three for apple from the moderate climate zone of Europe and two for peach from the sub-tropical area.

In WP 4.2 twelve research institutes and universities, from seven EU countries cooperated in the development of a prototype of a multi-cultivar DSS for quality indices for apple and for peach and in the development of a prototype of portable, non-destructive multi-sensor device for the rapid assessment of fruit quality indices. Participants came from different climatological zones in Europe which is necessary for future use all over Europe. Furthermore the company AgroFresh had an advisory role in Workpackage 4.2.

Comments on participants

The structure of Pillar 4 and the choice of participants was adequate to execute the tasks which have been formulated in Annex 1.

The Workpackage 4.1 on sustainable non-chemical alternatives for pesticides (Safer fruit and environment by replacing pesticide treatment against post-harvest rot, *NON-CHEMFRUIT*) was lead by Paolo Bertolini from the University of Bologna (UNI.BO).

The role of UNI.BO and IRTA was to work on alternatives for post harvest rot in peaches. The ultimate goal was to select the best options to resist postharvest rot, not only with separate treatments like BCA, GRAS and Hot Water but also looking for the best combinations for a specific fruit, variety, fruit chain or fungus. This could lead to a combination of treatments, the so called hurdle technology. Both participants developed and used strains of Biological Control Agents (antagonists).

The consortium of a few renowned institutions on fruitquality was a fruitful combination which lead to specific and succesful solutions. In apple only three research institutes (WUR-PPO, Glemboux and IHC) were involved in the heavy apple task. This consortium turned out to be too small to get entire solutions in the total fruitchain for apples in different countries or areas. As mentioned in the introduction it was impossible to get practical solutions, especially for apple. More effort is necessary to achieve a total solution for application in the fruitchain for apples.

Regarding the development of the DSS system the structure of involved participants was adequate to do the work as described in annex 1. Almost all research institutes are represented in EUFRIN (European Organisation of Fruitresearch Institutes). Experimental set ups, persons and equipment to determine fruitquality are well known. This organization level of the participants was an important advantage for a fast start of the joined experiments in case of data collection for the DSS and the ND device.

As WP 4.2 leader Ria Derkx from WUR-PPO managed and organized the whole workpackage 4.2 *(Optimal Quality).*

RIPF from Skierniewice Poland had a central role in the development of a prototype of a multi-cultivar DSS for quality indices for apple and for peach, as RIPF provided the modeller.

This was a logical choice because RIPF had adequate personnel for constructing the DSS interface and for modelling. The modeller could profit from the European network of modelling on fruit quality.

DSS models were developed for peach and for apple. IRTA was task leader of the peach part, WUR-PPO took charge of the apple part. Within WP4.2 there was close cooperation between the modeller, the WP leader, and the research partners who were involved in the development of the DSS (DIAS, ACW, IRTA, UNIBO, WUR-PPO, RIPF, KOB, UdL, CTIFL, LAIMBURG) in order to set up experiments required for DSS development. This cooperation worked very well. Well before the start of a new harvest season schemes with proposed experiments were made by the WP leader, based on discussions with the modeller and partners could decide what was realistic to do. After completion of experiments datasets were sent to the modeller. Extensive analysis followed, resulting in many sets of models' parameters and implementation into the DSS. Besides biochemical work was done by IRTA and UDL to better understand the underlying principles of quality changes and consumer acceptance.

Close cooperation also occurred in the development of a prototype of portable, non-destructive multi-sensor device for the rapid assessment of fruit quality indices. In 2006, 2007 and 2008 IRTA, UPM, CTIFL and CEMAGREF did collaborative experiments to determine the effectiveness of different non-destructive (ND) techniques in estimating changes in quality and maturity/ripeness in peach/nectarine. IRTA coordinated this task. In apple ND work was coordinated by UNIBO.

The role of the SMEs was limited. Fecoam provided fruits to Spanish partners. SACMI was involved in the discussions on the development of a ND device. The SACMI NIR case was tested by several partners, but was more suitable for laboratory use than for use in the orchard. INOVAFRUIT decided to stop its activity in ISAFRUIT for internal reasons.

Table 2. List of Participants in Pillar 4 and their engagement in work packages and tasks

#	Participant name	Short name	Country	WP engagement	Tasks engagement
	SCIENTIFIC PARTICIPANTS				
1	Danish Institute of Agricultural Sciences	DIAS	DK	4.2	4.2.1, 4.2.3, 4.2.4
2	Agroscope Chängins-Wädenswil	ACW	CH	4.2	4.2.1, 4.2.2, 4.2.3, 4.2.4
3	Danish Institute for Food and Veterinary Research	DFVF	DK	4.2	4.2.1
4	Institut de Recerca i Tecnologia Agroalimentŕries	IRTA	E	4.1 and 4.2	4.1.1, 4.2.1, 4.2.2, 4.2.3, 4.2.4
6	Università di Bologna	UNIBO	I	4.1 and 4.2	4.1.1 4.2.1, 4.2.2, 4.2.3
8	Wageningen UR- Applied Plant Research	WUR-PPO	NL	4.1 and 4.2	4.1.1 4.2.1, 4.2.2, 4.2.3, 4.2.4
9	Wageningen UR- Plant Research International	WUR-PPI	NL	4.2	4.2.3
11	Research Institute of Pomology and Floriculture	RIPF	PL	4.2	4.2.1, 4.2.2, 4.2.3, 4.2.4
13	University of Glemboux	UGEM	B	4.1	4.1.1
17	Kompetenzzentrum Obstbau-Bodensee Bavendorf	KOB	D	4.2	4.2.1, 4.2.3, 4.2.4
23	Universitat de Lleida	UdL	E	4.2	4.2.1, 4.2.2, 4.2.3, 4.2.4
24	Universidad Politécnica de Madrid	UPM	E	4.2	4.2.2
27	Centre Technique Interprofessionel Fruits et Legumes	CTIFL	F	4.2	4.2.1, 4.2.2, 4.2.3, 4.2.4
31	Land- und Forstw. Versuchszentrum Laimburg, Auer	LAIMB	I	4.2	4.2.1, 4.2.2, 4.2.3, 4.2.4
39	CEMAGREF	CEMAGREF	F	4.2	4.2.2
41	East Malling Research Station	EMRS	UK	4.1	4.1.1
43	University of California, Pomology Department, Davis	UCD	USA	4.2	4.2.1, 4.2.2, 4.2.3, 4.2.4
	SMALL MEDIUM ENTERPRISES (SME) PARTICIPANTS				
51	ISOLLCELL	ISOLCELL	I	4.2	
52	SACMI	SACMI	I	4.2	4.2.2
53	Fecoam	Fecoam	E	4.2	4.2.2
63	Inova Fruit BV	IF	NL	4.2	4.2.1, 4.2.2

Comment on organisation matters

WUR-PPO was responsible for the coordination of Pillar 4 and representation in the Management Committee. The two workpackages in Pillar 4 had no mutual cooperation on worklevel so it was not necessary to organize combined meetings. Nevertheless each year a Pillar meeting was organized for organizational matters. This was always combined with the workpackage meetings of WP4.2 as indicated below.

For Workpackage 4.1 with only five participants and one continuous task, organization of activities was easy. For peach cooperation between UNI.BO and IRTA was close and well adjusted. The work on peach was well organized and at the end of the project an effective protocol for application was defined. In apple Glemboux, WUR-PPO and EMRS studied different applications. Glemboux provided antagonists to WUR-PPO to be combined with HWT. At EMRS HWT application was combined with GRAS substances. Collaboration between the partners was sufficient. In apple the research was more complex than expected. Despite the efforts of the participants results mainly included knowledge for further research.

WP4.2 was a well-organized workpackage within ISAFRUIT, although DSS development faced quite some problems. First, start of programming the DSS was delayed due to the lack of programming software and problems with hiring a well qualified programmer. As a consequence the interface of DSS has been prepared for evaluation with a few months delay. The second reason for delay in the development of DSS were the experimental data which had to be collected from new experiments. The analyses of datasets existing before the start of ISAFRUIT (available for analyses in summer 2006) and the results of first experiments showed that for development of reliable models new types of experiments had to be conducted both for apples and peaches. As a result the development of such models could start after the second season of experiments, and the whole process of testing and validation had to be postponed. High variation of data collected by some partners resulted in very time-consuming data analysis by the modeller, much longer than ever planned. In 2010 data analysis greatly progressed as a result of significant help by Dr. Pol (L.M.M.) Tijskens of WUR (Netherlands). ISAFRUIT Management allocated additional funds for that purpose. Dr. Tijskens scrutinized the modelling procedures developed previously by RIPF, and used them on numerous datasets, which were not analysed before. All successfully analysed or re-analysed datasets were implemented into the DSS.

Within WP4.2 there was close cooperation between the modeller, the WP leader, and the research partners who were involved in the development of the DSS. As a result experiments were well planned based on requirements of the modeller. After completion of experiments datasets were sent to the modeller for analysis and implementation into the DSS.

Development of a prototype of portable, non-destructive multi-sensor device for the rapid assessment of fruit quality indices in peach was well organized. Organization of unique collaborative experiments and workshops/meetings for data analysis of the huge amounts of data collected illustrate this.

Once or twice a year workpackage/pillar meetings were organized within WP4.2: 2006: Randwijk (NL), 2007: Montpellier (F), Bologna (I), 2008: Wädenswil (CH), Girona (E), 2009: Lisse (NL), Angers (F), 2010: Lisse (NL). In these meetings progress was discussed and plans were made.

Besides, other meetings were organized within WP4.2 in which small groups of partners participated. Examples are a meeting in Bologna in 2008 on non-destructive devices and a meeting in Lleida in 2007 on peach experiments for DSS development. The modeller had several meetings with the Pillar coordinator and with the Workpackage Leader on DSS development, in which progress was discussed as well as problems defined. This resulted in the help from an expert from Wageningen University, so that DSS development could be finished before the end of the ISAFRUIT project.

Comment on reporting and dissemination of results

During the whole period of ISAFRUIT Pillar 4 significantly contributed to dissemination of information about the project. Results were described in a range of deliverables and activity reports. Moreover, pillar 4 contributed to the ISAFRUIT mid-term evaluation. Results were disseminated at ISAFRUIT general assemblies, various symposia, conferences, congresses and meetings with growers. Pillar 4 and 5 organized the general assembly in Girona and the ISA Fruit symposium at the International Horticultural Congress in Portugal (IHC Lisboa 2010.) Besides many scientific and applied papers have been prepared and published or have been submitted.

In relation to the DSS two special websites have been created. At http://wiki.peaple-dss.pl/ a user can find information on fruit storage. Via www.peaple-dss.eu and www.peaple-dss.pl everybody can download the Peaple software, and find basic information about the way it works.

Relationships between scientific, technological objectives and Pillar 4 WP's

Objectives	Work package	Relationship
A. Increase fruit quality	4.1	The control strategies that were set up provided a significant reduction of the losses caused by rots, particularly on peaches, where postharvest chemical treatment is not allowed in the E.C., thus increasing the overall quality of fruits. The Hot Water Treatment also improved firmness of the fruits in some cases.
	4.2	A decision support system (DSS) for apple and peach can simulate quality changes along different supply chains. As a consequence the best supply chain for a batch of fruits can be selected, resulting in a better fruit quality and thus consumer's demands will be better met. Guidelines for a portable non-destructive multi-sensor device for rapid assessment of fruit quality have been developed. In apple and peach it was demonstrated that the non-destructive DA-index could be able to monitor the progression of fruit ripening on the tree and to predict the optimal harvest time. Moreover, harvested fruits could be graded into homogeneous classes of ripening with different cold storage (apple) and shelf life (peach) potential and quality at consumption. Fruit quality changes during cold storage could be monitored.
B. Increase fruit safety	4.1	It was proved that post harvest (PH) pathogens causing rots in apples, peaches and nectarines can be prevented without pesti-cides, by natural substances, biological and physical methods. Allyl-isothiocyanate, a substance extracted from the seeds of cruciferous crops, controlled brown rot on peaches and nectarines when PH applied as a vapour. Trans 2-hexenal, a component of aroma compounds of various fruit, prevented *Penicillium* rot in apples. Among the generally recognized as safe substances (GRAS) Na-molybdate, alcohol ethoxylate and peroxyacetic acid had the highest efficacy on apple rots. HWT was the best performing treatment to control and even to eradicate orchard established infections of peaches, nectarines and plums. The main innovations introduced through the experi-ments were the use of high temperatures (60-65°C) with a short dipping time (from 20 to 30 seconds) making this technology compatible with the speed of the working lines and with a limited impact on precooling, as only few outside layers of tissue are warmed up.

		The replacement of synthetic fungicides with natural substances, biological and physical methods greatly improved the safety of fresh fruit, that can be eaten with their skin. Moreover hot water dipping, used to control PH rots, removed up to 40% of orchard applied pesticides. Also the population of food born pathogens can be sensibly reduced by applying alcohol ethoxylate and peroxyacetic acid.
C. More convenience	-	
D. Better availability	4.2	Insight in quality changes along supply chains as provided by the DSS and the non-destructive devices, will result in better post-harvest handling and thereby reduce losses of fruits and losses of quality, thus increase availability of high quality fruits appreciated by consumers.
E. Raise consciousness	-	.
F. Lower Fruit price	4.2	A more efficient planning in the supply chain as a result of the DSS and quick non-destructive quality assessments by the new devices will result in a lower fruit price. Moreover losses of fruits and losses of quality will be reduced, which will also contribute to a lower fruit price.

Description of achievements within each work package during the course of the project

WP 4.1 Safer fruit and environment by replacing pesticide treatment against post-harvest rot

(*NON-CHEMFRUIT*)

Leader: Paolo Bertolini, 6. UNIBO; other partners: 4. IRTA; 8. WUR-PPO ; 13. UGEM; 41. EMRS.

In Annex 1 the specific goals to be achieved within Pillar 4 were formulated for each work package and their achievements are described:

The objective of this WP 4.1 was to provide a strategy for rot prevention, through the post-harvest chain, without pesticides, thus reducing contamination of the environment and providing consumers with safer fruit that can be eaten with their skin. To achieve this objective different means (natural substances, biological control agents, generally recognized as safe - GRAS - substances, physical methods) were combined into an integrated approach. The control strategies were applied to fruit coming from integrated disease management (IDM) or organically grown orchards. Preliminary tests evidenced that hot water treatments (HWT), given their eradicating activity on recently established orchard infections, could be one of the basic means to be combined with biological control agents, natural or GRAS substances. The possibility of HWT to remove the pesticides applied in the orchard, was also evaluated, although it was not included in Annex 1. In the last phase a large scale commercial test, with the involvement of a commercial packing house, was executed.

Task 4.1.1 Integrating the most effective methods

APPLES

Hot water treatments (HWTs)

The experiments conducted in UK led to the following recommendations:

- Golden Delicious can be safely treated up to 52°C for 40 seconds and at 54°C and 56°C for 20 seconds.
- Braeburn can be safely treated up to 56°C for 40 seconds and at 58°C for 20 seconds.
- Royal Gala can be safely treated up to 52°C for 40 seconds and 54°C for 20 seconds.
- Queen Cox can be safely treated up to 54°C for 20 seconds.

A temperature of 52°C up to 40 seconds was safe for all UK cultivars tested, no adverse effects on skin and firmness were detected.

Rotting caused predominantly by *Nectria* sp. and to a lesser extent by *Gloeosporium album* (syn. *Phlyctema vagabunda*) was significantly reduced by 52°C for 20 or 40 seconds and by 54°C for 20 seconds, whereas no effect was detected on *Botrytis* rot.

The experiments conducted in B and NL combining 4 temperatures and 2 dipping times showed that:

- No symptoms of heat damage were detected in Elstar apples, whereas a slight skin browning was found on Golden Delicious and Topaz depending on year, orchard and ripening stage. HWTs were effective to prevent rots caused by *Gloeosporium* sp. and *Nectria* sp. but were ineffective on *Botrytis cinerea*. In NL and B conditions, the treatments with temperatures effective against PH pathogens should be considered as unsafe given the damages caused on fruit depending on orchard, ripening stage and year.

HWTs combined with GRAS or BCAs

The following cultivars were tested: Golden Delicious (reference cv), Royal Gala, Braeburn, Queen cox, Rubens, Elstar and Topaz. In UK experiments HWT at 52°C for 40 seconds was combined with the GRAS (generally recognized as safe) treatments Na-molibdate, chitosan, K-metabisulfite, peroxyacetic acid, acetic acid, K sorbate, Ca chloride, Na bicarbonate, alcohol ethoxylate. Combined treatment did not improve the biocidal activity of the single treatments. Therefore in the following years only single GRAS treatments were tested. The most effective were Na-molybdate, peroxyacetic acid and alcohol ethoxylate.

In NL and B experiments HWts of Elstar, Topaz and Golden Delicious followed by dipping in a suspension of two biological control agents (Prestop and NEX 0101) did not improve the fungicidal activity, compared with single treatments. Single treatments with BCAs proved to be very effective for the control of *Penicillium* and *Botrytis* rot in apples. In both organically and traditionally grown apples fungicide residues on the fruit from preharvest treatments, sometimes reduced the activity of the antagonistic micro organisms, e.g. copper and sulphur residues on organically grown apples had a negative impact on BCAs, depending on dose, time of treatment and rain.

PEACHES AND NECTARINES

Hot water treatments (HWTs)

The experiments were conducted in Spain and Italy combining 4 temperatures and 4 dipping times. 17 cultivars were tested: Yellow peaches – *Springbelle, Elegant Lady, Rich Lady, Jesca, Placido, Tardibelle, Fayette, Symphony*. White peach – *Benedicta*. Yellow nectarines –

Big top, Venus, Guerriera, Stark Red Gold, Sweet Lady, Autumn Free. White nectarine – *Caldesi 2000*. Clingstone peach – *Andros.*

All the cvs were damaged by dippings at 70 and 65°C. In some cultivars slight symptoms of heat burning were detected after HWTs at 60°C for 40 seconds and particularly for 60 seconds. An excellent control of *Monilinia laxa* (Brown rot) was obtained at 60°C for 20, 30 or 40 seconds (efficacy index ranged from 82.3 to 100 % in peaches and from 71 to 100% in nectarines). The efficacy increased when the dipping time was prolonged up to 60sec, but sometimes the fruits were damaged. The efficacy index was usually lower in fruit coming from organic orchards as a result of heavier pre-harvest infections. The best treating condition to prevent brown rot, was 60°C - 65°C for 20 or 30 seconds. The mechanism of action of HWTs was partially elucidated and related to the biocidal activity of HW and the activation in peaches of disease resistance by the production of lytic enzymes active against fungi.

Some of the improvements that were developed in the laboratory were introduced (year 2009) in the dipping machine for pallet boxes (240 Kg each) that was used in the packing house of APOFRUIT Cooperative in Cesena (Italy). The working capacity of the dipping machine is 40 pallet boxes per hour and the result of this practical experiment was in line with the laboratory experiments.

Although not foreseen in Annex 1, two plum cultivars were additionally tested by the dipping machine. After two months of storage plus shelf life, the percentage of rots in HWT plums was reduced by 57%.

HWT also proved to be very effective to remove preharvest applied pesticides on peaches. The residues of preharvest applied captan and trifloxystrobin were reduced by about 40% by dipping in HW at 60°C for 30 seconds. Information on current achievements have been transferred to the industry by producing "Postharvest guideline for minimizing Brown rot of peach and nectarine by safe control methods ".

HWTs combined with GRAS or BCAs

The experiments were performed on the cultivars Big Top, Rich Lady, Elegant Lady, Venus and Tardibelle. In the experiments conducted in Spain three antagonistic micro organisms were evaluated in combination with HWTs at 60°C for 30 seconds. These strains were active on recently established infections but when they were applied on orchard infected fruit, subsequently stored at 0ºC, the efficacy was variable. To eradicate orchard infections a HWT was needed. When the fruit were pre treated with HW and then dipped in a suspension of BCAs, rot control was only improved in some cases. Because of the increased costs double treatment was not further tested.

In the experiments performed in Italy the GRAS substances allyl-isothiocyanate (AITC), *trans*-2-hexenal, carvacrol and citral, *trans*–cinnamaldehyde, hexanal, (-)-carvone, eugenol, 2–nonanone and *p*–anisaldehyde, sodium bicarbonate were tested as single treatments. The best control of brown rot was obtained with allyl-isothiocyanate (efficacy ranging from 87.2% to 100% depending on cv), *trans*-2-hexenal (efficacy ranging from 46.2% to 80.3%, depending on cv), whereas citral and carvacrol provided a lower efficacy, 40% and 32.9%, respectively. However the fumigation with *trans*-2-hexenal at concentrations that stopped decay were phytotoxic and produced off-odors or off-flavors in peaches and nectarines**.** No side effect was detectable after fumigation with allyl-isothiocyanate.

MAIN ACHIEVEMENTS Work package 4.1

The experiments performed on apples evidenced the possibility to apply HWTs at industrial level for the control of some of the most damaging rot agents like *Nectria* and *Gloeosporium*.

Treatments at 52°C for 40 seconds on Golden Delicious, Royal Gala, Braeburn and Queen Cox apples were safe for the fruit and very effective on rots. The best performing GRAS treatments on apples were Na-molybdate, peroxyacetic acid and alcohol ethoxylate, however it should be noted that the use of these substances should undergo EU registration. The fungicidal activity was not improved by combining GRAS treatments with HWTs.

The activity of BCAs on organically grown Dutch Elstar apples was reduced by the residues of fungicides containing copper and sulphur. As a consequence these BCAs cannot be applied in pest management strategies in which these fungicides are applied.

HWT was the best method to control and even to eradicate orchard established fungal infections of peaches, nectarines and plums. The main innovations introduced through the experiments were the use of high temperatures (60-65°C) with a short dipping time (from 20 to 30 seconds), making this technology compatible with the speed of the working lines and with a limited impact on precooling as only few outside layers of fruit tissue are warmed up. Some of the improvements that were developed during the laboratory tests were introduced in the dipping machine for pallet boxes (240 Kg each) that was used in the packing house. The working capacity of the dipping machine was 40 pallet boxes per hour.

Among the GRAS substances evaluated on peaches and nectarines the best results were obtained with allyl-isothiocyanate.

HWT also proved to be very effective to remove preharvest applied pesticides on peaches. The residues of preharvest applied captan and trifloxystrobin were reduced by about 40% by dipping in HW at 60°C for 30 seconds. Postharvest guideline for minimizing Brown rot of peach and nectarine by safe control methods was produced and has been transferred to the industry.

Further developments in HWTs: water heating is the prevailing cost in HWTs. Large quantities of HW could be obtained from the cooling plants of the cold stores. During summer the temperature of the hot compressed vapor coming out of the compressors reaches 90-100°C. This heat is lost through the condensers, instead it could be used to warm up water. A project to save energy in HWTs of fruit is in the pipe.

In conclusion Workpackage 4.1 contributed very well to the main objectives of ISAFruit regarding fruit safety and sustainability. Based on the progress which has been made in peach by application of the HWT and GRAS this workpackage delivered remarkable results.

WP4.2 Control of fruit quality from harvest to point of sale (*OPTIMALQUALITY)*

WP leader: Ria Derkx (WUR-PPO)

Contractors involved: 1. DIAS; 2. Agroscope ACW; 3. DFVF; 4. IRTA; 6. UNI.BO; 8. WUR-PPO; WUR-PRI; 11. RIPF; 17 KOB; 23 UdL; 24. UPM; 27 CTIFL; 31. LAIMBURG; 39. CEMAGREF; 43. UCD; 51 ISOLCELL; 52. SACMI; 53. FECOAM; 63. INOVA

Twelve research institutes and universities from seven EU countries cooperated in WP 4.2 in the development of a prototype of a multi-cultivar DSS for quality indices for apple and for peach and in the development of a prototype of portable, non-destructive multi-sensor device for the rapid assessment of fruit quality indices. Besides the University of California, Davis had an advisory role. Four SMEs were involved in WP4.2.

The overall objective is to achieve a better fruit quality chain management in consumer driven apple and peach chains. To attain this, a prototype of a decision support system (DSS) for fruit quality chain management and a prototype device for multi non-destructive quality assessment will be developed. This requires a multi-disciplinary approach of the necessary activities. The major challenge is to incorporate existing and new technological, scientific and practical knowledge into parameters and calculation rules for DSS decision making and to design equipment for non-destructive quality assessment. The WP is organized in 4 tasks:

1. Development of a prototype of a multi-cultivar DSS for quality indices for apple and for peach (work period: month 0-48).
    a. DSS design
    b. DSS programming
    c. Decision making
2. Development of a prototype of portable, non-destructive multi-sensor device for the rapid assessment of fruit quality indices. (work period: month 0-48).
3. Set up of DSS databases containing data and guidelines for decision making in fruit quality management. (work period: month 0-48).
4. Validation of the concept DSS in present day and simulated apple and peach chain. (work period: month 20-48).

Work performed and end results:

1. Development of a prototype of a multi-cultivar DSS for quality indices for apple and for peach and 3. Set up of DSS databases containing data and guidelines for decision making in fruit quality management.

In order to achieve a better fruit quality chain management in consumer driven apple and peach chains a prototype of a Decision Support System (DSS) for fruit quality chain management has been developed. The DSS is a software intended for producers, wholesalers and managers of auctions or storage facilities. The DSS can simulate quality changes of apples and peaches along different supply chains, starting at harvest, throughout storage to point of sale and is thus a total chain approach. The DSS has been made as a multi-cultivar system which allows the user to design virtually any supply chain, containing unlimited number of supply chain steps, with technological parameters of each step, i.e. duration, temperature, storage atmosphere, use of 1-MCP. At the end of the project it can be stated that the DSS, named "Peaple" has been developed, is available for all users via a created website and works well. The DSS contains models for different quality attributes in apple and in peach. For description of firmness changes a logistic equation is always used. The changes of acidity and soluble solids content are always described by an exponential equation. The color of peaches and apples is simulated by different models, depending on the color coordinate/attribute. The DSS contains 127 sets of model parameters for apple and 16 sets for peach/nectarine.

Upon the provided information on initial fruit status the DSS gives a user the possibility to select one or more of the available 143 sets of models' parameters characterizing changes in different fruit quality indices of batches of apple and peach fruits of several cultivars cultivated in different regions of Europe in different years. Apple cultivars include 'Golden Delicious', 'Elstar', 'Elshof', 'Pinova' and 'Rubens' and peach cultivars include 'Rich Lady', 'Big Top' and 'Zee Glo'. Different chain players can use the DSS in order to plan their own commercial strategies. It can also be used as a training tool to investigate the influence of various environmental conditions on dynamics of fruit postharvest senescence. As a result users may build their own collection of different supply chains e.g. for different fruits, locations, orchards or clients and make selections out of it when starting to make simulations for a new cultivar or for a new client.

In order to build the DSS huge datasets have been collected in 2006-2010 on effects of ripening stage, storage duration, storage temperature and shelf life on several quality attributes in apple and peach, including fruit firmness, titratable acidity and the non-destructive I_{AD}-index. Moreover, effects of 1-MCP, hot water treatment and Dynamically Controlled Atmosphere have been included in apple, as they may alter storage behavior, and reduce losses, thereby increasing quality. Data were collected in a range of peach and apple cultivars, in different countries, in different orchards and in different years. These experiments characterized the most important quality parameters by which the DSS model had to be built and gave very valuable information on true processes during the pre-and post-harvest period. As a result of better insight in fruit quality changes in the supply chain, planning can be improved resulting in a better fruit quality for the consumer, a better availability and a lower price.

In order to better understand the underlying principles of quality changes and consumer acceptance in peach, some biochemical studies were performed.

It was demonstrated in peach and nectarine fruit that flavour is a major attribute determining consumer acceptance after storage, whereas firmness has apparently an indirect influence on consumer's satisfaction. As a consequence special attention has been given to different biochemical factors underlying flavour-related volatile biosynthesis and fruit softening. Results show that the different biochemical parameters studied are strongly altered in response to all post-harvest procedures considered, including storage temperature, atmosphere and period, 1-MCP treatment, hot water treatment, calcium dip, and CO_2. Traditionally, the fruit industry focused on firmness and appearance, but consumer satisfaction and thus repeated purchases are dependent on flavour. The final goal of the ISAFRUIT project is to increase fruit consumption. This goal can only be achieved if aroma/flavour is taken into account when determining harvest date and when implementing post-harvest procedures and commercialisation chains.

Additional biochemical studies were also performed to understand the difference in behavior of different cultivars with regards to temperature, more specifically chilling injury inducing temperatures. It appears that the ethylene metabolism is involved in this process. A sharp induction of the ethylene metabolism, likely related to the induction of chilling disorders can be observed at chilling injury inducing temperatures (6ºC - 12ºC) in chilling sensitive peach, whereas it is not present in insensitive nectarine.

2. Development of a prototype of portable, non-destructive multi-sensor device for the rapid assessment of fruit quality indices

Peach

In order to develop a rapid, portable, non-destructive multi-sensor method for fruit quality measurement collaborative experiments were carried out in peach in 2006, 2007 and 2008. In these experiments different non-destructive devices from different partners were tested on one common fruit sample and correlations with reference parameters such as firmness and acidity were established. In 2006 the cultivar 'Rich Lady' was used, in 2007 'Ryan Sun'. As the change in cultivar emphasised the fact that the model was cultivar dependent, three cultivars ('Rich Lady', 'Ryan Sun' and 'O'Henry') were used in 2008 to refine the model. Data collected on the different cultivars during the different years were extensively analysed to build one overall model. This has led to the establishment of an optimized model that can effectively estimate non-destructively firmness and soluble solids content in peach. It combines measurements of optical (pigments) and firmness properties of the peach, with existing commercial portable equipment. This model is a good basis for a physical ND prototype. Additional work will be required to adapt the NIR, impact and acoustic technology in collaboration with commercial companies specialized in ND technology.

Besides, it was demonstrated that the Index of Absorbance Difference (I_{AD}) (a combination of optical absorption in two different wavelengths), measured by the new portable non-destructive device DA meter, is a reliable tool to assess the fruit ripening stage of several peach cultivars. It turned out to be very useful to predict the optimal harvest date and to enhance the homogeneity of a batch of fruit. Therefore, the application of the user-friendly non-destructive device DA-meter in a total chain approach to monitor the fruit ripening from plant to consumption would strongly improve the homogeneity and the final quality of fruit at consumption. Further optical procedures using the same and improved optical indexes and spectral imaging have been developed, for portable and for on-line equipment with potential for packing-house grading of peach according to maturity.

Apple

Also in apple it was demonstrated that the I_{AD}, measured by the new portable non-destructive device DA meter, is a useful tool to assess apple fruit ripening. In fact, in several cultivars good correlations have been observed between the non-destructive index, the ethylene production and traditional quality parameters, like ground colour, titratable acidity and some-times firmness. Therefore, this non-destructive index could be useful to monitor the ripening evolution of apple fruit both pre- and post-harvest and, consequently, to predict the optimal harvest date and to define the best storage strategy. For example, the use of the I_{AD} can provide essential information for the application of 1-MCP, indicating the limit of effec-tiveness of the product. Moreover, it was demonstrated that fruit characterized by different I_{AD} values are recognized as qualitatively different by consumers.

3. Validation of the concept DSS in present day and simulated apple and peach chain.

The DSS was tested and validated by several WP4.2 partners, who provided data for DSS development. The DSS generally works reasonable to good for the known cultivar/orchard/season combinations (143 models' parameters sets). However, when non-analyzed 'new' datasets were tested differences were observed between measured and predicted values in several datasets. These differences might be attributed to differences in model parameters, like upper asymptote and kinetic rate values between analyzed datasets and 'new' datasets, as it was demonstrated that huge differences might exist between model para-meters of one cultivar from different locations or different seasons. As a consequence over- and underestimations are possible every time simulations are made on a new batch of fruits, using sets of parameters estimated for other fruits. A user has to be aware of that. In order to help a user in the selection of sets of model parameters a procedure has been included in the DSS.

Also other explanations have been given for over- and underestimations, like the use of handheld equipment by different operators, resulting in different input values for the DSS and the fact that some datasets were rather erratic. It is absolutely necessary that datasets are collected very carefully and uniformly with a sufficient number of measurement dates. More-over, it can be expected that use of a universal testing machine for firmness would give better kinetic and batch model parameters. However, due to the enormous number of measurements that had to be done for DSS development, handheld equipment was the only option for firmness determinations within ISAFRUIT.

The biggest challenge for future improvements is to find a method (new type of meter/new index or quick analysis of traditional measurements) that gives information on storage poten-tial", which allows to assess the batch model parameters, like upper and bottom asymptotes of each new batch of fruits. The potential of non-destructive indexes, like the I_{AD}, was extensively studied in this respect, but this approach failed.

Assessment

Workpackage 4.2 contributes to several objectives of ISAFRUIT as indicated in the table. The contribution to the objective fruit quality is especially important in order to meet consumer's demands. The DSS system aims to predict quality from production to point of sale and is therefore a total chain approach. But also particular elements of the total system like improved storage systems (1-MCP and DCA storage), non-destructive measurement of fruit quality, better determination of harvesting time etc, are important contributions to fruit quality.

Workpackage 4.2 also contributes to a better availability. The DSS system and the other developments will improve prediction and realization of good fruit quality throughout the season. The variability of fruit quality will decrease, which means that good and stable quality fruit is available on the market over a longer period.

The innovations in Workpackage 4.2 will also reduce losses of fruit, which will contribute to the objective of sustainability.

Pillar 5. Quality, safety and sustainability – Improved preharvest chain.

Summary

The pre harvest fruit production chain was addressed by Pillar 5, with special focus on points that are critical for the provision of apple and peach quality sustainably production in European growing zones. With reference to knowledge development and transfer, the Pillar was thought to create knowledge and methods that refer to 3 levels: i) ready to be transferred to extension services for final local adaptation and transfer into practice, ii) ready to be taken up by private industry (spin-off), and iii) new fundamental knowledge for further R & D to reach practical application (pre-competitive research). New knowledge and methods were developed by a network of the 19 public research institutions from 11 countries and 7 SMEs from 3 countries.

ECOFRUIT aimed at developing systems that would ensure efficient use and drastical reduction of pesticides and nutrients used in fruit growing and by that improve the inherent fruit quality for making fruit more attractive and safe as demanded by markets and consumers. The Crop Adapted Spray Application (CASA)-group developed a CASA-sprayer, integrating a Crop Identification System (CIS) and an Environmentally Dependent Application System (EDAS), while the development of a Crop Health Sensor (CHS) for the on field identification of the tree health status resulted far advanced but not yet in a stage of integration into the CASA-sprayer (European and Polish patents). The CIS enabled to reduce consistently (up to 82%) the spray volume application rate (l/ha) with respect to the reference method, while maintaining biological efficacy on target organisms of the pesticides applied. With regard to pesticide residues, the CIS produced less pesticide variation between individual fruits. EDAS gave over 80% reduction of drift outside orchard. First field tests showed that the CASA-system can produce lower residue levels in apples compared to a standard spraying machine. On a European demo-tour, the sprayer performance was demonstrated at 8 locations in Denmark, Italy, the Netherlands, Germany, France, Poland and Spain to advisers, press, extension services, sprayers' manufacturers and fruit growers. The Crop Adapted

Fertilizer Application (CAFA)-group addressed 3 aspects of tree nutrition: a tree demand based model for predicting nutrient uptake by apple, nutrient application technology preventing from nutrient loss mainly looking at Fe in peach orachards, and improving tree nutrition in organic orchards. The model has been developed for apple cultivars Fuji and Gala and is available on the ISAFRUIT website. Formulations for iron fertilizers in peach, which help reducing dosages in peach, or innovative methodologies for chelate analysis using HPLC coupled to time-of-flight mass spectrometry were developed, which help monitoring in fertilized plants. Fruit quality and yield changes of pear and peach trees affected by iron deficiency chlorosis were characterised. For improving tree nutrition in organic apple orchards, several approaches were tested in the field involving the use of compost, commercial N-fertilizers and foliar N-fertilizers. According to the results, soil fertility should be more in the focus than fertilization, since data showed that soil fertility conditions could not be compensated by the fertilization inputs.

SMARTFRUIT aimed at meeting the fruit quality standards required by the market and consumers through an optimised, i.e. respectful and well-directed (smart) resource use (genotype, light, water, nutrients) including an optimised crop load management (resource genotype). A range of studies was performed generating fundametal precompetitive knowledge, knowledeg ready to be transfered to private industry and konwledge publically available to extension serivces. Produced outputs are summarised as follows: Trans-European crop load regulation studies provided quantitative evidence of common principles and demonstrate that permanent adaptive research is required in each fruit growing zone for the fine tuning of methods and for optimally meeting local quality standards. Crop load regulation strategies for integrated and organic production including zero-residue thinning methods were studied. Significant influence of management practices on the allergenicity was found for apple (shadowing, elevation and storage reduce expression) and peach (enhancing the light radiation and decreasing crop load reduce transcription). The reduction of water loss was studied using peach tree orchards in dry Mediterranean climates as a model system, including the water use x genotype x fruit quality interaction. Positive effect of reflective mulches on cumulative peach yield, average fruit weight and size, ripeness and phenolic compound contents were found. Fundamental studies for understanding apple fruit abscission as provoked by shading orchards were performed as well as field trials for developing practical protocols including economical aspects to use this phenomenon for practical crop load regulation (fruit thinning). Models on peach fruit growth, for predicting apple fruit size, and for forecasting apple June drop for supporting thinning decisions were developed and showed promise in first verification tests. By using e-nose techniques and NIR-analysis on growing fruits, progress was made in developing tools for forecasting ripeness in apple, for discriminating in the orchard between fruits subjected to different crop management interventions and for reducing fruit quality heterogeneity at tree level. For conceptualising an apple abscission model candidate genes for abscission were identified. A 30K microarray can be used for the evaluation of thinning agents and the characterization of genotypes with interesting self-thinning attitudes.

PROFITFRUIT aimed at providing knowledge and methods for a sustainable management of integrated and organic apple farms. Crop management and economical data were collected in a transdisciplinary approach in apple growing zones in the North of Germany, the South of France, the Netherlands and some parts of Switzerland. The profitability of integrated and of organic apple growing was determined and compared. Key factors for successful sustainable farm management were identified for these growing zones. A user-friendly IT-model (PROFITFRUIT) was developed and delivered in English, German, French and Dutch for simulating production cost at the orchard and variety level. Additional costs, labour demand, and high yields of first quality fruit were identified to be critical success factors for the success of the introduction of innovative techniques. Technical, social and economical attributes of a sustainable fruit farm management were identified. A user-friendly, IT-based decision tool was developed for supporting fruit growers to take the right decision for producing good fruit quality and if converting their fruit farms from the integrated to the organic system or vice versa. Besides these objectives, PROFITFRUIT was the back bone of ISAFRUIT for evaluating innovations of Pillars 4 and 5 in terms of effects on production cost. The work package was also mandated by the Management Committee to develop a study on comparing fruit prices paid to farmers at the farm gate with the fruit prices to be paid by consumers at the point of sale. The respective report is published on the ISAFRUIT website.

The Pillar's activity was developed based on strong interactions between work packages of Pillar 5 and with other Pillars (1, 2 4, 6 and 8). The Pillar devoted a major effort in contributing to the **Vasco da Gama-process** for strengthening as science-based approach to support interdisciplinary research (see Pillar 8).

General objectives as defined in Annex 1

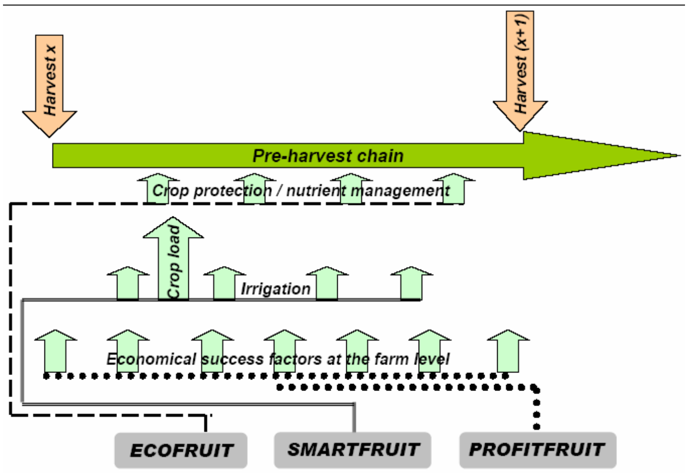
The overall goal of the Project was increasing fruit consumption in Europe through the improvement of fresh and processed fruit quality and safety and a better understanding of consumers' needs and behaviour.

In Annex 1 (p. 6) scientific and technological objectives were established, which in the case of pillar 5 covered the following topics:

- Increase the quality of fruit and fruit products meeting the consumer preferences for fruit quality, improving quality of selected processed fruit products, improving the chain management regarding quality aspects, generating knowledge on the genetic basis of fruit quality and developing and implementing new cultivars that are better suited to meet consumer preferences.
- Increase fruit safety by developing new production systems aiming at decreased levels of residues, enhancing safety of fresh and minimally processed fruits, improving chain management regarding safety aspects (providing safer alternatives to actual pre- and post-harvest treatments or crop-tailored spraying techniques) and by identifying low allergenic cultivars.
- Raise consciousness of consumers by generating and disseminating knowledge on short- and long-term beneficial effects of regular fruit consumption on human health and the relationship with prevention of diseases and over-weight.
- Contribute to the possibility of lower price through analyzing and benchmarking fruit chains, improving efficiency of low-input and organic production systems and developing possibilities for improved profitability of more sustainable production systems.

To achieve planned goals each work package was divided into tasks, which were assigned to competent partners working for the project (Table 1). Some partners were engaged in completion of two or even three work packages and numerous tasks. The tasks are listed in Annex 2 and results reported in the deliverables listed in Annex 3.

Basic information, contractors, work performed, end results Pillar 5



Pillar 5 was about to address key factors and bottlenecks of the pre-harvest chain to improved fruit quality in a way which responded to consumer demand (see figure from Annex 1). The desired fruit quality had to be produced in a sustainable way. By this, Pillar 5 was to contribute significnatly to the overal proeject goal to augemnt fruit consumption in Europe.

Numerous partners were involved in Pillar 5 in different roles (e.g. ACW: coordinating and scientific participant in Pillar 5, also scientific participant in Pillar 1, 2, 4, 6 and 8, but with different organisational units involved). Furhermore, the Pillar joined specialists of many research institutes and SMEs representing quite different steps in the technology transfer and echange chain from fundamental science to application in the field. There were scientists involved in rather fundamental work (e.g. molecular analysis of gene expression related with fruit abscission) while others were involved in technologcial transfer studies (e.g. CASA-spayer field tests).

This was a somewhat ambitious conceptual setting of Pillar 5, i.e. a challenge for consistently addressing the mentioned ISAFRUIT-goals, while it opened also the possibility for numerous unique interdisciplinary interactions and opportunities. This is, because Pillar 5 developed since the beginning of the project a strong interest in integrating the science and activities developed by the many disciplines and participanting institutions involved in ISAFRUIT. Consequently, Pillar 5 started to develop an intercation plan, which was constantly updated and reported as a deliverable of the Pillar5. Furthermore, after the 1st project year it became clear, that interdisciplinary interaction does not happen by itsself even if the scientists work for the same project. A conceptual framwork adn methodology was needed for taking advantage of this inter- and transdisciplinary setting of ISAFRUIT. Based on an inititaive of Pillar 5, the Vasco da Gama-initiative was launched (see deliverables Pillar 8) for bringing together social and natural scientists and developing a methodology for linking disciplines and orienting research to assure consumer-orientation in ISAFRUIT's pre- and post harvest research. The tool developed by a cross pillar team, the ISAFRUIT House of Quality, was tested in several case studies.

The Pillar's activity aimed at impact and innovation evidenced e.g. by the high number of SMEs involved in the Pillar activites or the European patents obtained for some of the technological innocations (pesticide spraying technology), the European and Polish patenting of CASA-sprayer components and the high output in technical publications.

Contractors/participants

In Pillar 5 participated 19 research institutions (institutes and universities), representing 11 EU countries. Several of the prinicipal partners were also heavily involved in other Pillars (e.g. RFIP, WUR-PPO, UNIBO), reporting to other Pillar coordinators. This stimulated interactions, but was challaenge to maintain the overview.

In the pillar 5, 7 SMEs representing 3 countries were involved.

Comment on participants and relationships between participants

The scientists involved in research carried out by represented different specialties, there were agricultural technology engineers, phytopathologists, fruit tree and fruit physiologists including specialsits for tree nutrition and intergarted and organic production, molecular biologists, agronomists and agricultural economists and consumer scientists also relating to food scientists and food chemists. The team was indeed interdisciplinary and, by involving also stakholder representatives and organisations, transdisciplinary.

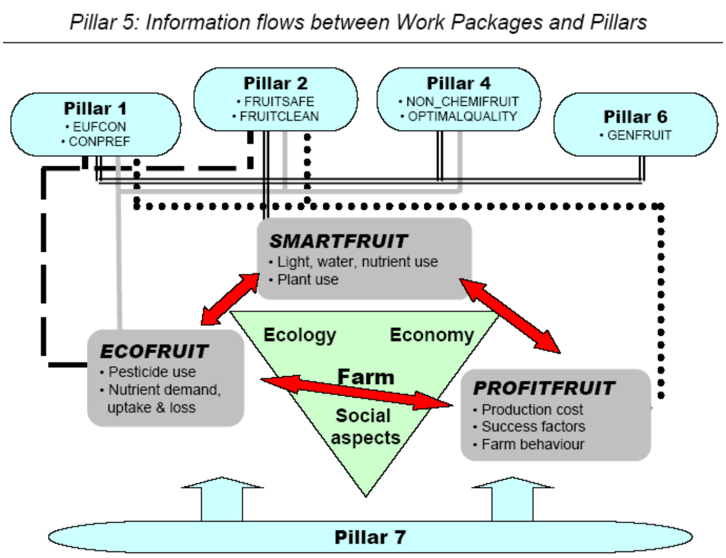
Some of the involved scientists were also involved in other Pillars, particularly Pillar 4. This, and in particular the heavy involvement of Pillar 5 in the Vasco da Gama inisiative (mentioned above and below) created a truely interdisciplinary environment, which indeed beared fruit by achieving not only selected results of high scientific value, but outputs which were validated in a quite holistic way and qualifying by this for a high impact potential.

Some of these relationships between partners were developing by itsself, particlulary if the based on relationships that were aleady existing before the project started. Other such relationships were stimulated by the project, often initiated and later strengthened by workshops at the General Assemblies or other special occasions.

The partners involved were motivated and engaged, even if some delays in supplying deliverables had to be accepted for jusified reasons. The reasons for delays were always commented in the activity reports. Some partners were excellent in basic research, others in practical approach but all contributed a valuble element to the final output. Only with two scienific partners, it seemed during project execution consistently difficult to obtain the reports needed. Also, considerable efforts were needed for focusing some of the partners on the major trait to be addressed by Pillar 5: fruit quality. These management challenges related to the fact, that the project was very big. Annex 1 designed a multiple disuipline programme with institutions across Europe which were not all necesarily collaborating or knowing each other from the beginning of the project. This was a team budilding challenge. It was not possible and in case, the contribution of a partner was small even if essential but small, not justified to bring all partners physically together. The results of the team building efforts however seems quite positive if looking at the knowledge and technology developed and the final project output.

Some difficulies were experienced in collaborating with small SMEs, which are explained further below.

Specific interactions between participants



Interactions developed along the lines planned at project start (see figure from Annex 1) and stimulated even further direct linkages (e.g. between WPs 4.1 and 5.3).

The were strong interactions between the participants within Pillar 5 and also with work packages from other Pillars. Particularly. The economical expertise of WP 5.3 was instrumentalised for evaluating in economical terms technological innovations developed by the project (particularlyl by Pillars 4 and 5).

Particular interactions were: Tasks 5.2.1 (Non-alternating orchard cropping patterns) with WP 4.2: altering fruit cell growth dynamics to improve fruit storability), WP 2.3 (allergenic properties of apple); Task 5.2.3 with WP 4.2 forecasting fruit quality (ripening); Task 5.3.2 (PROFITFRUIT-model) with WP 5.1 (post harvest hot water treatment), Tasks 5.1.7 (organic tree nutrition), 5.2.1 (CASA-sprayer), 5.2.2 (thinning by shading, mechanical thinning).

Interaction plan and Vasco da Gama-initiative

The interactions were stimulated and discussed during workshopa at the 1std , 2nd and 3rd Gerenal Assembly. Essential elements of the substantial interaction activity of Pillar 5 was the interaction plan (a specific deliverable) and the strong involvement in the Vasco da Gama-initiative, as explained above already (see also deliverables Pillar 8).

Comment on organisational and administrative matters

Du to the high numebr of partners involved, Pillar 5 concentrated on Pillar meetings at the General Assemblies. Specific workshops were organised in the context of the Vasco da Gama-process.

Project administration procedures, requested by the financial guidelines of FP6 projects were too heavy for small SMEs with small project budgets. This became clear during project execution with two SME partners involved in Pillar 5. One left the project in its first year. Another steped out in the 4th project year by giving back the project money it obtained. This was not a nice experience and required considerable management efforts and resources, resulting in a quite bad balance between effort need and impact generrated.

If SMEs continue to play an important role in the EU FRP-research programme strategy, it seems necessary to simplify considerable the respective administrative procedures.

Comment on reporting and dissemination of results

Pillar 5 achieved a high output in partical, technical and scientific publications, oral presentations and other communications (see list "all publishabel results" and list "dissemination of knowledge"). It was a challenge to produce a significant scientific, impact-oriented output in peer-reviewed journal within the 4.5 years of the project: Apple and peach are perennial crops (1 harvest per year), i.e. in most cases several (at least 3) growing seasons are needed for achieving significant scientific results. The output of practical and technical publications and communications of Pillar 5 was high during the entire project, while outputs in scientific journals was initially low, but increased steadily during project execution achieving by the end of the project a respectful level. A unusual, particular effort was realised for promoting the CASA-sparyer in a trasn-european demo-tours at 8 locations in Denmark, Italy, the Netherlands, Germany, France, Poland and Spain including demo materials in 8 language versions (GB, IT, DE, ES, PL, FR, NL, DK). Also, Pillar 5 contributed significantly to the concluding project symposium at the International Horticultural Congress IHC 2010 in August 2010 in Lissbon by organising sessions on the pre-harvest actuivity and the Vasco da gama-process.

Table 1. List of Participants in Pillar 5 and their engagement in work packages and tasks

#	Participant name	Short name	Country	WP engagement	Tasks engagement
	SCIENTIFIC PARTICIPANTS				
1	**Danish Institute of Agricultural Sciences**	**DIAS**	**DK**	5.2	5.2.1
2	**Agroscope Changins-Wädenswil**	**ACW**	**CH**	5.1, 5.2 & 5.3	5.1.2, 5.1.4, 5.1.7, 5.2.1, 5.2.2, 5.3.1, 5.3.2, 5.3.3
3	**Danish Institute for Food and Veterinary Research**	**DFVF**	**DK**	5.1 & 5.3	5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.1.7
4	**Institut de Recerca i Tecnologia Agroalimentàries**	**IRTA**	**E**	5.2	5.2.1, 5.2.2, 5.2.3
5	**Institut National Recherche Agronomique**	**INRA**	**F**	5.2	5.2.1, 5.2.3
6	**Università di Bologna**	**UNIBO**	**I**	5.1 & 5.2	5.1.5, 5.1.6, 5.1.7, 5.2.1, 5.2.2, 5.2.3
7	**The Norwegian Institute of Agricultural and Environmental Research**	**NCRI**	**N**	5.2	5.2.1
8	**Wageningen UR-Applied Plant Research**	**WUR-PPO**	**NL**	5.1, 5.2 & 5.3	5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.1.5, 5.2.1, 5.2.3, 5.3.1, 5.3.2, 5.3.3
9	**Wageningen UR-Plant Research International**	**WUR-PRI**	**NL**	5.1 & 5.2	5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.2.1
10	**Wageningen UR-Agricultural Economics Research Institute**	**WUR-LEI**	**NL**	5.1, 5.2 & 5.3	5.1.4, 5.1.5, 5.2.1, 5.2.3, 5.3.1, 5.3.3
11	**Research Institute of Pomology and Floriculture**	**RIPF**	**PL**	5.1	5.1.1, 5.1.2, 5.1.3, 5.1.4
14	**Research Institute of Organic Farming**	**FiBL**	**CH**	5.1, 5.2 & 5.3	5.1.5, 5.1.7, 5.2.1, 5.3.1, 5.3.3
20	**Obstbau Versuchsring Jork**	**OVR**	**D**	5.3	5.3.1, 5.3.2, 5.3.3
26	**Consejo Superior de Investigaciones Científicas, EE Aula Dei**	**CSIC**	**E**	5.1	5.1.5, 5.1.6
28	**Groupe de Recherche en Agriculture biologique, Avignon**	**GRAB**	**F**	5.1 & 5.3	5.1.7, 5.3.3
31	**Land- und Forstw. Versuchszentrum Laimburg, Auer**	**LAIMB**	**I**	5.1	5.1.7
32	**DEIAFA Turin**	**DEIAFA**	**I**	5.1	5.1.1, 5.1.2, 5.1.3, 5.1.4
33	**University of Padova**	**UNIPD**	**I**	5.2	5.2.1, 5.2.3
40	**Agricultural Institute of Slovenia (KIS)**	**KIS**	**SL**	5.2	5.2.1
	SME's PARTICIPANTS				
44	**Andermatt Biocontrol AG**	**ABAG**	**CH**	5.1	5.1.7
45	**Hauert HBG Dünger AG**	**HAUERT**	**CH**	5.1	5.1.7
46	**Schweizerischer Obstverband**	**SOV**	**CH**	5.3	5.3.1, 5.3.2
50	**Intrachem Bio Italia S.P.A.**	**IBI**	**I**	5.1	5.1.7
61	**Sistemi Elettronici Industriali**	**3B6**	**I**	5.1	5.1.1
62	**Netzteam**	**NT**	**CH**	5.2	5.2.2
66	**AGROCOM Polska**	**AP**	**PL**		5.1.3

Relationships between scientific, technological objectives of ISAFRUIT and how they were reached yb WP 5.1, 5.2 and 5.3 for the period 2006 –2010

Objectives	Work package	Relationship
A. Increase fruit quality	5.1	The results showed that variable rate application performed by CASA sprayer with CIS system, may lead to increase of both external and internal fruit quality compared to the use of conventional sprayer. The precise (more uniform) spray deposition; obtained with a reduced 50% dose of PPPs resulted in less fruit damage caused by diseases (apple scab and powdery mildew) and less fruit with high pesticide residue (less variation of pesticide residue between individual fruits). Also, technology has been developed for improving tree nutrition in organic apple-growing for producing better quality. Plant demand driven fertilisation techniques were developed, i.e. formulations for iron fertilizers, which help reducing dosages in peach, or methodologies for iron chelate analysis developed, which help monitoring in fertilized plants. The developed crop protection spray and fertilisation technology requiring less chemical input into the environment contributes very much to a healthier environment, which is nowadays an intrinsic, market relevant fruit quality trait.
	5.2	The results pointed out that alternate bearing continues to be an important syndrome that affect fruit quantity and quality. This phenomenon creates more problems in pome as compared to stone-fruit. Control of this phenomenon was obtained by innovative physical method, such as pruning (bud extinction, etc) as well as by the use of novel fruit thinners. In pomefruit, thinning is a fundamental chemical management operation for obtaining high quality fruits. The availability of different thinners classes (ATS, Ethephon, etc; fruitlet stage: auxins, cytochinins) allows to obtain good results. In stone fruit, thinning becomes also essential although the lack of effective chemical thinners creates difficulties. However in the last years of the project the availability of new molecules (Abscisic acid, auxins) opened interesting possibilities.
	5.3	By setting up the concept for a close collaboration between growers, extension and research, the fruit quality was and will still be further increased (OVR – 5.3.1). Results from a survey in Switzerland showed that apple growers with larger apple surface and mainly fruit production achieve higher yield of the top quality class than apple growers with less surface and intercrop farms (ACW – 5.3.1). By using the model PROFITFRUIT, apple growers can evaluate effects of different techniques on their income. Quality (e.g ranking results, but also aspects like reduced use of chemicals) affects price and, therefore, has a strong impact on the labour income. To improve labour income, apple growers need to produce high quality fruit. The model shows the effect on returns and costs (WUR-PPO – 5.3.2). In future, growers who want to switch to organic production could use an ISAFRUIT decision tool. By using this multi-attribute model, growers can check if they should convert to organic or not, taking into account technical, economical and environmental parameters.
B. Increase fruit safety	5.1	Use of CHS for detection of diseases on apple leaves in early stage of development allows to introduce a new crop protection strategy with drastically reduced use of PPP. Also CIS has a potential to reduce chemical input by 50% when performing variable dose rate application. This leads to essential reduction, or even elimination, of PPP residues in fruits.

	5.2	Use of environment-friendly chemical and alternative methods for fruit thinning such as light reduction and mechanical approaches were tested for a better, more effective exploitation of natural resources.
	5.3	In WP 5.3, the costs and benefits of the use of ISAFRUIT technology, e.g. of the CASA sprayer and of the hot water treatments, were calculated by using the model PROFITFRUIT. If we assume, that the apples will be safer by using less pesticides, then WP 5.3 contributed to this ISAFRUIT objective with helping to value the feasibility of ISAFRUIT technology for safer fruit by the mentioned calculations..
C. More convenience	5.2	This ISAFRUIT objective is not in the centre of this work package. However, if convenience of fruit products for consumers is appreciated in its large sense, and if consequently, fruit size and extrinsic fruit quality parameters such as reduced resource use, avoidance of chemical and not nature identical products such as thinning agents of mineral fertilizers are considered as being part of a “convenient” fruit, the work package has contributed with advancing and developing respective production methods making possible a “convenient” fruit.
D. Better availability	5.2	Implicitly, the work package has contributed in making possible a better availability of fruit with fruit quality characteristics as desired by the consumer by developing production methods which meet these quality standard. However, many other factors, which could not be influenced by ISAFRUIT, play an eventually decisive role determining how much quality fruit is available to the consumers at the point of sale.
E. Raise consciousness	5.1	Dissemination of knowledge and project results, especially with tools available for consumers (press articles, internet, demonstrations) and in the native languages of the consumers is the most efficient way to raise the consciousness. Such tools were used across Europe to convince consumers that sustainable fruit production with innovative crop adapted pesticide and fertiliser application systems ensures supply of a safer fruit on the market and preserves a healthier environment for the present and future generations.
	5.2	Dissemination of knowledge and project results, especially with path-ways and tools available to stakeholders of fruit-growing and, since public, also to consumers (publications, meetings, webpage, open days with extension and consumers representative) offered in English or the respective national languages of the consumers were the way how to raise consciousness. These pathways were used across Europe to make public that sustainable fruit production with innovative methods for crop load control and more effective resource use contributes to safer fruit on the market while preserving a healthier environment. Whether the level of consciousness has been indeed influenced by this is difficult to verify.
	5.3	The PROFITFRUIT model can increase the consciousness of apple growers regarding the impact of measurements and techniques, deve-loped in several (ISAFRUIT) projects, on their income (WUR-PPO – 5.3.2).
F. Lower Fruit price	5.1	Fruit price depends on fruit production costs. At the relatively large fruit growing farms the higher price of advanced spray application tech-nology, like CASA sprayer, may be compensated by drastically reduced chemical input and hence considerably decreased annual costs of PPPs and labour. Besides implementing environmentally friendly production methods may allow growers benefit from the financial assistance and incentives. These extra profits for fruit growers will certainly be reflected in lower price of fruit for consumers.
	5.2	Production techniques (and a higher income/kg fruit) does not

		necessarily, but may in certain cases influence the fruit price at the point of sale, depending on the cost of the production technique. If a new technology is more expensive than the prevalent one, the technology might not be adapted easily and it may influence the price at the point of sale (e.g. fruit from IP or organic systems). Some technology, developed by this work package has been studied with regard to its impact on production cost. The thinning by shading technique was economically analysed (by WP 5.3), based on this analyses, it could be concluded, that the technique is particularly applicable and competitive for organic production systems. In general terms, the work package addressed production techniques which should make production more efficient, which potential may (but must not) contribute to a lower price.
	5.3	In order to achieve a lower fruit price in long term period, the economic sustainability of the apple production is very important. If apple growers go bankrupt, the produced quantity will drop and the price will rise. To ensure that growers will produce apples on a long term period, their decision should base on technical and managerial knowledge. With extension services as suggested in task 5.3.1 (OVR) quantity and quality of production could be on high standards entailing acceptable income as a consequence. Within WP 5.3, methods and decision tools were developed in order to help apple growers to make rational decisions, and, thus, to guaranty their production in a long term period.

Description of achievements within each work package during the course of the project

General remark: The specific goals to be achieved by Pillar 5 were formulated in Annex 1 of the ISAFRUIT contract for each work package. Their fulfilment is described in this report in a summarised way. Extensive reports of each work package are published on the internet, as long as the ISAFRUIT webpage is maintained.

WP 5.1 - Safe European fruit from a healthy environment *(ECOFRUIT)* Leader: 11: RIPF (Grzegorz Doruchowski) ; other Partners : 2. ACW; 3. DFVF; 8. WUR-PPO; 6. UNIBO-DCA; 9. WUR-PRI; 10. WUR-LEI; 14. FiBL; 26. CSIC; 28. GRAB; 31. LAIMB; 32. DEIAFA; 44. ABAG; 45. Hauert; 50. IBI; 61. 3B6. (work period: 0-48 months).

Objectives

The overall objective of this work package is to improve the inherent fruit quality for making fruit more attractive and safe, i.e. meeting quality standards required by markets and consumers, particularly though a drastically reduced exposure of trees and fruits to pesticides and tree nutrients, being sustainably produced top quality fruit a prerequisite of better meeting consumer demand and therefore contributing to increasing fruit consumption. The specific objectives of this work package, all to contribute to an improved fruit quality, are:

- To develop a Crop Identification System (CIS) for adjusting the pesticide application equipment to the target characteristics as an integral component of a novel pesticide application system.
- To develop a Crop Health Sensor (CHS) for the on field identification of the tree health status as an integral component of a novel pesticide application system.

- To develop an Environmentally Dependent Application System (EDAS) for adjusting the pesticide application equipment according to the environmental circumstances an integral component of a novel pesticide application system.
- To coordinate the development of the CIS, CHS and EDAS, validate their effects and integrate them into novel system.
- To develop a model for the prediction of nutrient demand rates of orchards needed for top quality fruit.
- To develop site-specific, highly efficient fertilizer application methods to achieve top quality fruit and minimized nutrient losses.
- To optimize tree nutrition in organic fruit production to achieve top quality fruit while increasing the efficient use of nutrients and improving tree performance.

In order to meet these objectives the works have been carried out by two working groups:

- Crop Adapted Spray Application (CASA)
- Crop Adapted Fertilizer Application (CAFA)

Activities and results

The CASA-group

The specific objective of the activities within CASA working group was to develop a Crop Adapted Spray Application system (CASA) that would ensure precise, efficient and safe spray application in orchards according to actual needs and with respect to the environment. The CASA system consists of three sub-systems: Crop Health Sensor (CHS) - identifying the health status of fruit crops to apply chemicals only when necessary; Crop Identification System (CIS) - identifying the tree canopy size and density to apply spray precisely at relevant doses; Environmentally Dependent Application Systems (EDAS) - identifying environmental circumstances and navigating the sprayer to adjust application parameters accordingly so that spray drift is minimised and direct water contamination is avoided. The development of CASA system was realized within the 4 tasks.

Ad 1: Task 5.1.1- Development of a Crop Identification System (CIS) to adjust spray application to the target characteristics

In order to enable an orchard sprayer to automatically adjust nozzles flow rate and spray vertical pattern according to the presence and to the characteristics of canopy target (size and density), ultrasonic sensor and software were developed by 3B6 Company. The sensor can acquire information on (a) distance between vegetation and sensor, (b) index of vegetation density. For integration of CIS with the other CASA sub-systems it was configured to communicate with them according to ISOCANBUS protocol.

Several series of measurements were carried out in 2006 in the laboratory and in apple orchard (Golden Delicious, spacing 4.5 x 1.5 m, max tree height 4 m).

In October 2007 first field tests with the CIS sprayer prototype were carried out in orchard 2, to evaluate spray distribution on the leaves.

Based on data acquired during 2007 season several simulations were carried out with the Crop Identification System (CIS) software in order to define the thresholds values in terms of tree width and density which enable to modify the spraying parameters (number of active nozzles and operating pressure in each spray band).

Between May and September 2008 field tests were made in 3 apple orchards: orchard A - cv. Gala – tree spacing 3.8 x 1 m - average tree height 2.2 m; orchard B - cv. Red Chief – tree spacing 3.8 x 1 m - average tree height 4.0 m; orchard C - cv. Gala – tree spacing 3.8 x 1 m - average tree height 4.0 m

Starting from mid May 2008, the sprayer prototype equipped with the Crop Identification System (CIS) was employed for making spray deposition tests in orchards.

All the tests were made spraying a solution of water and yellow dye (Tartrazine E102), 5% v/v; samples of sprayed leaves were picked from 7 areas in the trees in order to assess the homogeneity of distribution in the plants. In the test made at BBCH 91 growth stage, also sprayed fruits were sampled. Results obtained in the first round of tests (BBCH 71 growth stage) pointed out that even if the volume application rate was nearly halved when the CIS sensors were activated, the average spray deposit on the leaves in the whole tree was pretty the same as that obtained applying the conventional volume of 850 /ha (CIS sensors off), while the uniformity of spray distribution within the tree resulted the same for the two treatments examined.

In the same Gala apple orchard, biological tests were made. Between 14 May and 2 August 2008, 10 spray applications were made using 10 different pesticides (sometimes mixed and applied in the same treatment) mainly facing apple scab, powdery mildew and aphids (see Table 1). Assessments of biological efficacy of pesticide applications made in the six experimental plots were made on 27 June 2008 and on 25 September 2008.

Reached objectives / conclusions

- Test results showed that the CIS sprayer prototype with the ultrasonic sensors switched on adapted the spray volume (and consequently the pesticide doses sprayed per hectare, as tests were carried out spraying mixtures at constant PPP concentration) to the vegetation development, and therefore reduced by about 33% the overall amount of pesticides applied during the season
- It enabled to obtain the same biological efficacy of treatments made applying the constant reference volume of 850 l/ha, either using the conventional air-assisted sprayer or the CIS prototype with sensors switched off.

Ad 2: Task 5.1.2 - Development of a Crop Health Sensor (CHS) for identification of tree health status determining pesticide application

To develop a Crop Health Sensor for the detection of apple scab in apples it was decided to use spectral analysis of apple leaf tissue as a starting point. First the available spectral analysis device to be used for health detection of apple leaves was updated in hardware and software. The data security and robustness were improved.

An inventory of literature on spectral analysis of apple leaves showed little information could be found.

In 2006 spectral analysis measurements were performed on individual apple leaves from different cultivars (Elstar, Jonagold, Rubens, Wellant, Autento).

In 2007 spectral analysis measurements were performed on individual apple leaves from two cultivars (M9 rootstock, Gala) (in cooperation with WUR-PPO) to evaluate the time of infection with apple scab and the spectral reflection in time from the apple leaves.

In 2008 the data analysis of the measurements performed in 2006 and 2007 is being continued. Assessment of leaves of the apple cultivars Elstar, Jonagold, Autento, Wellant and Rubens on the spectral reflectance showed that the apple varieties could be discriminated from each other based on spectral reflectance, expressed as Normalized Differential Vegetation Index (NDVI) for typical wavelengths. Every apple variety has typically its own typical NDVI value. Healthy parts of the leaves can be distinguished from diseased parts of the leaves on the mm^2 level. Results of the spectral reflectance measurements showed a difference in reflection between infected leaves and healthy leaves in the apple varieties Elstar and Jonagold.

From the 2007 leaf infection tests it was found that with spectral analysis apple scab can be detected as early as 2 days after infection. Standard evaluation of apple scab in practice only can detect scab 10-12 days after infection. Due to severe problems in the data acquisition part and afterwards in the data handling of the spectral analysis of scab infected apple leaves a severe delay was found in the development of the CHS. To secure the algorithm a new analysis on the data was performed making use of neural networks.

In 2010 the software for the CHS is developed and tested. From the data analysis with the neural network three discriminating wavelength were determined. Testing this wavelength on earlier gathered information from healthy and infected leaves showed more than 90% correct classification. A simple algorithm was developed for dose adjustment based on the CHS signal (virtually based on in software developed sensor). Unfortunately the hardware development of the sensor was running out of time, budget and capacities. This was a too complex part to be solved within the project given time limits at the moment of identified discriminating wavelength at the end of the project.

Reached objectives / conclusions

- The prove that a CHS can function for apple scab on apple leaves has been delivered. This development will open windows to new crop protection strategies for apple scab and other diseases and pests in practice.
- It was not possible, however, to build a CHS sensor-in-the-box to be integrated into the CASA sprayer and verify its operation in the orchard. There is the perspective that this can be possible in a flowing project.

Ad 3: Task 5.1.3 - Development of Environmentally Dependent Application System (EDAS) for adjusting the spraying equipment according to the environmental circumstances

Development of the airflow adjustment system.

In 2006 the adjustable air discharge concept for EDAS sub-system has been built on HARDI Arrow 1000 sprayer with a double radial fan (air output 18 000 m^3/h). The air collector with air vanes was constructed and mounted on the fan outlet. The new equipment was extensively tested.

In 2007 a diaphragm leaf shutter was designed and fixed on the inlet of radial fan to restrict air flow sucked by the fan and hence compensate for the undesired increase of air velocity on one section once the other section was closed. Preliminary measurements of air velocity for different positions of the leaf shutter and the air vane showed that desired control of air is possible with this arrangement. Both air vane and diaphragm leaf shutter were adjusted with electric actuators.

The set of equal mechanisms was produced for DEIAFA to be mounted on the CIS sprayer. The products were delivered to DEIAFA together with documentation in May 2007.

Air flow distribution measurements

In July-August 2007 extended laboratory test of the EDAS air discharge system was carried out. With a set of 8 hot film anemometers and 8-channel data logger a simultaneous measurements of air velocity at all the air spouts of one section were made for different settings of the air vane and leaf shutter. The air velocity was measured for all possible combinations of:

The measurements were made in 5 replications for each combination, separately for left and light section. Thus, 660 runs of anemometer setup were made. The values were extracted from 5280 measurements and air distribution was analysed.

The results of air velocity measurements showed that by the manipulation of the leaf shutter and air vane it was possible to adjust air velocity individually for each section.

The air spouts with nozzle holders were produced for RIPF by DEIAFA. The final setup of the spouts and nozzles as well as the control system for the nozzles was decided for CIS and EDAS.

Development of spray adjustment system

Assembling of nozzle control system started in September 2007. The system consists of 16 pneumatic valves that control nozzle batteries of each individual air spout. These valves are in turn controlled by electric valves. With the system it is possible to alter the nozzles depending on the wind situation and sprayer location. By the end of 2007 the final setup of sprayer was completed.

Development of EDAS software

The company AGROCOM POLSKA (SME, ISAFRUIT partner 60) developed a controller and software to control the operation of GPS navigated sprayer and automatically adjust sprayer working parameters (spray quality and air-jet volume) according to the environmental circumstances (sprayer location identified by GPS and wind situation measured with an ultrasonic anemometer) according to the concept of EDAS. The software allows creation of orchard map with sensitive areas and defining zones. The entered data are used to display the map on the screen of spray computer. The software was tested in orchard. Satisfactory performance of sprayer controlled by software was observed.

Field trials

In 2008 and 2009 the drift and deposition trials were carried out for different low drift application strategies, being combinations of airflow settings and nozzle settings. The objectives of these trials were:

- to evaluate the drift reducing potential of different spray application scenarios,
- to find out if the coarse spray and reduced airflow affect spray deposit.

DRIFT

Drift experiments were performed in a pear orchard (Conference cv. – hedgerow -10-year old; 5 rows x 160 m). Three spray quality scenarios (fine and coarse spray nozzles) combined with three air flow adjustment scenarios (9 treatments) applied with EDAS sprayer were compared with a standard application made by reference sprayer Munckhof – (M) (Fig. 29).

After the experimental plot (5 rows of trees) was sprayed on the drift collectors, collectors were taken and analyzed for spray ground deposit. The weighted mean deposit for different downwind distances were calculated as % of dose applied. Measurements were repeated 8 times during the full foliage stage (BBCH 71-79).

The results of the experiments showed that ccoarse spray and air flow reduction on the edge rows of orchard considerably reduce drift compared to the standard method with fine spray and two-sided airflow applications; the air flow is a much stronger factor accounting for over 60% of the total treatment variation while spray quality accounted only for 8.8% of total variation. There was no significant interaction between the airflow and spray quality; combination of coarse spray and one-sided air flow application on the two outer tree rows of orchard reduced ground deposit at the downwind distance 5-25 m (drift to adjacent sensitive objects) to nearly 1% of applied dose which is a safe level as far as surface water contamination is considered; and all the treatments with coarse spray on two outer tree rows gave over 80% reduction of drift outside orchard (downwind distance 3,5 – 25 m).

DEPOSITION

Experiments were performed in an apple orchard (Apples- Honeygold cv. – hedgerow -10-year old; plot: 5 rows x 25 m). Treatments were the same as in the drift experiments.

All the treatments were repeated 5 times during the fruit development period (BBCH 73-79). The deposition of BSF tracer on leaves was evaluated on seven locations within each of the three trees per row (replicates), in the three rows (row 1, 2 and 3) at the edge of orchard.

The results of the experiments showed that in average all the treatments with EDAS sprayer produced similar or higher deposition then that obtained with a reference standard sprayer; the treatments with coarse spray applied on the 1st row and on the 1st and 2nd row gave in general quite uniform deposition on all three rows; and within the treatments with fine spray switching off the air flow on the inner side of the 1st row and additionally reduction of the air flow on the inner side of the 2nd row gave significantly less deposit on the 1st row compared to row 2 and 3. This was not, however, significantly different from deposits obtained with a standard sprayer.

<u>Reached objectives / conclusions</u>

- Use of coarse spray nozzles and reduced air flow on the edge rows of orchard can reduce drift by more than 80% compared to standard application technique
- Applying spray with EDAS sprayer according to drift reducing strategies (coarse spray and reduced air flow on boundary rows) did not significantly reduce deposition on the boundary rows which shows the potential of practical use of these strategies.

Ad 4: Task 5.1.4 - Evaluation of the effects of the crop adapted spray applications techniques

For 2006-2009, several elements of the system to be established were tested in close collaboration with Tasks 5.1.1-3 for being prepared for the finals tests to be performed in the last year of the project.

In 2010 the performance of the CASA-system was tested in the orchard for specific conditions in the Netherlands (or Northern Europe) – spindle trees. The system was tested for deposition, biological efficacy, drift and residue trials.

<u>Drift trials</u>

The CASA-sprayer was adapted to operate in a Dutch orchard system, and drift trials were performed in an apple orchard with the variety Elstar. In order to show the relevance of the

CIS system; switching on/off nozzles depending on canopy in front of nozzles and one/more nozzles on canopy volume and the EDAS system; switching between fine and coarse nozzles depending on position and adapting air assistance settings depending on wind speed and direction it was necessary to adapt the measurement protocol to these features.

As the CASA system was expected to reduce the variation in spray drift because of tree adaptation we wanted to show this effect by measuring spray drift apart from the standard setup (ISO22369) also alongside the downwind orchard field edges. As nozzle and air settings vary depending on the position/path in the orchard a larger area of orchard had to be sprayed only the outside 20 m of the orchard as according to the ISO22369 standard for spray drift field measurements. Therefore an orchard area (100 m length and 14 rows (40 m) width; location Randwijk, the Netherlands) with its variation in settings of upwind and downwind area (14 m) and a standard middle area was sprayed in fixed settings and a fully operational CASA setting to show stepwise contributions in drift reduction of the CIS, EDAS-air and fully CASA settings compared to a standard cross-flow fan orchard sprayer (Munckhof sprayer). Measurements were performed in the full leaf stage of the orchard. For these spray drift measurements the following sprayer settings were identified in order to show the relevance of the partial systems (CIS, EDAS).

The specific settings of the sprayers used are reported in ISAFRUIT publications and in respective documents on the ISAFRUIT webpage.

Due to several constraints, the CIS had to be excluded from the field test, while the EDAS system was tested.

Conclusions

- The CASA sprayer with the EDAS system in use reduces spray drift.
- The CASA sprayer equipped with Albuz TVI 800075 nozzles and the EDAS system in use reduces spray drift at surface water distance in the Netherlands with 95%..

Drift trials with the CASA-system






Spray deposits on leaves and fruits, ground losses

During 2010, spray deposit trials were performed during three periods of the growing season (25^{th} May, 22^{nd} June, 27^{th} July): Assessment of spray deposits. Sampling procedures were as much as possible the same as in Italy in 2008, i.e. after ISO 22522.

Conclusions

- Spray deposition in the top of the tree is lower for the CASA sprayer using the CIS system independent of nozzle type.
- The CASA sprayer equipped with Albuz ATR Lilac nozzles gives in the middle and bottom section of the tree a lower deposition than of the Munckhof sprayer equipped with the same nozzle type.
- The CASA sprayer equipped with Albuz TVI 800075 venturi nozzles gives in the middle and bottom section of the tree a higher deposition than of the Munckhof sprayer equipped with the same nozzle type.
- The CASA sprayer equipped with Albuz ATR Lilac nozzles and used in combination with the CIS system gives a lower spray deposition on apples in all sections of the tree compared to a Munckhof sprayer equipped with the same nozzle type.
- The CASA sprayer equipped with Albuz TVI 800075 venturi nozzles and used in combination with the CIS system gives a higher spray deposition on apples in all sections of the tree compared to a Munckhof sprayer equipped with the same nozzle type.
- Biological efficacy: In 2010 the disease pressure during the ascospore season was very low, due to the very dry conditions. Only one infection period was observed. This resulted in very low disease incidences in the untreated control plots (5% infected leaves). In the treated plots disease incidences varied from 0.0%-0.3%, with no differences between the treatments.

Pesticide residues on fruits

In a field trial in 2007 the effect of droplet size (nozzle type) on residues in apples was evaluated in a commercial orchard. The orchard was divided in two experimental plots. Each plot was sprayed for the whole season with one (the same) nozzle type, according to a standard commercial spraying scheme with insecticides and fungicides, following label directions. The canopy effect was evaluated by dividing the apple tree (spindle type) into four distinct zones: top, middle, lower outside and lower inside. Four apples were collected from five trees per nozzle type and tree zone.

In general, the mean residue levels (average of 80 fruits) for the coarse and fine droplet applications did not differ significantly. However, large variations in residue levels were observed between the individual apples, either sprayed with coarse or fine spray quality nozzle types. These large variations were also present between fruits within the different zones. Apples from the middle or lower outside position showed the highest residue levels. The results indicated that the residue levels appeared to be independent of fruit size or weight.

Conclusions

- No differences exist in average residue levels between fine and coarse spray quality applications.
- Large variations exist in residue levels between individual fruits, independent of the spray quality of the spray nozzles.
- Difference in maximal concentration compared to mean concentrations are between order magnitudes of 2-5 times.

In 2010 residue trials were performed with the CASA-system (CIS and EDAS) in order to measure the effect of adapted spraying volumes and nozzle type (fine versus coarse droplet application). The field trials were carried out at the Applied Plant Research, Randwijk, The Netherlands.

The trial orchard was divided in two experimental plots. Each plot consisted of 3 apple tree rows of the apple variety Elstar of 100m length (with a tree height of 2.25 m, 3 m row distance, and 0.80 m tree distance). Plots were sprayed four times in the summer season with one sprayer and nozzle type, according to a standard commercial spraying scheme with insecticides and fungicides, following label instructions. Dose rates were therefore kept equal for all sprayer type tested.

The canopy effect was evaluated by dividing the apple tree (spindle type) into four distinct positions: top (T), middle (M), bottom outside (BO) and bottom inside (BI). Three apples per position were collected from four trees per sprayer-nozzle combination. Selection of the trees and apples per position was randomized out of a tree row (variety Elstar) in the middle of the experimental plot. A few days before the start of the (commercial) harvest, single fruit samples were taken (16 August). The total weight of each individual apple was determined before the residue was analysed.

Total residues per fruit and residue concentrations ($mg.kg^{-1}$) at harvest were determined according to standard analyzing procedures and methods (LC-MS-MS) in the laboratory (National Food Institute, Denmark; Mette Poulsen), and expressed for individual fruits and mean values per sprayer, nozzle type and tree position.

All data were subjected to analysis of regression using GenStat Release 9.2 statistical package (Lawes Agricultural Trust, Rothamsted Research, UK). The amounts of residue on the fruits appeared to have a lognormal distribution. Therefore data were analysed using a GLM (Generalized Linear Model) based on a lognormal distribution using a log transformation. Significant F-tests ($P<0.05$) were followed by a Least Significant Difference (LSD)-test for pair wise comparison of treatment means using LSD0.05 values. Treatment means were estimated on the transformed scale and later back transformed to the original scale.

Results

- No statistical significant correlation between the fruit weight (size) and residue level was observed, showing that the residue level were independent of the fruit size.
- None of the results for the pesticides residues measured in the apples exceeded the MRLs (European Commission, 2010). However, there was a big variation in the residue levels between the individual apples for all analyzed pesticides.
- There was a trend of less variation in the residues of the individual apples for the CASA sprayer equipped with standard ATR hollow cone nozzles (C-ATR), especially for the insecticide applications imidacloprid and fenoxycarb.
- Only for the insecticide Imidacloprid no statistical differences were found regarding the mean residue values in the apples for the four treatments. For the other pesticides, lowest residue levels were found in the apples sprayed with the CASA-CIS system, equipped with standard ATR hollow cone nozzles (C-ATR) being equal or lower than the reference (M-ATR). The C-TVI system resulted in comparable or higher residue levels than the C-ATR however still equal or lower than of the reference (M-ATR). For the standard sprayer (M) there were no clear differences between the nozzle types; sometimes a higher deposition was found for ATR and sometimes for TVI.
- The effect of spray technique on residue per application date is identical. Although the levels of residue differ the ranking of the residue levels per technique remains the same.
- *Sprayer-Nozzle-Tree Position effects:* The trees were divided into 4 positions: bottom inside (BI), bottom outside (BO), middle (M) and top (P). The residue levels for the different positions in the tree for the different application systems were analyzed. When the positions where the apples were picked are compared, the following was observed. For the BI-position lowest residues were found in apples sprayed with the C-ATR system (CASA). In contrast highest residue levels were found for the C-TVI system (CASA). There were no differences between the M-ATR and M-TVI systems (conventional sprayer). The same trends were found for the BO-position, although there was a tendency of higher residue levels for the M-ATR and M-TVI systems (conventional sprayer). For the M-position there was also a tendency for higher residue levels for conventional sprayers, for the T-position this tendency was even more pronounced.

Conclusions from residue trials

- In that respect the CASA-system resulted in lower residue levels in apples compared to the standard spraying machine. The higher residue levels in the lower part of the tree (BI and BO) could be explained by the fact that a higher spray volume was applied in the bottom part of the tree due to the tree canopy characteristics (spindle form).

- It should be realized that residue levels measured a certain period after the spray application do not reflect the initial depositions. The initial depositions will be reduced in the growing season due to sun light, wash off by rain and other factors. So, initial levels in the top of the tree could be high, but at harvest residue levels in the inner part could be higher because of less photolysis and wash-off.

Reached objectives / overall conclusions

From trials in Italy before 2010 it was concluded that regarding the Crop Identification System:

- The Crop Identification System enabled to reduce consistently (up to 82%) the volume application rate with respect to the reference one, especially when operating at early orchards growth stages.
- Spray deposits on the target and evenness of spray distribution on vegetation (leaves and fruits) using CIS generally resulted higher with respect to those obtained employing the conventional axial fan sprayer.
- Spray deposits on the target using CIS and air induction nozzles resulted consistently lower in comparison with those obtained operating CIS and conventional nozzles, but the values were similar to those obtained using the conventional axial fan sprayer.

The trials performed in 2010 in the Netherlands showed that:

- The CIS-ATR (CASA) results in less pesticide variation between individual fruits.
- The total reduction in pesticide use depends on standard spraying volumes applied.
- The CASA-CIS system could improve the pesticide distribution in the tree, and therefore on fruits.
- CASA-system resulted in some respects in lower residue levels in apples compared to the standard spraying machine. At some instances higher residue levels in the lower part of the tree (BI and BO) could be explained by higher spray volumes applied in the bottom part of the tree due to the tree canopy characteristics (spindle form).
- Residue levels measured a certain period after the spray application does not reflect the initial depositions. The initial depositions will be reduced in the growing season due to sun light, wash off by rain and other factors. Initial levels in the top of the tree could be high, but at harvest residue levels in the inner part could be higher because of less photolysis and wash-off.

For use of the CASA-CIS system adjustments and calibration to specific tree shapes and orchards is highly important.

Demonstration activities of tasks 5.1.1-4

DemoTour

In order to present the advantages of the crop adapted application technique to advisers, press, extension services, sprayers manufacturers and fruit growers the performance of CASA sprayer was demonstrated at 8 locations in Europe: Denmark, Italy, the Netherlands, Germany, France, Poland and Spain. The dates, locations and number of spectators of demonstrations are given in the table.

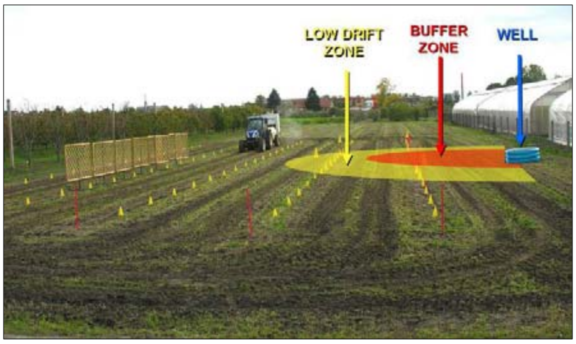
Table: The dates, locations and number of spectators of DemoTour demonstrations in 2009

Date	*Location*	*Number of participants*
9 June	Aarslev, Denmark	800
9 July	San Michelle, Italy	150
13 August	Randwijk, the Netherlands	200
26 August	Jork, Germany	30
10 September	Lanxade, France	25
1 October	Randwijk, the Netherlands	100
14 October	Skierniewice, Poland	110
23 October	Casell del Remei, Spain	300

The demo materials in 8 language versions (GB, IT, DE, ES, PL, FR, NL, DK) were elaborated to facilitate the provision of information about ISAFRUIT project and the concept of CASA system during the demonstration of the sprayer.

The scenario of demonstrations was elaborated which would ensure the best and the most attractive form of sprayer presentation. The demonstration site was arranged to simulate spray application in orchard with the sprayer controlled with CIS and EDAS systems.

Demo-site layout



The CAFA –group

The Crop Adapted Fertilizer Application (CAFA)- group addressed 3 aspects of tree nutrition which were considered to be critical for the advancement of modern sustainable fruit-growing: tree demand based nutrient demand for an adequte nutrient appliucation to prevent from nutrient loss, nutrient application technology preventing from nutrient loss, and improving tree nutrition in organic orchards.

Ad 5: Task 5.1.5 - Development of a model for the prediction of nutrient demand rates

The aim of this task was to develop a model predicting nutrient uptake by apple trees during the vegetative season. This is the first step toward the developing of a low environmental impact fertilization technique to limit nutrient losses from the soil. The basic idea of the model is that the amount of nutrient absorbed by trees is a function of the tree annual growth and the nutrient concentration in tree organs.

For the preparation of our model we chose to focus our attention on the cultivar Gala and Fuji. The relationships integrated into the model have been obtained by carrying out tree excavations and collecting data of trunk diameter, pruning wood and abscised leaf weights for several vegetative seasons.

During the first year of the ISAFRUIT project we collected the literature available on trees nutrient demand rate, apple uptake and partitioning. These data were integrated with those derived from previous projects of our group for the cultivar Gala to develop the first draft of a general model of nutrient uptake. The first version of the “Model of nutrient demand rates” has been delivered (D 5.1.8) during the first months of the 2007. During the first year of ISAFRUIT we started to collect data on the cultivar Fuji and continued to measure trees of the cultivar Gala. Excavations of whole trees were performed in winter 2006/07 both in the Experimental farm of the Bologna University and in a commercial farm (D5.1.10, D5.1.11 and D5.1.22).

Trunk and branch diameters were measured and the dry weight of single organs determined (orchards both in Emilia Romagna and South Tyrol). The allometric relationships between trunk and branch diameter and their weight, already based on historical data, were corroborated by new excavations of five Gala trees of different size. At the end of winter 2005/06, 10 trees of cv. Gala and Fuji were selected at the University experimental farm. Tree data were assessed as well as fruit biomass per individual tree. Soil N has been estimated with soil sampling or soil solution collection (by lysimeters). The effect of nitrogen fertilization rates on nitrogen leaching (**D5.1.12**) has been studied in experimental and commercial apple orchards. Data indicated that not all the supplied nitrogen had been absorbed by the trees and that it is necessary not only to determine the amount of the nutrient required by the trees, but also soil availability and the timing of nutrient absorption. Results revealed differences among different locations, probably due to differences in soil type and climatic conditions that should be taken into account when planning orchard fertilization. (**D5.1.23**, Fertilization strategies for reducing leaching assessed)

During the second year of ISAFRUIT we mainly focused on the Fuji cultivar, for which data were scarce, while we continued to collect data on the Gala cultivar as well (excavation of Fuji trees carried in February 2007). The analysis of tree organs collected during the extirpations and by samplings provided us a database of nutrient concentration in tree organs. During the second part of the year, we began to collect biometric information on Fuji and Gala in new orchards in Bologna, Ferrara and Bolzano provinces (fruit biomass, abscised leaves and pruning wood). In December 2007 five Fuji trees have been excavated and branches, trunk, rootstock and coarse roots collected. During the first months of 2008 the samples were prepared for analysis, analysed and data added to the preexisting database. Further data were collected in 2008 (pruning wood at the end of the vegetative season, petals, abscissed fruitlets, ripe fruits and abscissed leaves, length and diameter of twigs and branches, trunk and rootstock diameters).

The collected data allowed updating the electronic spreadsheet presented as **D5.1.8** (Model of nutrient demand rates). Since the beginning of the ISAFRUIT project 376 samples have been collected of which 14 from whole tree excavation (and 2 excavation of only above ground biomass) and a total of 690 trunk and rootstock circumference data have been obtained. A total of 208 analysis of macronutrient concentration have been performed and at least other 23 will be performed before the end of the year.

During the 4th year of ISAFRUIT the detailed analysis of the data collected in the last years by our group revealed that there is a sufficiently robust base on which to build a nutrient demand rate model for apple trees. The model, released with **D5.1.8**, has been upgraded and verified.

For the whole duration of the project there was an intense and profitable interaction between CSIC and UNIBO. Two young scientists from CSIC visited the group of UNIBO to practice with the techniques used for the project and to discuss the progression of the research, as demonstrated by the **D5.1.9** (Protocol of samples collection and analysis delivered).

Reached objectives / conclusions

- The model is now working for cultivars Fuji and Gala only, but algorithms might be changed and adapted to other cultivars as well. The model is based on the prediction of yearly growth and of allocation of newly formed biomass within the tree. The amount of nutrients yearly absorbed by the tree is derived from their amount contained in the annual growth as calculated by the biomass increases and nutrient concentration of single organs.
- Data of biomass increase derive from relationships obtained from our laboratory, while nutrient concentration derives from a dataset including our own data.
- At present, the model is running through spreadsheet software available on the ISAFRUIT website (www.isafruit.org).
- A parallel work has been developed in peach orchards by partner CSIC (partner 26) on a nutrient demand rate model.

Ad 6: Task 5.1.6 - Development of site-specific fertilizer application methods for minimized nutrient loss

During the project we have developed novel advanced methodologies (using HPLC coupled to time-of-flight mass spectrometry –HPLC-TOFMS-) that allow for the determination of Fe-chelates and chelating agents.

First, we developed, validated and published a method for the simultaneous determination of most commercial synthetic Fe-chelates used in agriculture in irrigation water, fertilizer solutions, nutrient solutions and plant fluids such as xylem sap. The method is very sensitive and well suited to satisfy the needs of researchers, growers and the industry.

A specific and sensitive HPLC-TOFMS method was also designed, validated and published to determine the amounts of the commonly used chelate Fe(III)-*o,o*EDDHA in plant tissues, where deleterious analytical matrix effects are very strong, using a structural analog as internal standard. Furthermore, several mass spectrometry techniques were used to obtain typical MS-MS fingerprints of the most common polyaminocarboxylate Fe-chelates (see Orera *et al.*, 2010). This information will constitute a useful tool for monitoring known active agents in plants and in the environment. This methodology has also permitted to identify unknown impurities in commercial chelates. Both a scientific overview and a technical note for growers on Fe-chelate analysis have also been published (see Orera *et al.*, 2009a, Orera *et al.*, 2009c).

The development of the new HPLC-TOFMS based methodology to analyze Fe-citrate complexes has allowed observing, for the first time, a citrate-Fe complex ($Fe_3Citrate_3$) in the xylem sap of two model plants. This is a major breakthrough in the field, since the existence of these complexes was hypothesized many years ago, but a direct proof of their occurrence was not found until now.

We have used a dual-stable Fe isotope tracer method to study the uptake, xylem transport and distribution of Fe and chelating agents from two different Fe(III)-*o,o*EDDHA stereoisomers in Fe-deficient plants (Orera *et al.*, submitted). This is the first time such a method has been used in plants. This work includes the use of HPLC-TOFMS for chelating agent determination, as well as ICP-MS for stable Fe isotope analysis.

Regarding foliar fertilization, several Fe compounds were tested in peach in combination with one surfactant and different adjuvants, and significant differences in formulation effectiveness were found. Several Fe compounds were tested in peach with different surfactants, and major differences were found again between formulations. A scientific review was written to discuss the state-of-the-art of foliar Fe fertilization (see Fernández *et al.*, 2009) and a dissemination report for growers was also published on the same issue (see Toselli *et al.*, 2009). This information will be used in the future to find more efficient formulations.

Furthermore, a study was to unravel the basic characteristics of Fe-deficient leaves, since these would undoubtedly affect Fe fertilization efficiency. We have investigated the photosynthetic characteristics of Fe-deficient pear and peach trees (see Larbi *et al.*, 2006), obtained information on the morphological –by scanning electron microscopy- and chemical changes –including wax contents- in pear and peach leaf surfaces caused by Fe deficiency (see Fernández *et al.*, 2008b) and studied the organic acid composition changes in leaves (see López-Millán *et al.*, 2009), xylem sap and apoplastic fluid (see Larbi *et al.*, 2010) of model plants, as an intermediate step to carry studies in fruit trees.

Relating the advances in understanding Fe deficiencies and Fe fertilization to fruit quality

We have characterized the fruit quality and yield changes in pear (*Pyrus communis* L.) and peach (*Prunus persica* L. Batsch) trees as affected by iron deficiency chlorosis (Álvarez-Fernández et al., submitted). We have also designed and validated a new HPLC-TOFMS method for the determination of carboxylates (organic acids) in plant tissues, including fruits (López-Gomollón et al., submitted)

Deliverables **D5.1.9** (Protocol for sample collection and analysis of nutrient losses), **D5.1.12** (Data on leached N and Fe assessed) and **D5.1.23** (Fertilization strategies for reducing N and Fe leaching assessed) have been released, and **D 5.1.44** (Results of fruit quality evaluation as affected by Fe application) were released by project month 57 (Sept 2010).

Reached objectives / conclusions

- The research undertaken has allowed a step forward towards understanding nutrient demand rate models in trees, Fe deficiency in plants, Fe fertilizers and Fe fertilization.
- Significant steps for dissemination of the new knowledge to a scientific and technical audience have been undertaken (see ISAFRUIT publication records: 16 scientific papers so far, with several more submitted or in preparation). We expect further such activities to follow in the next years.
- The development of the new HPLC-TOFMS based methodology to analyze Fe-citrate complexes is a major breakthrough in the field, since the existence of these complexes was hypothesized without direct proof many years ago.
- Regarding foliar fertilization, significant differences were found in Fe formulation effectiveness. The state-of-the-art of foliar Fe fertilization was discussed and published which will be instrumental in the future to find more efficient formulations, improve sustainable fertilization techniques and improve fruit quality.
- The results of this research can have a great importance for European agriculture. Excessive or insufficient fertilizer addition in orchards affects growth, fruit yield and quality or represents a source of environmental pollution and an economical loss for the farmer. Our findings contribute to fertilization practices that are based on the understanding and forecasts of nutrient uptake and tools to predict tree nutritional needs or economical issues should be available to farmers, technicians and agriculturists.

Ad 7: Task 5.1.7 - Optimizing tree nutrition in organic fruit production to achieve top quality fruit while increasing the efficient use of nutrients and improving tree performance

The goal of this Task was to optimize tree nutrition in organic fruit production to achieve top quality fruit while increasing the efficient use of nutrients and improving tree performance (work period: months 0-24).

Study of solitary and interactive effects of novel fertilization agents and nutrition strategies on tree performance (yield and quality, the latter referenced to WP 1.2) in organic full-factorial field and pot experiments, testing a) composts, b) new solid and liquid organic N-fertilizers applied on soil or c) on leaves and d) commercial bacteria products. Valuing the effect of improved quality and yield on production cost, market price and demand (link to WP 1.2 and WP 5.3).

Selected results and conclusions are presented in this report. More detailed results can be obtained from Deliverable 5.1.24 (Final reports on experimental and dissemination activities accomplished).

Four experiments were conducted by this Task:

- A long-term full factorial experiment in a commercial organic orchard in Switzerland (KOB trial) with use of compost, commercial N-fertilizer and foliar N-fertilizer in 11 different combinations.
- In Italy an assessment of the nitrogen-mineralisation pattern of a large number of different commercial, organic-approved N-fertilizers with laboratory incubation studies and trials with potted plants;
- A field trial with different novel, organically-approved fertilizers in a apple tree nursery in France.
- A longer-term trial in a commercial organic apple orchard in Switzerland to test a novel biological product with several bacteria strains that should increase the soil born nitrogen mineralisation

The studies were lead by partner FiBL (no. 14) within the two long term trials: (a) KOB-trial (installed in 2001) on the nutrition effect of compost amendments, organic commercial N-fertilizer and foliar N-fertilizer on scab resistant apple trees, cv. Topaz; (b) BactoFil-trial (installed in 2003) on the effect of Bactofil2 Professional on apple tree nutrition. BactoFil is an innovative commercial product including different Azotobacter bacteria strains to fixate air borne N and make it available for plant uptake - applied twice a year to the soil.

The laboratory trials were set up by partner LAIMB (no. 31) to evaluate N-mineralization of organic fertilizers under standardized conditions. Three evaluation cycles were done from February till May 2006. The effect of microbiological products (BactoFil, EM, Euromix etc.) added to the organic fertilizers on their mineralization rate was studied.

The comparison trial in the apple nursery was set up by partner GRAB (no. 28). The innovative commercial organic products, such as Rhizopus and BactoFil (applied by hand) and Biorga (applied with irrigation system), were compared with a standard organic fertilizer. The effect of the fertilizers was evaluated based on the measurements on tree growth, stem diameter, and soil N contents.

All 4 main experimental and dissemination activities of the Task will be continued as planned without conceptual or methodological changes. The participants were willing to do this on their own costs.

KOB trial with use of compost, commercial N-fertilizer and foliar N-fertilizer in 11 different combinations

The trial has been installed in March 2001 in a commercial, certified organic orchard planted in 1999 on a site without fruit production before with the scab resistant cultivar Topaz on Rootstock M27 (planting distance: 1 m in row, 3 m between rows) at Remigen, Switzerland. The KOB trial is a full factorial, fully randomized tree nutrition trial where the factors i) compost (from green wastes), ii) commercial organic N-fertilizer and iii) foliar organic N-fertilizer are tested in all possible combinations (8 treatments).

Additionally, there are 3 sub-treatments included: a „control"-plot without N-fertilizer, however with the application in mineral form of K, P, Ca, Mg equivalent to the compost variant. The two other sub-treatments address the question of effects by bio-dynamic (bd) methods: in both bd-treatments the compost used is made from the same basic material as in the main treatments, however, it has been prepared by the specific bd-method (5 bd preparations are added when the compost is 4 weeks old; from then on the compost is not turned around anymore); in the bd-treatment "Kbd—Prep." also the bio-dynamic spray-preparations "500" and "501" are sprayed on soil and leaves, respectively.

The experiment is divided in 3 field blocks each containing all 11 treatments randomly arranged (33 plots; each 18 m long with 18 trees from which 5 representative trees had been chosen).

Economical considerations

The costs of each fertilizer treatment were calculated. It became evident, that the different fertilization strategies are combined with high differences up to a factor 5.8 regarding the costs per hectare.

The total expenses are composed by the costs for the fertilizers, and machinery plus labour costs for their application. The cost range varies – apart from the untreated control with 0.- SFR/ha - from 174.- SFR with the only-compost treatments such as K--, Kbd--, Kbd-- + prep. up to 1016.- SFR/ha in the –OB treatment (here, the costs for the bio-dynamic preparation of the bd-compost are not considered).

Generally, treatments with commercial organic soil (O+) and/or foliar N-fertilizers (B+) cause high costs ranging from 556.- to 1016.- SFR/ha.

Principal results

- The long-term effects of the treatments on tree performance (growth, yield, specific yield), fruit quality, mineral concentration in leaves and fruit, and finally on soil fertility parameters were in most cases of minor importance.
- Possible reasons for non-effects of the fertilization factors applied
    - As apparent with the N_{min}-content measurements during the season, due to its low nitrate contents the compost apparently fixated some of the N_{min} that has been mineralised from the soil reserves or came from the applied N-fertilizer.
    - The obvious soil improvements by the compost in the first 5 cm became "diluted" in the quantitative assessments we carried out in the 0-25 and the 12-5-25 cm layer, respectively.
- The reasons for a non-effect of the commercial organic fertilizer may be:
    - Relatively good basic soil fertility, while additional fertilizer provoked little effect.

  - Careful management practices, while moderate quantities of additional fertilizer could not provoke striking effects.
  - Slow release of the commercial organic fertilizer without “flush” of plant available nitrate.
- Possible reasons for the non-effect of the foliar N-fertilizer were:
  - Bi-annual bearing of trees provoked by Vinasse in 2002 with reduced accumulated yield performance compared to the treatments without foliar N-sprays.
  - The foliar N-products seem neither capable to enhance the N-intake of these basically not N-deficient trees.

<u>Conclusions</u>

- Soil improvements before planting are more efficient, since compost only provoked a positive trend but no significant difference. It would have been more efficient to improve, before planting, the soil conditions and to deepen the rootable soil layer.
- Soil improvement by considerable compost quantities is necessary. Small amounts as they are allowed by e.g. by the Swiss legislation will hardly have the desired effect.
- Soil fertility should be more in the focus than fertilization, since data showed that soil fertility conditions could not be compensated by the fertilization inputs. N_{mic} seems to be an interesting parameter to observe.
- Financial considerations lead to compost and precision fertilization**.** When looking at the costs of the tree nutrition strategies studied, we would advise growers to cover the reconstitution supply of P, K, Ca, Mg by green waste compost. Using compost for this purpose costs only half the price than buying organic-approved mineral fertilizers and even incorporates some extra carbon into the soil.
- The findings of our study are of considerable practical and scientific relevance. Organic fruit growers can or must learn, that in an organic orchard system sub-optimal tree performance which is nutrient deficiency induced (mostly by nitrogen) cannot simply be compensated by and increased application of organically approved N-fertilizer.

<u>Nitrogen-mineralisation pattern of a large number of different commercial, organic-approved N-fertilizers with laboratory incubation studies and trials with potted plants</u>

This study describes an approach to test the mineralization of various commercial organic fertilizers with the aim to improve fertilizer efficiency and thus to reduce environmental impact, such as nitrogen leaching and economic loss.

Nitrogen mineralization is a microbial process and as such dependent on environmental conditions. The main factors affecting mineralization are temperature, soil humidity and chemical/physical soil conditions.

The incubation trials were carried out under standardized laboratory conditions at constant temperatures of 8 and 16°C.

<u>Principal results</u>

- Organic fertilizers have generally lower rates of nitrogen release than mineral fertilizers which moreover extend over a longer period of time.

- Microbial products, which according to the manufacturers should enhance the mineralization of organic matter, led to no significant increase of the rates of mineralization.
- The increments of nutrient elements other than nitrogen during 60 days of incubation were rather limited. The highest increments were generally recorded in the case of the microbial products.
- In all incubated samples at 8°C an increase of salinity was noticed, ranging from 13 mg/kg (Geovis) to 188 mg/kg (Ecoferro 250 Plus).

Reached objectives / conclusions

- Large number of different commercial, organic-approved N-fertilizers.
- This investigation about the nitrogen mineralization at 8 and 16°C gives indications about the optimal timing of application of each product, depending also on climatic conditions and nutrient demand. Results need to be confirmed under orchard conditions.

A field trial with different novel, organically-approved fertilizers in an apple tree nursery in France

This study was to test the mineralization of various commercial organic fertilizers in different types of soil at the relatively low temperature of 12°C with the aim to improve fertilizer efficiency and thus to reduce environmental impact, such as nitrogen leaching and economic loss.

General results

- Organic fertilizers have generally low rates of nitrogen release. In this trial, of all tested products, Nutristart and Biogas slurry showed the highest percentages of release. The lowest values could be observed in the variants treated with Agrobiosol. The best results of mineralization of the latter, but also of the other products were recorded in loam and silt soils. Sand, in contrast, showed the lowest values, both after 14 and after 60 days of incubation.
- The increments of nutrient elements other than nitrogen during 60 days of incubation were rather limited. The highest increments were generally recorded in the soils treated with Nutristart, Biogas slurry and slurry as well as in some cases with Lysofert and Bioilsa.

Conclusions

This investigation about the nitrogen mineralization in different soil types at 12°C temperature gives indications about the optimal conditions of application of each product, depending also on climatic conditions and nutrient demand.

Test a novel biological product with several bacteria strains that should increase the soil born nitrogen mineralisation

The product tested in this study, BactoFil ® Professional 2, is a commercial microbial biofertilizer for soil improvement and plant nutrition in liquid form (since 2008 also in powder form).

BactoFil Prof.2 contains strains of:

- *Azospirillum lipoferum*
- *Azotobacter vinelandii*
- *Bacillus megaterium*
- *Bacillus circulans*
- *Bacillus subtilis*
- *Pseudomonas fluorescens*
- *Micrococcus roseus*

As this trial started already in 2004 with 2 yearly applications of the product and the measurement described here started two years later, we can assume that the soil/tree root system had sufficient time to get inoculated by the microbial biofertilizer (BactoFil Professional-2) and could get into a balanced equilibrium.

Principal results

- The correlation between the total and species-wise abundance of the product bacteria with N_{min} contents in the field and with the in-vitro N-mineralisation was in no case significant.
- Some tendencies for positive correlations could be seen if ignoring the zero values. There, the closest correlation occurred with *Bacillus circulans*, the second most abundant species found here – also in 3 (of 8) of the non-treated plots.
- Our leaf and fruit mineral analyses – acting as sinks for the nutrients - show that the biofertilizer treatment did not create a exuberant N-supply situation for the trees, because mineral contents in young and ripe leaves or fruits didn't reveal treatment differences.
- In contrast to the fact, that no treatment effects on the "assessable" N-supply (N_{min} in soil, N in leaves and fruits) could be found, the biofertilizer treated trees performed significantly elevated yields. Another indication for a good or even better tree nutrition situation in the biofertilizer treated plots is the fact that fruit quality as sugar content and fruit flesh firmness, in spite of significant higher yields, did not decrease in the biofertilized plots.

Conclusions

BactoFil-Professional-2 microbial biofertilizer may be recommended since:

- It can inoculate and/or enrich the soil with the desired bacteria species that are favourable for N_2-fixation from air-borne nitrogen.
- It contributes to a yield increasing effect which is not combined with any fruit quality loss or exuberant N_{min} contents in the soil that could - trough leaching - negatively affect the ground water..

WP 5.2 Optimised resource use for top quality fruit *(SMARTFRUIT)* Leader: 6: UNIBO-DCA (Guglielmo Costa); other partners : 1. DIAS; 2. ACW; 4. IRTA; 5. INRA; 7. NCRI; 8. WUR-PPO; 9. WUR-PRI; 10. WUR-LEI; 14. FiBL; 33. UNIPD; 40. KIS. (Work period 0-48 months).

Objectives

The general objective of WP5.2 activities was to meet fruit quality standards demanded by markets and consumes with production technology based on an optimised, i.e. respectful and well-directed resource use such as water, light, nutrients and the plant genotype. The major objectives of the work package were:

- To better exploit natural plant resources via innovative and environment-friendly approaches for crop load regulation.
- To exploit natural resources such as water, light and naturally occurring nutrients more efficiently and/or more effectively.
- To define and implement decision-support tools for properly steering the production process along the above-mentioned lines.

Activities and results

SMARTFRUIT developed a wide range of activities and addressed many problems of the pre-harvest production chain. In order to facilitate a proper appreciation of the research performed, it is subsequently categorized not only according to its thematic focus (evidenced by different tasks as documented in Annex 1) but also to its focus with regard to the level of knowledge development and transfer.

Knowledge development and transfer level

New knowledge and methods have been developed by a network of the many research institutions and SMEs involved in various countries. This knowledge and these methods are now at the disposal for the interested parties, however at different levels of knowledge and technology transfer.

The knowledge and methods created refer to 3 levels:

1. ready to be transferred to extension services for final local adaptation and transfer into practice
2. ready to be taken up by private industry (spin-off)
3. new fundamental knowledge for further R & D to reach practical application (pre-competitive research)

Outputs of SMARTFRUIT were classified according to these categories (see table below) for making it easier to overlook and value the activities of this Work Package.

Focus of the activities

Activities centered on the first objective were addressing an improved exploitation of the plant genotype's quality potential under different European growing conditions by regulating crop load. The knowledge is at a quite practical level. However, local extension services in the respective fruit-growing zones need to make the transfer to the growers for creating maximum impact.

The activities centered on the second objective task aimed at developing innovative technologies for exploiting water, light and naturally occurring nutrients more effectively for fruit crop management purposes in integrated and organic production systems. In particular, this task focused on i) investigating the methodologies to adapt full irrigation requirements for peach orchards grown under hailing-nets or above reflective mulches, ii) investigating the effect of a bio-stimulant product, for improving the plant uptake of limited naturally occurring nutrients in deprived soils, iii) investigating the relationships between fruit quality development and the orchard environmental conditions in peach, and iv) a new method for the regulation of apple fruit load based on the use of shading.

The activities centred on the third objective aimed at developing models at the mathematical, molecular or physiological level for an improved understanding of growth and quality regulating processes. Mathematics, molecular biology and non-destructive biochemical measurement technology was used for studying fruit growth and quality. The task should provide conceptual support for activities of Tasks 1 and 2.

Since abscission, fruit size and ripeness of a fruit are factors of highest importance with regard to optimizing fruit quality, being addressed also by Tasks 1 and 2, the developed models were addressing these issues.

Table: Overview of activities developed by SAMRTFRUIT, including the resource use addressed and the knowledge development and transfer level.

Chapter	**Title**	**Addressed resource use**	**Knowledge transfer level ***
	Task 5.2.1 (genotype: crop load regulation)		
5.2.1-a	Validating molecules for fruit chemical thinning for apple and pear	Genotype	1
5.2.1-b	The development of methods to overcoming alternate bearing in apple and plum in northern climates		
5.2.1-b1	– Effects of different crop loads and thinning times on yield, fruit quality and return bloom in *Malus x domestica* Borkh. cv. 'Elstar' under Nordic conditions	Genotype	1
5.2.1-b2	– Managing alternate bearing of 'Opal' plum (*Prunus domestica* L.) trees with GA_3 applications by increasing fruit size, and normalizing return bloom and yield in a Nordic climate	Genotype	1
5.2.1-c	The development of zero residue methods	Genotype	1
5.2.1-d	Innovative pruning methods	Genotype	3
5.2.1-e	The allergenicity of apple and peach as influenced by crop load and pre-harvest factors	Genotype	3
	Task 5.2.2 (water-nutrients-light-genotype)		
5.2.2-a	Irrigation requirements for peach orchards grown under hailing-nets or above reflective mulches	Water, light	1, 2
5.2.2-b	Improving the plant uptake of the limited naturally occurring nutrients in deprived soils	Nutrients	1, 2
5.2.2-c	Investigating on the relationships between fruit quality development and the orchard environmental conditions in peach.	Light	1, 2
5.2.2-c1	– Effect of environmental variations on daily growth, vascular flows and carbohydrate metabolism of peach	Light	3
5.2.2-c2	– Effects of crop load on daily fruit growth and vascular flows	Genotype	3
5.2.2-d	Developing a new method for the control of apple fruit load based on the use of shading as a natural thinner		
5.2.2-d1	– Protocol adjustment	Light	1
5.2.2-d2	– Biochemical studies	Light	3
5.2.2-d3	– Biophysical studies	Light	1, 3
5.2.2-d4	– Practicability tests	Light	1, 2
	Task 5.2.3 (models)		
5.2.3-a	Mathematical model (Peach)		
5.2.3-a1	– A mathematical model of peach fruit growth, which can be used to assess the fruit growth potential	Genotype	3
5.2.3-a2	– An intelligent virtual fruit focusing on quality attributes (peach)	Genotype	3
5.2.3-a3	– Simulation of the effect of water availability on the virtual fruit	Water	3
5.2.3-a4	– Impact of a carbon requirement mutation on virtual peach fruit	Genotype	3
5.2.3-b	Prediction of the apple fruit weight two months before harvest	Genotype	2
5.2.3-c	Physiological and biochemical models (apple)		
5.2.3-c1	– Forecasting the June drop	Genotype	3
5.2.3-c2	– Forecasting fruit quality (ripening)	Genotype	3
5.2.3-d	*The apple abscission model derived from transcriptomic and metabolomic data*	Genotype	3

* The following types are distinguished:

1: ready to be transferred to extension services for final local adaptation and transfer into practice

2: ready to be taken up by private industry (spin-off)

3: new fundamental knowledge for further R & D to reach practical application (pre-competitive research).

Ad 1: Task 5.2.1 Non-alternating top quality fruit orchard cropping patterns

The experiments carried out in this Task were established to develop methods for overcoming alternate bearing and increasing the security of achieving top fruit quality by innovative techniques of manipulation of crop load, by integrated and organic production technique and by developing zero-residue thinning methods.

<u>The main achievement by the different partners concerned:</u>

- Validating molecules for fruit chemical thinning
- The development of methods to overcoming alternate bearing in apple and plum in a northern climate.
- The development of zero residue methods friendly chemicals, Mechanical thinning
- Innovative pruning methods

<u>5.2.1-a Validating molecules for fruit chemical thinning for apple and pear</u>

Fruit thinning represents the most effective tool in controlling alternate bearing and enhancing fruit quality (size as well appearance and internal parameters). While in apple the results obtained are quite interesting and the chemicals available for thinning are still numerous (Ethephon, ATS, Line sulphur, NAA, BA, etc), in stone fruit the situation is more complicated and difficult. In fact, Ethephon is practically the only chemical thinner available, although in the recent years some other compounds have been tested, as some oil (soybean oil, pinolene, etc) but the effectiveness of the operation is not as good to convince the growers to use commonly such a practice.

Abscisic acid, a well known hormone, has been prepared in a formulation whose cost seems affordable for field conditions and some interesting evidence has been obtained,

<u>Apple</u>

The cell division enhancing agent BA was the most studied among the partners (this work has been reassigned from Task 5.2.3 (Annex 1) to 5.2.1 as based on the better integration into the work of this Work package).

Trials focused on different varieties, environments and methods. With regard to varieties, Gala, Fuji, Golden Delicious, Elstar were tested, some of them grown in different environments (low and high altitude). Time of application and concentrations study was performed by different partners.

<u>Reached objectives / conclusions</u>

- The efficacy of BA was dependent upon time of application (the window is from 9 to 14 mm king fruit diameter) and temperature and humidity of the environment tested.
- While a temperature of at least 18°C is required, high humidity shows a strong effect on the response; in high altitude locations, treatments resulted effective even at a later application stage (15-16 mm king fruit diameter).
- With regard to fruit quality traits, the soluble solid content is the most influenced parameter, showing an increase in treated fruits in the geographical areas studied, while other parameters i.e. flesh firmness, and peel red color, are differently altered depending on altitude.

- Trials showed that a direct increase on final fruit yield related with crop load was observed in all the experiments. This increase in yield was similar on the majority of 9 experiments, because from 2 to 15 fruits/cm^2 an increase on final yield of around 4 times was observed.
- This increase on crop load was directly related with a decrease on fruit weight, although correlation coefficients were lower than fruit yield. Globally it seems that cultivars characterized by a high fruit size (Golden and Fuji) show a low correlation in comparison with a medium fruit size (Gala).
- A slight crop load effect on fruit quality was observed, although the highest crop load involves a diminution on total solid soluble (TSS) and titrable acidity, which would imply a reduction of consumer acceptance.
- Another important result to be pointed out concerns the effect of crops load on return bloom particularly in some cultivars like Fuji. This cultivar usually shows an alternate bearing performance which is increased after a high crop load.
- Another trial carried out in Slovenia quantified the efficacy of chemical thinners with regards to different crop load (Golden Delicious, Elstar) and showed the strong interaction between crop load, chemical thinner and fruit drop und return bloom.

Peach

Also in peaches the project carried out work aimed to find solutions suitable for fruit thinning to increase fruit quality and avoid alternate bearing, in view of the relatively few interesting results obtained due to lack of appropriate, well-performing chemical thinners.

ABA might represent an innovative tool: an interesting, although preliminary, thinning effect was found, in addition to its capacity to interfere with water assumption.

On Red Gold nectarine, trials were carried out in field conditions and also on excised shoots maintained in controlled conditions in greenhouse for all the length of the experiment. Plum varieties Precoce d'Ersingher and Shiro were also studied under field conditions.

Reached objectives / conclusions

- Precoce d'Ersinger pointed out the capability of ABA to induce fruit abscission.
- Controlled environmental conditions in greenhouse on Red Gold bearing excised shoots, very interesting preliminary results were obtained. Interestingly the water used by the control shoots was 3 orders of magnitude higher than that taken from the ABA treated shoot.
- The experiment evidenced variation in the fruit drop response possibly related with the physiological performance of the tissues reflected in the water use metabolism. The principles behind are not yet understood.

5.2.1-b The development of methods to overcoming alternate bearing in apple and plum in northern climates.

5.2.1-b1 Effects of different crop loads and thinning times on yield, fruit quality and return bloom in *Malus* x *domestica* Borkh. 'Elstar' under Nordic conditions

The objective of this study was to investigate the most appropriate crop load for 'Elstar' apple trees in order to achieve a reliable annual yield of high quality fruit grown under Nordic conditions. A 4 year field experiment was carried out in a commercial orchard near Bioforsk Vest Ullensvang, Norway, using the apple cultivar 'Elstar'/M9 rootstock in 2006 and 2007.

Reached objectives / conclusions

- Thinning at FB improved fruit size and fruit quality at the same crop load in ‘Elstar’ apple trees. Low crop loads gave larger, more coloured fruit with higher SSC.
- The amount of return bloom declined with increased crop load in the previous year and by thinning at 20 mm - fruitlet stage.
- Thinning at FB to a level of 2 - 4 apples cm^{-2} TCSA gave an annual crop of high quality.

5.2.1-b2 Managing alternate bearing of ’Opal’ plum (*Prunus domestica* L.) trees with GA_3 applications by increasing fruit size, and normalizing return bloom and yield in a Nordic climate

The effects of Gibberellic Acid (GA_3) applied during Stage I of fruit growth was studied on ’Opal’ plum trees (*Prunus domestica L.*), a cultivar especially prone to alternate bearing, when grown under temperature conditions of Nordic countries. In 2008, 9-year-old ’Opal’ plum trees, growing in a research orchard at Ullensvang, Norway were selected as ”off-year” trees if they were high yielding in 2007 and exhibited very little flowering in 2008. Some of these trees were sprayed to the point of run off with 0, 50 or 100 ppm Gibberelllic acid (GA_3), five weeks (June 16, 2008) or ten weeks (July 21, 2008) after full bloom (WAF). Some trees were sprayed only once on each of these dates, other trees were sprayed on both of these dates at the same application rate used on July 21, 2008 (double application).

In 2008, yield quantity and quality was recorded. In 2009, flower and fruit set were recorded.

50 ppm GA_3 applied once at 5 WAF in 2008 increased average fruit size (41 g) significantly at harvest in 2009 compared to untreated control fruit (35.7 g) and all other treatments.

One application of 50 ppm GA_3 5 weeks after full bloom (WAF) in 2008 had a significant positive effect on fruit size at harvest in both 2008 and 2009 when compared to controls and all other treatments. Furthermore, fruit from these same trees had significantly higher soluble solids in 2009 than all other treatments and untreated controls.

Reached objectives / conclusions

50 ppm GA_3 applied once, 5 weeks after full bloom has much potential for increasing fruit size, normalizing both return bloom and yields in alternate bearing ’Opal’ plums in mesic, temperate climates of Nordic countries.

5.2.1-c The development of zero residue methods

The thinning experiments were carried out in 2006-2008 at the Department of Horticulture, Faculty of Agricultural Sciences, Denmark. Elshof (Elstar clone)/M9 trees at a spacing of 1 x 3.5m from the same planting were used for the experiment, while different experimental trees were selected each year dependent on a high flower density. Chemical treatments were applied at 1000 l/ha. In 2007, Kaolin (lime sulphur) and a similar clay compound containing 70% silica algae called Absomol were applied in a non-aqueous form by the use of a fan blower. All treatments were intended to be applied twice, the first application was aimed at flower clusters on spurs when these had reached the full bloom (FB) stage, and the second application was carried out when flower clusters on the annuals were at FB. All data were subjected to statistical analysis.

In 2006, spring was cool with prolonged periods of rain and wind causing the flowering period to be long. The weather during bloom was much better in 2007 and the general fruit set was high. In 2008, the weather was perfect for thinning, with relatively warm and sunny weather.

Generally there appears to be consensus that the effect of NaCl is highly concentration dependent, and then the use of 2%, as in our experiment, is pushing the limit, and certainly higher concentrations than that must be avoided or over-thinning / crop loss is possible.

Reached objectives / conclusions

- NaCl was clearly the most promising of the thinning compounds tested in the experiments. It was able to thin Elshof in two of the three years, and it consistently improved return bloom to an extent that may enable consistent cropping in this often very alternating variety.
- Weather conditions may be of importance. Over-thinning may occur when temperatures at time of thinning and in the days afterwards exceed 20°C. No negative effect of the NaCl application was observed in a cool Nordic climate.
- The two clay mineral compounds (Kaolin and Absomol) were thought to thin based on a combined action of their desiccation properties and ability to deter insects. The clay compounds proved very difficult to work within the windy and rainy springtime of 2006 and insufficient coverage may account for some of the lacking thinning effects. In 2007 the trees remained well covered in clay dust during the bloom period, but this did not in any way deter bees and other pollinating insects and again no thinning effect was achieved. It can therefore be concluded that clay dusting is not a feasible thinning method. The addition of 2% rape seed oil did contribute some thinning effects, but not enough to achieve sufficient thinning.

5.2.1-d Innovative pruning methods

This ISAFRUIT study aimed at assessing an innovative training system based on precision spur-pruning management, the Centrifugal Training (CT), with regard to its impact on fruit quality.

This system was compared to two other training systems. The first one is the Solaxe system (OS), used as a French reference, with the bending of branches and no renewal pruning. The second one is a more contrasted training system, the Vertical Axis (VA), used as a worldwide reference, with renewal pruning of side branches and well-established tiers. In both OS and VA systems the control of fruit load is carried out *via* chemical and hand-thinning whereas in Centrifugal Training the extinction procedure aims at adjusting the spur load on each individual branch before flowering. Therefore, in CT, chemical and/or hand-thinning are only a complementary means to fit fruit load to branch and tree development.

Two cultivars with contrasted bearing characteristics: a regular bearing cultivar, ‘Ariane’ with scab resistance (INRA) grafted on M9, studied in 2006 and 2007, and an alternate bearing cultivar, ‘Jubilé’, on M9, were used for the trials. Both cultivars were 6-year old at the beginning of the experiment. For practical reasons (availability of mature trees) CT *vs* OS was carried out on ‘Ariane’ only, and CT *vs* VA was carried out on ‘Jubilé’ only. Chemical thinning on all trees: 6BA (150 ppm), one time of application (stage 10-12 mm of king fruit). Complementary hand thinning if necessary after June drop. The standard is one fruit per flower cluster; 0 to 2 fruits if needed to fit the desired crop load.

Summarised principal results

Two experiments showed that in a given year and for a same crop load CT improved fruit size and/or colour compared to OS ('Ariane' 2006) and VA ('Jubilé' 2008). Moreover, the CT *vs* OS experiment on 'Ariane' showed that CT made it possible to increase the fruiting potential of trees, *ie* to increase crop load, without altering fruit size and colour. The CT *vs* VA experiment on 'Jubilé' showed that CT not only had higher crop load but also higher fruit size homogeneity.

In general, there were no significant differences in the internal fruit quality traits between tree training systems for a given cultivar.

The effect of the training system on regularity of bearing seemed strongly related to the cultivar. In 'Ariane' CT improved regular bearing and made it possible to increase fruit load without detrimental effect on regular bearing and fruit quality. However, on 'Jubilé' there was no consistent effect of the training system on alternate bearing.

Conclusions

Combining the results obtained in this ISAFRUIT task and previous ones (literature and from our research team) our conclusions are:

- The range of alternate bearing patterns characterizing apple cultivars and the underlying mechanisms described at morphological (*e.g.*, length of annual shoots, branching density) and physiological (*e.g.*, classical hypothesis of GAs produced by apple seeds inhibiting flower induction for the following year in adjacent buds) level clearly support the idea that the genetic part plays a fundamental role in regularity of bearing.
- Training and pruning procedures would only modulate these intrinsic patterns. Present results, and others both in growers field and experimental stations, suggest that cultivars may be divided in two groups:
    - *plastic cultivars* for which training and pruning may improve fruit quality and regularity of bearing,
    - *Non/less plastic cultivars* for which training and pruning may improve fruit quality without a consistent effect on regularity of bearing.
- Cultivars such as 'Ariane' and others such as 'Golden delicious' and 'Gala' clones belong to the first group whereas 'Jubilé' and others such as 'Fuji' and 'Golden Extreme' belong to the second one.
- Our study emphasized the need to develop collaborative work between ecophysiologists and morphologists on one hand, and geneticists and breeders on the other hand, to select not only cultivars with high fruit quality and resistance to pests and diseases but also with a high ability for regular bearing.

5.2.1-e The allergicity of apple and peach as influenced by crop load and pre-harvest factors

Since allergenicity is becoming an quality trait of increasing concern, its relation with crop load, one of the most important crop management factors influencing quality, wanted to be know. The aim was therefore to set up molecular tools in Real-time PCR experiments to assess fruit allergenicity and apply them to shed light on the environmental and endogenous factors affecting the allergenic potential in fruits of different species (i.e. apple, peach, and kiwi).

The results would give important information to breeders, growers and consumers for the selection of hypoallergenic genotypes, the adoption of agronomical practices decreasing the allergenic potential, and eventually the offer of fruits with reduced amount of allergens.

The relation between crop loads and fruit allergenic compounds was characterised clarifying preliminary data pointing at higher allergenicity at high crop loads. Molecular work performed:

- Preparation of molecular tools to assess fruit allergenicity: Nucleotide sequences encoding all known allergens in apple, peach, and kiwi were recovered from public databases. Specific primers were designed and optimized for Real-time PCR experiments.
- Setting up of molecular tools to be used in marker-assisted selection and evaluation of new thinning agents: A 30K microarray was set up starting from all apple ESTs (expressed sequence tags) available at the time when the research began. A dedicated bioinformatic pipeline was set up to cluster and assemble the sequences in order to reduce redundancy. 30,419 clusters and singletons were used out of a total 41,927 for microarray construction by means of the Combimatrix platform.
- Identification of endogenous and exogenous factors affecting fruit allergenicity: In order to identify the factors that may affect the fruit allergenic potential both exogenous (i.e. the environment, the pre- and post-harvest practices) and endogenous (i.e. the genotype, developmental stage, organs, and tissue) conditions were taken into account.

Concerning apple, hypoallergenic candidates were found. Moreover, shadowing, elevation and storage were shown to significantly affect the transcription of the genes encoding allergens, whereas water stress slightly influenced the expression of only two genes.

Concerning peach, the expression profiles of some selected genes encoding major allergen isoforms were studied during fruit growth and development, and upon different fruit load and light radiation regimes.

The results indicate that the majority of allergen-encoding genes are expressed at their maximum during the ripening stage, therefore representing a potential risk for peach consumers. Enhancing the light radiation and decreasing the fruit load achieved a reduction of the transcription rate of most genes and a possible decrease of the overall allergenic potential at harvest.

As far as kiwi, a preliminary evaluation of allergen-specific transcripts in kiwifruit was carried out. Hayward and KiwiGold varieties were assessed. Differential expression patterns were detected among the different tissues and between the two varieties. According to this first trial, the primers were shown to give a specific amplicon, thus representing an important molecular tool for allergen-related transcripts detection in kiwifruit. The genes encoding the thaumatin-like protein and the phytocistatin were expressed at higher levels in the cv Hayward kiwifruits, whereas no significant difference was detected for the actinidin gene.

Reached objectives / conclusions

Apple

- This oligonucleotide 30K microarray was validated. It can be used as a tool for the evaluation of thinning agents efficacy and the characterization of genotypes with interesting self-thinning attitudes.

- Shadowing may represent an important cultural practice aimed to reduce apple cortex allergenicity. Elevation and storage may be combined to reduce the allergenic potential of apple fruits.

Peach

- Expression profiles of selected genes encoding for major allergen isoforms of peach established.
- New growing practices should be tested with hypoallergenic genotypes in order to obtain a significant reduction of the allergenic potential by enhancing the light radiation and decreasing the peach fruit load.

Kiwi

- Evaluation of allergen-specific transcripts in kiwifruit cv. Hayward and KiwiGold varieties was carried out.
- Differential expression patterns detected among the different tissues and between the two varieties point at genotypic and tissue specific expression patterns.

Ad 2: Task 5.2.2 efficient use of naturally occurring limited resources

Main objectives

This task aimed at developing innovative technologies for exploiting natural resources for fruit crop management purposes in integrated and organic production systems.

In particular, this task focused on ...

- investigating the methodologies to adapt full irrigation requirements for peach orchards grown under hailing-nets or above reflective mulches.
- investigating the effect of a bio-stimulant product, for improving the plant uptake of the limited naturally occurring nutrients in deprived soils.
- investigating the relationships between fruit quality development and the orchard environmental conditions in peach.
- developing a new method for the control of apple fruit load based on the use of shading as a natural thinner.

5.2.2-a Irrigation requirements for peach orchards grown under hailing-nets or above reflective mulches

Tree light exposure is one of the most important components of plant transpiration. Therefore it is evident that by reducing tree light exposure something would happen with tree water consumption. The initial assumption was that the use of hailing protecting nets in peach orchards should reduce irrigation water requirements. With different rates of applied water and light exposure, fruit quality may also be modified.

To analyze these effects a three year experiment was set in one peach orchard located in Corbins (Leida-Spain). During the two first years, hail nets were deployed during the fruit growing season (mid stage II of fruit development until harvest). However, for the last year, any possible deleterious effect occurring during the previous two years was attempted to be reversed by the removal of hailing nets and the deployment of reflective mulches located on the ground.

Stem water potential measured at midday (SWP) as an indicator of plant water balance and to manage irrigation according to these measurements. Midday stem water potential (SWP) were measured in selected trees of both treatments and irrigation was adjusted on a weekly basis in

Net trees to maintain similar SWP in Net and Control trees. Reference evapotranspiration (ETo) was obtained from the closest weather station less than 5 Km from the experimental plot. Irrigation schedules were twice a week loaded to an irrigation controller. The experiment consisted in a randomized block design with tree replications for each treatment.

Net use

During the first year of the experiment (2006) the effects of the nets on water requirements was estimated by progressively reducing applied water in trees under nets as compared to control trees. The second year (2007) the approach was to adapt applied water and dynamically re-schedule (every 3 days) irrigation in peach trees growing under. In the third year (2008) it was attempted to revert the pre-history of shadow in the net trees by installing reflecting mulches.

The results of the first two years of the experiment were on line with the initial assumptions. When tree light exposure was reduced, so did happen with tree water status and water consumption. The two methods of modifying tree light exposure (nets and reflective mulches) were functionally applied after fruit thinning was applied in order to avoid any direct effect of the shadow on the reproductive behavior the very same year of their deployment.

In the initial year of experiment (2006), it was proven that nets reduced incoming radiation by 18 % as indicated by the manufacturer (and evaluated directly on the field). Recalculating ETo considering a reduction of incoming radiation by 20% it was obtained that ETo was reduced by 15%. This sets ground for a substantial reduction in irrigation water requirements in trees grown under nets.

With regard to fruit quality, the results indicated that the composite effect of Nets and reducing irrigation produced an undesirable effect on reproductive growth. Fruit size and yield at harvest was reduced, mainly as a result of water stress during phase C. It was also found an advance in maturation in Net trees since fruits had more reddish skin than Sun fruits. Therefore, the water stress effect would be added to the shadow effect.

Net trees, in 2007, exhibited a significant delay in maturation (this is contrary to the first year) and had yield and fruit quality reduced (less soluble solids). However, the yield reduction was not related to fruit size, as in the previous year of experiment in which Net trees ended up water stressed, but to lower fruit number. Lower fruit numbers in Net trees could not be significantly related by the same year reduction in lower fruit set or flower density. However, this still remained the most likely explanation, since in the following year (2008) flower density and bloom return was significantly reduced in Net trees.

Reflective mulches

If the use of nets had resulted negative for fruit production (maturation delay, less sugars, reduced fruit number per tree), the use of reflecting mulching, perhaps could counterbalance such effects. In the third year of experiment, trees which had been grown under nets for two consecutive years, had the nets removed and reflective mulching deployed, instead.

Reflective mulching increased exposure to radiation by 18%, and this implied an ETo increase of 11%. In order to maintain similar SWP between reflective mulching and Sun trees irrigation had to be increased by an average of 16 % in mulching trees.

Increasing light exposure had a positive effect on fruit quality (larger fruit size, higher soluble solids and relative dry matter) and advanced maturation. In the fruit quality sampling at harvest, fruits on mulching trees were softer because of the advanced maturation, but also had redder skin.

A further analysis comparing average fruit fresh weight at harvest with fruit number per tree normalized by trunk cross sectional area (TCSA) revealed that fruit size in reflective mulching were less sensitive to crop load than Sun trees. This means that, irrespective of cropping changes related to previous year treatments, a genuine positive mulching effect on fruit quality may be very likely.

- If nets have to be deployed for hail protection, slight losses in yield and fruit quality have to be expected. A possible compensation for these losses is the significant decrease in irrigation requirements.
- In the prevailing climate in which the experiment took place, we recommend managing irrigation reductions according to the evaporimentric demand of the period considered.
- The use of nets delayed maturation, reduced fruit soluble solids, and after two years it also reduced return bloom and fruit number. A possible way to overcome this limitation on fruit quality is by using reflective mulching which increased tree light exposure and irrigation requirements by 10%.
- It can be expected that for an average commercial crop load (i.e. 300 fruit per tree), trees on reflective mulching are expected to produce larger fruits. Further studies are needed for final conclusions.

<u>Basic studies on the effect of modified environmental conditions in the orchard (i.e. obtained by applying shading net and reflective mulches) on fruit quality</u>

The effect of modified environmental conditions in the orchard on the biophysical and biochemical mechanisms of fruit growth and on the final crop quality has been studied in peach. The physiological basis of fruit quality was studied by comparing the effect of different crop loads on the mechanisms of fruit growth. The results obtained provide new insight for improving fruit quality through innovative approaches in the orchard management.

<u>Summarised results / conclusions</u>

- The high surface conductance of peach and nectarine fruit is a key feature to promote passive phloem and xylem imports, thus allowing the fruit to accumulate dry matter and grow.
- Therefore, conditions promoting fruit transpiration, as those determined by reflective mulches increase final fruit quality as they increase phloem and xylem imports.
- In absence of water limitations, a crop management aimed at increasing the vapour pressure deficit at tree level will result in higher fruit quality at harvest.
- Heavy / low crop load: At low crop conditions, higher carbon imports by phloem flow and lower specific transpiratory losses provoke higher fruit size and dry matter content. However, at some times during the day, sinks developed under conditions of high competition (heavy crop load) may be more “active” in attracting water and assimilates than fruit in low cropped trees. This phenomenon could be exploited for trees with higher crop load to produce better quality, needs to be better understood, however.

See also chapter 5.2.2-c1 and 5.2.2-c2.

5.2.2-b Improving the plant uptake of the limited naturally occurring nutrients in deprived soils

The aim of the study was to verify the effect of a bio-stimulant product, the Actiwave® (Valagro S.p.A.), based on natural compounds such as alginic acid, kaedrin and betaine for improving the plant uptake of the limited naturally occurring nutrients in deprived soils and for increasing the plant resistance against biotic and abiotic stresses.

The experiments were carried out on apple trees (cv Fuji), grafted on M9 rootstock. To study the effect of the bio-stimulant on alternate bearing, it was applied on trees carrying a different fruit load that was artificially imposed *via* differential hand thinning, at the experimental station of the Bologna University (Cadriano, BO, Emilia-Romagna, Po Valley area).

Also, to study the effect on the photosynthetic rate, further experiments were performed on model plants (strawberries, cv Garriguette). These plants were grown in enclosed transparent containers connected to a gas analyzer and a flow meter. The CO_2 assimilation and the transpiration were monitored in real-time for several days after the treatment.

These data will be used for a comparative study with the experiments carried out in the Northern Europe climatic conditions.

Results / conclusions

It was observed, that Actiwave:

- did not significantly increase shoot growth, fruit yield of thinned trees (but it did on unthinned trees, even if to a minor extent than the fertilization treatment did), fruit weight,
- increased towards the end of the growing season the chlorophyll content, but to a very minor extent as compared to the fertilization treatment,
- increased soluble solids on thinned trees particularly in combination with the fertilization treatment (but did not on unthinned trees), fruit firmness on unthinned trees (but did not on thinned trees),
- seemed to have an additive effect on the fertilization treatment, even if the latter had the stronger effect.
- It can be concluded, that Actiwave might have some positive fertilising effect, but further studies are needed for verifying whether the effect is a nutrient mobilising effect or may be due to other reasons.

5.2.2-c Investigating on the relationships between fruit quality development and the orchard environmental conditions in peach

DCA-UNIBO carried out specific experimental trials on the evaluation of the effects on reproductive parameters and fruit quality of modified light environment at tree canopy level. For this purpose the enhancement of light avalability was obtained with the placement of reflective mulches in the interow space of a peach orchard. The light reflective mulches may change local environmental and growing condition: temperature, relative humidity and, specifically, the quantity and quality of light passed through the canopy or reaching the alley-row floor back into the fruiting canopy.

Experiments were carried out on the nectarine ‘Red Gold’ grafted on seedling rootstock (approximately 830 trees/Ha). Environmental condition was studied at tree canopy level (light quantity, temperature and relative humidity). Measurements: fruit load, fruit growth, phenolic compounds (potential antioxidant capability of fruits).

Generally, 3-4 °C higher values at intermediate canopy level were recorded for the maximum daily temperature in mulch treated trees. Relative humidity data showed limited influence of mulches on this parameter.

<u>*Results*</u>

- There were higher cumulative yield of mulch-treated trees compared to control trees and there was a tendency to earlier ripening (higher number of harvested fruit in picks 1 and 2, and necessity of pick 4 for control trees only). Average fruit weight in the first two picks was larger from mulch-treated trees.
- Mulch-treated fruits were larger and for the same pick (reported data are for pick 1) as a tendency more ripe. This was evident by considering all the internal quality parameters evaluated, such as: pulp firmness, titrable acidity and sugars
- Total phenolic amount was higher in peaches harvested from mulch-treated trees in both the analysed tissues, with concentration ranging from 8 to 11 mg/g DW in the control and mulch treated mesocarp respectively, and from 15 to 19 mg/g DW in the skin of untreated and treated fruits.
- As for the single classes of phenolics, cinnamic acids (mainly chlorogenic acid) and flavan-3-ols were the main constitutive compounds in the mesocarp and exocarp tissues. Flavonol glycosides (quercetin derivatives) were mainly detected in fruit exocarp. Relative concentrations of the reported classes of compounds were not clearly influenced by the environmental modifications introduced with the reflective mulch treatment.

<u>5.2.2-c1 Effect of environmental variations on daily growth, vascular flows and carbohydrate metabolism of peach fruit</u>

Previous studies report a positive effect of reflective mulches either on fresh weight, soluble solids content and dry matter percentage for peach fruit at harvest. Such results let foresee the possibility to improve fruit quality by modifying the orchard environmental conditions through new management approaches.

In 2007, trees environment was modified in the mid-season nectarine, cv Red Gold, by applying Extenday® reflective mulches. The microclimatic conditions of some fruit from the same cv. were also modified by enclosing them in holed plastic bags. In 2008, Extenday® and black shading nets were applied on trees of the early season cv. Alice-col. During both seasons, daily growth rate, vascular and transpiration flows were measured on four-five fruit per treatment during cell expansion stage. Phloem, xylem and transpiration flows were determined by comparing the daily patterns of intact, girdled and detached fruit whose diameter variations were continuously monitored using automatic, custom-built fruit gauges.

Extenday® mulches and shading nets increased and decreased the orchard VPD, respectively thus affecting fruit transpiration, which was higher for Extenday® fruit. Fruit growth rate and phloem flow were positively affected by reflective mulches but were reduced in shading and bagged conditions. Extenday® fruit showed higher SS, NI and SDH activities compared to shading, thus indicating increased sucrose and sorbitol catabolic activities which may respond to higher assimilates availability in these conditions. Daily phloem inflow was positively related to fruit water losses, during both seasons.

Results / conclusions

- Fruit transpiration may have a positive role in peach fruit growth, as water losses may decrease fruit water potential, thus increasing the necessary gradient needed for bulk flow phloem unloading to occur.
- Reflective mulches may regulate fruit transpiration and represent a potential tool to improve quality and growth in peach fruit.

5.2.2-c2 Effects of crop load on peach fruit daily growth and vascular flows

In 2007, the daily patterns of vascular and transpiration flows to and from peach fruit were compared between heavily thinned (LCL) and unthinned (HCL) trees, in order to assess when these flows were limited by resource availability (source-limited) or by the genetic potential of the fruit (sink-limited) during the day. Results from this study have been published in the ISAFRUIT special Issue of the JHSB.

At cell division stage of the fruit, no difference between treatments was found in any of the transpiration flows, suggesting that fresh matter (FM) import from phloem and xylem is sink-limited during early fruit development. At cell expansion state, HCL fruit were smaller and had higher specific transpiration rates during the day. Xylem flow did not show any source limitations due to high crop load. However, it was "sink-strengthened" in the afternoon, in HCL, as these fruit reached lower water potentials during the day. Phloem flow to HCL fruit was source-limited during the afternoon and at night due to fruit to fruit competition. However, HCL fruit appeared to take advantage of their lower water potential at midday, when they showed higher rates of phloem flow.

Reached objectives / conclusions

- Although daily growth in fruit on unthinned trees undergoes periods of source limitation, this study showed how, at certain times of day, fruit may be more active sinks in attracting resources in high cropping conditions than at low crop loads.
- This information can be used for improving fruit quality of trees with heavy crop load.

See also chapter 5.2.2-a.

5.2.2-d Developing a new method for the control of apple fruit load based on the use of shading as a natural thinner.

5.2.2-d1 Protocol adjustment

The protocol development for the use of shading for thinning in apple started in 2007 with a study on the physiological response of apple trees to shading, and the dynamics of apple fruit abscission. Results from this study have been published in the ISAFRUIT special Issue of the J. Hort. Sci. Biotech.

These results of the study with cv. Imperial Gala showed that shading could be used as an effective tool for apple fruit thinning. However, the method still missed a rational approach to identify the best time to apply and to remove the shading, also considering the environmental variability existing between different years and orchard locations.

Therefore, in 2008 the work focused on studying the effect of different shading periods on whole tree carbon balance and on fruit drop by applying both experimental and modellistic approaches, then, in 2009, different methods for identifying the time of shade removal were tested in the field. This study successfully identified two working methods for this goal in the growing region of Bologna, Italy.

From previous results it was known that early season shading of apple trees determines a first period of constant fruit drop, which is followed by a sharp increase in the fruit drop rates. Following this approach, shading was removed when the peak in daily fruit drop was detected. This method allowed reaching satisfactory thinning levels and fruit quality at harvest.

The second approach was based on the continuous monitoring of fruit relative growth rate (RGR). In natural conditions (control), apple fruit RGR decreases sharply during the first period of fruit development. These changes in the RGR pattern indicate that a significant reduction in the tree fruit number has occurred and can be used as indicators for shading removal. In the trial conducted, different crop load treatments were assessed before shading application, in order to test the RGR approach in a wider range of conditions. After shading imposition, fruit RGR was monitored at very short time intervals, and on each treatment shading was removed once its RGR pattern showed a steady state period or an increase. Also this approach allowed reaching satisfactory thinning levels and fruit quality at harvest.

Reached objectives / conclusions

- Two methods for determining time of shading removal have been identified: the monitoring of both daily fruit drop and fruit relative growth rates (RGR).
- This method is ready to be implemented in commercial orchards.

5.2.2-d2 Biochemical studies

The rapid shortcut of assimilates available for the growing sinks due to shading application may lead to a sudden lack of sorbitol and sucrose, which are the main translocated assimilates in apple, with consequent changes in the activity of fruit metabolic enzymes. To investigate on this issue, in 2007, leaves from control and shaded trees were compared for the activity of a key enzyme in sorbitol biosynthesis: sorbitol-6-phosphate-dehydrogenase (S6PDH) whereas fruit from the same trees were compared for the activity of sorbitol and sucrose metabolic enzymes and the activities of sorbitol dehydrogenase (SDH), sorbitol oxidase (SOX), sucrose synthase (SS), acid (AI) and neutral (NI) invertases were assayed on 4-6. The following year’s assays were performed not only during the shading period but also before and after shading application.

Results

- Results from this trial showed that sorbitol biosynthesis in leaves are negatively affected by shading, probably due to the reduction in whole tree carbon assimilation, as recorded on the same trees.
- This would lower the amount of sorbitol translocated in the phloem of shaded trees and available for fruit sinks.
- Such reduction is in accordance with the highly significant decrease in daily growth rate shown by shaded fruit.
- Furthermore, assimilate availability affect the activity of some sugar-related enzyme in the fruit, like SDH, that is known to be directly regulated by its substrate.
- Also, a positive linear relationship was found between SDH activity and fruit growth rate: the more sorbitol a fruit receives, the higher both its catabolic activity by SDH and its growth rate are.

Conclusions

- Shading in principle affects sugar biosynthesis negatively and therefore is a potential threat for fruit quality.
- If advantages of shading techniques (e.g. for thinning purposes) want to be exploited, the observed threat for fruit quality needs to be compensated in some way.

5.2.2-d3 Biophysical studies

In 2008, a further study on the effects of shading on the biophysical mechanisms of apple fruit growth was carried out. This study focused on how vascular and transpiration flows to/from the fruit are affected by shading-for-thinning.

At 30 days after full bloom, the 90% neutral shading net was applied to four trees of the cv. Gala, for seven days, while four more trees, chemically thinned, were used as control. Fruit vascular and transpiration flows were assessed from two days before, to several days after shading removal. The daily patterns of fruit relative growth rate and of phloem, xylem and transpiration flows to/from the fruit were determined by continuous monitoring of fruit diameter by automatic, custom-built fruit gauges.

Before shading, no differences between treatments were found for any of the parameters measured. Despite shading induced an immediate drop in canopy photosynthesis, both fruit daily relative growth rate (RGR) and phloem flow decreased gradually, until reaching 80% lower values than controls at the end of the experiment. No, or very small differences were found between treatments for transpiration rates and for the daily amount of xylem flow.

Reached objectives / conclusions

- This study shows how shading-for-thinning in apple affects fruit growth indirectly, by reducing the amount of assimilates fixed by canopy photosynthesis, and not by directly modifying fruit strength as sinks through changes in fruit micro-environmental conditions.
- This conclusion would suggest that the length of shading necessary to reach the desired thinning level might be simply determined based on the determination of missed carbon assimilation under shading conditions.
- However, the whole process is probably complicated by the remobilization of carbon reserves, which delay the effects of shading both on fruit growth and on fruit final drop (further studies needed).

5.2.2-d4 Practicability tests

Practicability tests of the shading for thinning method where made by partner ACW. In Swiss organic apple production, there is no agent available for fruit thinning. Trials in 2006–2008 were carried out in experimental orchards at Wädenswil and Güttingen, north and north-east of Switzerland, respectively. Focus of the trials during three years on different apple cultivars was to detect the effect of shading date and length on yield, external and internal fruit quality and flower intensity the following year under practical orchard situations.

In 2006 12 trees in three blocks of 9-year-old Elstar and Golden Delicious on P22 rootstock with a planting distance of 3.4 x 1 m were fully covered with a 3m-width shading cloth, which blocks 74% of solar radiation. Trees were shaded 25 days after full bloom (DAFB) for three days or until peak in fruit fall was registered, which happened after seven days.

Three days shading at 25 DAFB reduced crop load for Golden Delicious to 60 fruits per 100 flower clusters. This is an optimal efficacy compared with the chemical thinning (+hand thinning). Fruit quality regarding soluble solids and firmness was very good.

Results 2006

- For Elstar, seven days shading reduced crop load to an extent (66 fruits per 100 flower clusters) comparable to the commonly used thinning methods. With seven days shading, flower intensity the following year 2007 was not as good as after chemical thinning treatment plus hand thinning but still acceptable.
- Fruit weight, crop load and internal fruit quality were comparable to the commonly used thinning methods after seven days shading.

In 2007, trials focused on the practical shading technique and the length of the shading cloth with which material costs could be saved. Thus, 2 m and 3 m-width shading cloth of the same material as in 2006 was compared. Both treatments were applied at 19, 26 and 33 DAFB for three days on 12 trees in three blocks of 5-year-old Topaz and Golden Delicious on rootstock Fleuren 56 and a planting distance of 3.5 x 1.1m.

Results 2007

- Three days shading at 19, 26 DAFB (2 m and 3 m-width net) and 33 DAFB (3 m-width net) showed a good thinning effect for Golden Delicious. Crop load, fruit weight and internal fruit quality showed good results and were comparable to chemical thinning plus hand thinning.
- For Topaz, only three days shading at 19 DAFB (2 m and 3 m-width net) showed a significant thinning effect compared to the control as well as an effect on soluble solids and fruit weight. Flower intensity next spring was best after 19 DAFB with 6.6 (3 m net) and 5.5 (2 m net).

In 2008, selected repetitions of the trials 2007 (shading with a 2 m-width shading cloth at 19, 26 and 29 DAFB) were done on 11-year-old Golden Delicious on rootstock P22 with a planting distance of 3.4 x 1 m and 6-year-old Braeburn on rootstock M9 with a planting distance of 3.5 x 1.1 m.

Results 2008

- Crop load reduction in general was too high with *all* thinning treatments. However, for Golden Delicious, three days shading at 26 DAFB with a 2 m-width net led to a crop load equal to chemical thinning treatment plus hand thinning.
- Flower intensity the following year 2009 was very good when shaded 26 DAFB. Shading at 29 DAFB led to a good thinning effect. Internal fruit quality, crop yield and fruit weight were comparable to chemical thinning treatment plus hand thinning.
- For cv. Braeburn, natural fruit fall was very high in that year and therefore, all treatments in this trial led to overthinning.

Feasibility study for commercial orchards

Fixing the shading nets by hand is very time-consuming and therefore very costly. A mechanisation to fix the shading nets in the orchard needs to be found. A possibility might be using the machine “Fruitwrap”. Usually, this machine is used to cover cherries with a plastic film. In fact, this is still not possible in orchards with hail nets because the machine does not fit under the hail nets. We now check with the manufacturer whether a smaller machine could be constructed to fit the requirements of an orchard with hail nets.

A Fruit grower has invented a different system. He fixes the 3 m width shading net by hand using a self-steering hydraulic hoist. The hydraulic hoist can be used for different other work in orchards, e.g. pruning or harvesting.

The calculation of the costs has been done in an interaction with WP 5.3.

Reached objectives / conclusions

- Extensive practicability tests of the shading for thinning method were performed.
- With regard to the desired thinning effect, the technology satisfies its goals.
- The ISAFRUIT findings obtained for determining the shading removal (see 5.2.2-d1) should confronted with the shading periods used in the practicability tests.
- The development of suitable devices for an efficient application of the shading nets in orchards needs further efforts.
- Economical calculations, however, make clear, that the method is too expensive for standard integrated production. In the organic production, it is for the same reason not an alternative to the "thinning machine" (rotating brush) if trees are suitable for the thinning machine as it is the case in modern orchards. However, if thinning is done by hand at flowering stage, shading seems to be an interesting alternative to this option. Moreover, if varieties bloom at different stages, nets can be used more than twice in the orchard.

Reached objectives / general conclusions Task 5.2.2

The 4th objective mentioned at the beginning of this task chapter has been pursued both by implementing a workable protocol for the exploitation of shading for thinning in commercial orchards, and by improving the physiological knowledge on the effects of shading on apple fruit leaves and fruit.

The following main conclusions can be drawn:

- Shading can be used as an agronomical alternative to chemical sprays in apple fruit thinning. Two different approaches have been developed for determining the shading duration and the technology is ready to be implemented in commercial orchards.
- Shading induces sharp decreases in canopy carbon assimilation and negatively affects fruit daily growth by progressively reducing the amount of phloem flow to the fruit, which eventually drop. Shading also decreases sorbitol metabolism in leaves and fruit. However, as practicability test show, the methods does not provoke minor fruit quality at harvest.
- With regard to the impact of this technology on production cost, the method can be competitive particularly for organic orchards.
- The development of a technical device for an efficient application of shading nets in orchards still needs further efforts.

Ad 3: Task 5.2.3 Tools for an optimal exploitation of the quality potential of peach and apple

This task focused on mathematical models, molecular biology tools and non-destructive technology to study fruit quality aiming at an improved understanding of growth and quality regulating processes. The task should provide conceptual support for activities of Tasks 1 and 2.

Since abscission, fruit size and ripeness of a fruit are factors of highest importance with regard to optimizing fruit quality, being addressed also by Tasks 1 and 2, models developed were addressing these issues.

5.2.3-a Mathematical model (peach)

5.2.3-a1 Development of a mathematical model of peach fruit growth, which can be used to assess the fruit growth potential

Our initial objective was to develop a mathematical model of peach fruit growth, which can be used as a tool in assessing the fruits growing potential, and in forecasting fruit size at harvest. That objective was enlarged during the research progress to other criteria of fruit quality, such as sweetness.

One important step of the research has been to propose a model able to simulate the potential growth of peach fruit. In a second phase that model has been included in a more general model of fruit growth and quality, the model has been tested and used to simulate different scenarios of water availability. Lastly the model has been used to simulate the effect of a small change in the fruit growth (as due to a genetic mutation) in order to analyze the effect of that change on the whole shoot-bearing fruit system.

The model used in this study has been based on sink-source concepts. One important step of the modeling work has been to study the fruit demand for dry matter. To undertake this study, we used measurements of fruit growth obtained on peach trees (cultivar Zéphir) with very low fruit loads. After testing different mathematical equations, a logistic equation has been chosen to describe the potential fruit growth from 700 degrees-days after bloom to harvest (1800 dd). The fit to the data was good and the variability of growth within the tree is well explained by the variability of dry mass at 700 dd.

While first validation test overestimated fruit dry matter, the fit was improved if the parameter defining the maximal dry mass that the fruit can reach was modelled as a function n of the mean dry matter mass and the crop load.

Reached objectives / conclusions

- A mathematical model of peach potential fruit growth in dry mass has been proposed which allows representing the evolution of fruit growth on the basis of its early dry mass.
- The fit of the simulations to the studied population of peach fruits showed that the potential growth model gives good predictions of fruit dry mass.
- A method has been proposed to simulate the fruit growth in suboptimal growing conditions which seems to give good predictions.
- Such model can be used for harvest date prediction and harvest forecasts. Fruit weight and size are essential elements of fruit quality.

5.2.3-a2 An intelligent virtual fruit focusing on quality attributes (peach)

The above mentioned fruit growth potential model was integrated into a process-based model of fruit quality expressing the seasonal changes of several quality attributes of fruits (size, percent of flesh, water and sugar contents) on a fruit-bearing shoot (FBS).

We have specified the multi-criterion process-based model of fruit quality focusing on peach and on attributes interesting both growers and consumers. These attributes are the fresh weight, which determines the price paid to the grower, the percent of flesh, which determines the edible part of the fruit, the water and sugar contents, which determine the fruit taste that is a driving force of consumer satisfaction.

The model shows the time-course of quality attributes along fruit growth, either monitored or simulated by the modeled "virtual fruit", in the case of a mid-season peach, cv. Suncrest.

Reached objectives / conclusions

- A model "virtual fruit" was established.
- The model accounts for the increase of fruit growth during growing season addressing low and high fruit loads.

5.2.3-a3 Simulation of the effect of different scenarios of water availability on the virtual peach fruit

In a series of virtual experiments, a large set of time-scenarios of water conditions was simulated on peach fruits cv. Suncrest, starting from the idea that, classically, fruit crops encounter an alternation of dry and humid periods, the duration of these periods being quite variable because of unpredictable weather events or variable technical constraints.

This virtual experiment aimed at answering, in the framework of an alternation of dry and humid periods, the following question: is there a relationship between time-scenarios and fruit quality at harvest? If so, which trait or set of traits of the time-scenarios is responsible for such a relationship? To answer this question, two series of 100 time-scenarios composed of six successive periods with alternation of contrasted water conditions were set up. The periods had a random length, the sum of the durations of the six periods of a scenario being a fixed duration of fruit growth.

In the first series, the first period was dry (water deficit, represented by a mean stem water potential of -0.8 Mpa as input of the virtual fruit). In the second series, the first period was irrigated by irrigation system or rainfall (normal water conditions, represented by a mean stem water potential of -0.5 Mpa as input of the virtual fruit).

The time-course of quality attributes (fresh mass, sweetness) was highly fluctuating, indicating a very reactive behavior of the virtual fruit. The duration of each of the six successive periods, the total duration of the dry periods and the total duration of the humid periods were tested as candidate variables for explaining fruit quality at harvest.

Results / conclusions

- This exploratory analysis (verification) with this still model demonstrated that the fruit quality at harvest is essentially explained by the duration of the last period with alteration of contrasting water conditions.
- The correlation was positive for fresh mass and negative for sweetness in the case of scenarios starting by a dry period and ending by a humid period, and conversely negative for fresh mass and positive for sweetness in the case of scenarios starting by a humid period and ending by a dry period.
- The model still requires proper validation to make sure, that it may be used for explaining and forecasting fruit quality at harvest by means of water availability conditions.

5.2.3-a4 Impact of a carbon requirement mutation on the virtual peach fruit

The purpose of this analysis has been to show that our model can be used to compare and contrast the state of a virtual wild-type system and a virtual mutant, and analyze the impact of a single mutation on different processes (sensitivity analysis).

The virtual mutation tested was a single mutation which decreases the fruit's requirement for carbon. The simulation shows that mutation triggered large effects in several variables of the fruit development (growth, respiration and metabolism) and delayed the fruit developmental rate.

Such a virtual approach could lead to new ways of exploring the impact of management changes (mutations), or naturally occurring genetic variations, in silico under differing environmental conditions.

Results / conclusions

- The study showed the possible use of a multi-criterion process-based model of fruit quality (a virtual fruit), especially for analyzing environmental effects by means of virtual experiments.
- As it stands, the model is a useful contribution to SMARTFRUIT being a conceptual and methodological research tool for studying the improvement of fruit quality in support of the promotion of fruit consumption in Europe.
- The virtual fruit behaved like a system, and showed emerging properties that have theoretical and practical interests. The model needs to be finally verified and validated, however.

5.2.3-b Prediction of the apple fruit weight two months before harvest

At the inception of the project, pre-existing knowledge on fruit growth forecasting gained via modelling was specifically excluded from the Consortium agreement but the task was included in the Annex I, to test the possibility of providing real-time control and evaluation of the progress of apple fruit growth in orchards physically remote from the site where the models were being run.

This was achieved in a "soft" way by agreeing to a data gathering protocol to be adopted by some of the WP 5.2 partners in different European countries. This protocol constituted one of the first deliverables of this task. The data were normally gathered in the course of other ISAFRUIT trials involving fruit growth, in order not to duplicate the amount of work, but rather to make it possible that the data collected once could be used in more than one study. Since the trials involved early season crop-load manipulation, followed by best management practices until harvest, it was possible to collect the data from these trials because, after the setting of a determined crop load early in the season, the orchards resembled quite closely standard, commercial orchards and constituted therefore a highly representative sample for the proposed testing. The protocol was adopted by a different number of cooperators in the different years, particularly in Norway, Denmark, Slovenia and France.

As these assays were found to be satisfactory, i.e. simulations agreed with observed data, the following step, carried out in Italian commercial farms, has been to provide real-time guidance to growers of the apple cvs. Fujy and Pink Lady. These two high value varieties need careful management of the growth process in order to achieve marketable sizes, high quality and yields, while retaining good return bloom, in particular 'Fuji'.

The results have consistently shown that this objective is widely achievable. Only internal reports have been produced until this moment, in particular deliverable D 5.2.7 (Month 31), as the data are being processed for a publication that is foreseen after the completion of the project.

Results / conclusions

- Providing (simulated) forecasts of fruit size at harvest without any physical contact with the grower and the orchard has been successfully achieved.
- The comparisons between expected and actual fruit sizes in experiment trees in France and Denmark have shown a considerable precision of the method, despite the fact that the researchers running the models and providing the forecasts had no knowledge whatsoever of the actual orchards and the conditions under which the trees were growing.
- Fruit size predictions represent a useful real-time tool to test orchard performance, and provide an indirect, but very precise, assessment of crop load levels. The forecasts of course allow an evaluation of orchard performance and provide insight on whether or not to modify orchard management practices (i.e. modifying the irrigation regime, or adjusting crop load) in those instances where they show inadequate fruit size would be reached at harvest.

5.2.3-c Physiological and biochemical models (apple)

5.2.3-c1 Forecasting the June drop for apple

Shading and chemical thinning affect the fruit fall in spring. Thus, during the project, the question arose if it is possible to predict the June drop for apple. For the optimisation of fruit thinning, a forecast of the June drop would be very helpful for the growers. Crop regulation is crucial for an optimal yield and a good internal and external fruit quality.

This activity developed during project execution (not in Annex 1, but in later planning documents), was developed by a team (ACW) engaged in Task 5.2.2 and produced an additional deliverable. Due to the nature of the activity, however, it was for this report attributed to Task 5.2.3.

In spring 2010 the following possibilities for forecasting fruit fall were investigated:

- Greene's model: based upon the fruit growth rate after fertilization. A fruit is predicted to be abscising if the growth rate slows down to 50% or less of the growth rate of the fastest growing fruit. The model will be verified for different varieties, but outside ISAFRUIT.
- NIR- (near infrared-) Analyzer "Phazir" (Polychromix, Inc.): Measurements were made on three cultivars (Diwa, Rubens, Golden Delicious). On 10 trees per cultivar (5 unthinned and 5 chemical thinned trees), the fruits of 12 clusters per tree were marked After the June drop, the persisting and the dropped fruits were determined.

Results / conclusions

- On average, the method used succeeded in identifying correctly 73% of the fruitlets which abscise in June drop. This is a promising result and it would be interesting to continue these measurements with the "Phazir" in the future.

- The instrument, and especially the measurement head, is not adapted for small fruit before June drop. A modification with a smaller unit should be possible and might lead to more precise values.
- Referring to these first results, it seems to be very important to continue trials based on the model of Greene and on NIR-measurements for the development of a June drop prediction model. This would help the fruit growers to optimise the crop regulation.

5.2.3-c2 Forecasting fruit quality (ripening)

This part concerned the possibility to forecast the fruit quality as early as possible during the fruit growth and to adopt the cultural management techniques able to reduce the ripening heterogeneity that is normally present in trees.

This part was carried out in strict connection with the WP4.2 of the Pillar 4. Both electronic – nose and NIRs systems were explored to be used to determine early in the season differences in fruit as related to specific thinners used and environment.

As far as the electronic-nose is concerned, this was utilized to collect the different olfactory signatures that characterized the growth of ‘Galaxy’ fruit from set to harvest time (trees unthinned and thinned by 6-BA). The chromatographic profiles of volatile compounds obtained by SPME-GC analysis changed during fruit growth from 75 dAFB onwards. Clear qualitative and quantitative differences were observed already one week before harvest in 6-BA treated fruit.

Results

The e-nose analysis showed a good capacity to follow changes in the volatile compounds emission related to fruit phenological stage and to discriminate between fruits subjected to different crop management interventions (untreated and 6-BA-treated fruits).

Ripening homogeneity

A simplified VIS/NIR system (DA-Meter) was used. The instrument may give the possibility to follow “in orchard conditions” the ripening evolution of the fruit and decide if differences among them are related to different bearing shoots, the light exposure and to other situations or cultural management techniques. The DA-Meter could also work jointly with a GPS for identifying differences between trees in the same orchard, between different orchards in given areas, to decide what type of storage must be used for the fruit, or what type of markets (nearby or far away) should be chosen.

Results / conclusions

- The research results showed that reducing the heterogeneity at tree level, fruit of uniform ripening at packing house level, assured by using either portable or stationary on-line DA-Meter, have a positive impact at consumer level.
- The consumer test performed within a supermarket chain on apples, peach and apricot pointed out the preference of the” repurchase attitude” of the consumers according to a given ripening stage reached by the fruit. The provision of fruit ripeness information to the consumers by the supermarkets should help to better meet consumers’ expectations.
- This study shows the potential use of the technology explored. It requires further adaptive research for being applied in practice.

5.2.3-d The apple abscission model derived from transcriptomic and metabolomic data

Main objective: To isolate abscission-related genes in apple for contributing to the development of easy screening methods for chemical thinners and self-thinning varieties, for an eventually efficient exploitation of the quality potential of an apple genotype.

Purpose: Along with this activity, the task included also the setting up of an apple microarray to be used in screening for new thinning agents and new genotypes with self-thinning attitudes.

Isolation of abscission-related genes: A double approach was adopted to isolate abscission-related genes in apple. Initially, a cDNA-AFLP differential display was performed on abscising (AF) and non-abscising (NAF) fruitlets sampled from cv Golden Delicious/M9. AFLP was performed to point out the genes differentially expressed in the two populations and allowed to isolate 301 differentially expressed clones, which were deposited in dbEST and are publicly available at the following link: http://www.ncbi.nlm.nih.gov/dbEST/. The cortex showed the majority of differentially expressed genes, followed by the peduncle, and the seed. The second approach consisted in the setting-up of a custom apple microarray including more than 30,000 genes to be used for the identification of new abscission-related genes. BA was used to obtain fruitlet populations with different abscission potentials, to be analysed by means of the 30K oligonucleotide microarray set up by Combimatrix technology. Transcriptomic profiles of persisting and abscising fruitlets were tested for statistical association with abscission potential, allowing identifying molecular signatures strictly related to the fruit abscission potential.

Model development: A hypothetical model for apple fruitlet abscission was obtained by putting together available transcriptomic and metabolomic data.

Reached objectives / conclusions

- Candidate genes were identified by means of Real-time PCR validation to be tested for their potential use in MAS programmes.
- A 30K microarray can be used as a tool for the evaluation of thinning agents and the characterization of genotypes with interesting self-thinning attitudes.
- Based on the transcriptomic and metabolomic data, a hypothetical model for apple fruitlet abscission was obtained.

WP 5.3 High-quality fruit from profitable production systems *(PROFITFRUIT)* Leader: 2: ACW (Esther Bravin); other partners: Partners: 20. OVR; 8. WUR-PPO; 28. GRAB; 10. WUR-LEI; 14. FiBL. (Work period 0-48 months).

Objectives

The overall objective of this work package was to provide knowledge and methods for improving the capacity of farms in economical terms to deliver more fruits with quality standards required by the market and consumers for satisfying an increased demand and consumption.

The objectives of the work package were:

- To document and to compare the economical situation of fruit farms in selected growing zones and to identify success factors that are important for economical success while producing top quality fruit.

- To develop a user-friendly decision support tool for growers and extension services for analyzing production costs and profit, to apply the tool, and to indentify farm management factors that are essential in terms of their impact on success if changes are made while producing top quality fruit.
- To validate such economical farm management factors for an improved production of top quality fruit in an exemplary case study looking at how fruit farms behave in situations of change balancing out market and consumers' demands and own norms and values.

ISAFRUIT economical expert back bone

Besides these objectives, PROFITFRUIT evolved into something like the economical back bone of ISAFRUIT, i.e. if economical aspects of the ISAFRUIT technology under development seemed to be of importance, specialists of this work package were involved. By this, scientists of this work package interacted strongly with specialists from other work packages of Pillar 5 and also other Pillars.

This is not reported in particular at this place, but in the reports of the respective work packages.

Activities and results

Ad 1: Task 5.3.1 Transdisciplinary data collection and analyses and mutual learning (work period: months 0-48).

Task 5.3.1 Part 1

Partner: Matthias Görgens and Karin Fricke, Obstbauversuchsringes Des Alten Landes - OVR

The objectives of this task were:

- to establish a dynamic network involving research, extension and growers with mutual learning effects for the persons involved;
- to set up a fruit farm management database, and to analyze data;
- to compare the profitability of integrated production and organic farming;
- to identify key success factors;
- to encourage the participation of growers SMEs drawing on already existing networks and broadening the impact of the task.

Activity

The orchard management of four integrated and four organic farms was observed in the lower Elbe region during three years. The farmers got the opportunity to get detailed data of the yield and of the costs of their own orchards. From the advisor point of view, weak points of the farm management could be pointed out and improved to get the optimal fruit quality and yield and, thus, to increase the rate of return. Nowadays, it is very important for fruit growers to get very exact data of their orchards. During this project, additional data of the organic apple orchards could be determined.

Results

The results of Clever & Görgens (2006), namely that the yield of organic orchards was on average 30% lower than the one of integrated orchards, could be confirmed. In spite of lower yields and higher losses during storage, organic farmers get twice the higher producer prices and, therefore, higher proceeds. Beside the yield-depending harvest costs, organic farms have higher labour costs.

In this project, OVR could test an advanced extension model. Growers, advisers and researchers worked closely together in networks. Advisers or researchers visited frequently the parcels to consult the farmers in order to improve the profit of the orchard.

Reached objectives

- Establishment of a dynamic network involving research, extension and growers with mutual learning effects for the persons involved;
- Set up of a fruit farm management database, and analysis of data;
- Comparison of the profitability of integrated production and of organic farming;
- Identification of key success factors;
- Participation of growers SMEs strengthened drawing on already existing networks and broadening the impact of the task.

Task 5.3.1 Part 2

Partner: Daniela Mencarelli Hofmann, Adeline Kilchenmann and Esther Bravin, Agroscope Changins-Wädenswil Research Station ACW

Activity

In Switzerland, farmers increasingly face risks and challenges resulting from a changing environment. To stay in business, they have to search for new strategies which reduce costs and improve productivity. But the questions about which factors are critical and about which strategies work are not easy to answer. Subsequently, we conducted a representative survey (2008) for organic and for IP apple producers.

In the questionnaires, farmers were asked to assess qualitatively:

- The economic situation of their apple production.
- Which factors were particularly responsible for that outcome?
- Those strategies which they have implemented in the last ten years: production, input and technical strategies as well as implemented cooperation forms.

Quantitative information:

- Cultivars per ha.
- Type of output: dessert apples, apples for must and for the industry.
- Range of commercialisation

The sample was composed by:

Farm size	**Agricultural education**
25%: < 10 ha	19% apprenticeship
31%: 10 ≤ ha < 20	27% master certificate
44%: ha ≥ 20	22% university degree
Main production	**Average age = 49**
30%: Apples	40% are fifty or older
20%: Apples and pears	
10%: Other fruits	**Average work experience =**
40%: Mixed farms	25 years – only 6 of them have less than 15 years

IP apple farming

Farm size	**Agricultural education**
53%: < 20 ha	30% apprenticeship
34%: 20 ≤ ha < 40	30% master certificate
13%: ha ≥ 40	15% university degree
Main production	**Average age = 48**
21%: Apples	40% are fifty or older
24%: Apples and pears	
5%: Other fruits	**Average work experience =**
50%: Mixed farms	24 years – only 15% of them have less than 15 years of work experience

The data analysis was divided into two parts:

– Major factors and selected strategies as the famers had evaluated them ;
– Regression analysis - Logit model to evaluate the statistical significance of :
  - Factors and strategies
  - Farm size and apple orchard size
  - Region
  - Some social variables

Results

The first results show the farmers' evaluation of the key factors. The question is "Which factors are in your opinion important for success?"

Factors	Organic	IP
Commercialisation	76%	26%
Tree age and condition	38%	25%
Apple quality	**27%**	**25%**
Production costs	19%	14%
Yield	14%	14%
Investment	19%	9%
Work organisation	14%	15%
Employees' expertise	5%	7%

The results of the "success" model for organic production are:

- The variable "output" of dessert apples (kg/ha) explains the difference between the successful and less successful group (p-value = 0.006) (Nagelkerke R^2 =0.334).

- Fruit quality and all other factors are not significant.
- The same holds true for farm and orchard size (p-values = 0.360 and 0.425 respectively).
- All "spatial & social variables", as well as the variable "main production" are not significant.
- 74% of the model cases are correctly identified.

The results of the "success" model: IP productions are:

- The factor "production costs" is significant (p-value = 0.005) and its coefficient is negative.
- The variable "orchard size" is significant, meaning that a positive relationship exists between "success" and orchard size (p-value = 0.001).
- The variable "region" (p-value = 0.009) is significant.
- Direct commercialisation has a positive influence on success (p-value = 0.011), while selling to the distributive trades causes a negative effect (p-value = 0.005).
- Fruit quality and all other factors are not significant.

The model explains 44.1% of the variance (Nagelkerke R^2 =0.441); Model cases = 72% correctly identified.

In the survey farmers also evaluated the technique. The question is "Which strategies are in your opinion effective?"

Technique	Organic	IP
Several harvest passages	**81%**	**70%**
Confusion technique	**67%**	**45%**
Binding and forming process	**61%**	**60%**
Thinning by hand	**61%**	**76%**
Other strategy	39%	7%
Summer cut	36%	8%
Compost use before planting	36%	22%
Hail netting	**31%**	**31%**
Irrigation at planting	28%	36%
Pheromone case - prognosis	25%	21%
Working platform	22%	50%
Root cut	17%	19%

Result of the "Fruit quality" IP model:

- The variable which mostly contributes to explain differences in the evaluation of "fruit quality" is hail netting (p-value = 0.009).
- None of the "spatial and social variables" but "orchard size" is significant (p-value = 0.028) and the coefficient is positive.
- The model explains 34% of the variance (Nagelkerke R Square = 0.337).
- 74% of the model cases are correctly identified.

Reached objectives

- Establishment of a dynamic network involving research, extension and growers with mutual learning effects for the persons involved;
- Set up a fruit farm management database, respective data analyses;
- Determination of the profitably of integrated and organic farms;
- Identification of key success factors for successful sustainable farm management.

Task 5.3.1 Part 3

Partner: Esther Bravin, Agroscope Changins-Wädenswil Research Station ACW

Activity

We compared data of four main growing apple regions like the North of Germany, the South of France, the Netherlands and some parts of Switzerland. First, we identified and, second, we compared important production-related (e.g. yield and grading results) and economic (working hours and producer prices) dimensions between the above-named countries. Based on the enlightening method of SWOT analysis (S=strength, W=weakness, O=opportunities and T=threats) we developed different strategies for apple growers in these regions to proceed their apple production in a sustainable way.

Results

The chosen countries differ in strengths and weaknesses of the apple production. The Netherlands have high yield and quality but low grower prices. In the North of Germany, the apple quality, the large apple surface per orchard, and the low labour costs are strengths. However, the grower price is low. In France, apple growers have a large surface per orchard, high produced quality, and yield important as strengths. However, apple prices are low and labour costs are high. In Switzerland, the apple price for grower is high. This is the only strengths. Weaknesses of Swiss apple growers are: high labour costs, little apple surface per orchard, and too many cultivars per orchard.

Good=G Medium=M Poor=P	the Netherlands	Germany	France	Switzerland
Yield (t/ha)	S		S	
Prices	W	W	W	S
Quality	S	S		
Surface apple orchard		S	S	W
Cultivar/Orchard	S		S	W
Labor costs		S	W	W

As part of the analysis, we identified the following external factors of importance referring to the socio-economcial context of these growing zones:

Environment – nature, market, policy, consumers, trends, technics developement. The most important difference in the external factors is found between EU countries and Switzerland. Because of actual strict import reglementation for fruits in Switzerland, and because of the possible free trade agrement with EU, policy and market are great threats for the Swiss apple production.

Reached objectives

- SWOT-analysis of main growing apple regions like the North of Germany, the South of France, the Netherlands and some parts of Switzerland based on production key figures of these zones.
- Identification of external factors of importance referring to the socio-economical context of these growing zones.

- The results obtained provide more clarity of the strengths to build on and weaknesses to be addressed for supporting a positive perspective of apple growing in the European regions analysed.

Ad 3: Task 5.3.2 Decision support tool for fruit growers (work period: months 5-48).

Task 5.3.2 Part 1

Partner: Peter Roelofs and Marianne Groot, Wageningen UR/Praktijkonderzoek Plant & Omgeving B.V. WUR-PPO

The objectives of the task were:

- to conceptualize the development of a user-friendly IT-model for simulating economical scenarios at the orchard and variety level;
- to select European growing zones, for which the tools shall be tested and validated;
- to develop a first prototype;
- to verify the model and conduct sensitivity analyses with task 5.3.1 data, to develop risk profiles based on real world data for comparing farm management factors in terms of their impact on financial success if changes are made.

Activity

Innovative techniques were developed in order to create a more ecological sustainable way of fruit growing. To be implemented by fruit growers, these innovations should also be economically sustainable. Therefore, in the ISAFRUIT project an economic simulation model, PROFITFRUIT, was developed. The model calculates returns, gross margin, marginal gross margin, fixed costs as well as labour income of the apple grower.

PROFITFRUIT includes a database containing quantitative data on fruit growing in three European fruit growing regions: North Germany (Jork), Switzerland and The Netherlands. Data include costs, prices, yield and labour demand for apple growing. The model and these data were used to compare (marginal) gross margins for Elstar in these countries.

PROFITFRUIT is used as well to study the economic effects of three different innovations: the ISAFRUIT spraying machine (casa sprayer), the hot water treatment for prevention of storage rot, and the mechanical thinning apparatus. The study includes a sensitivity analysis for the critical factors: investment costs, quality and quantity of the production, storage loss and labour demand.

Results

Based on the input data returns, gross margin and marginal gross margin for apple production (Elstar) in the Netherlands, Germany and Switzerland were compared.

(EUR / ha)	The Netherlands	Germany	Switzerland
Yield x prices	14'932	11'385	14'934
Calculated costs	4'460	2'761	3'869
Gross margin	10'472	8'624	11'065
Marginal gross margin	1'850	2'770	7'793

The analysis of the chosen three different innovations has the following results:

Economically, the casa sprayer has the highest potential. It results in lower costs and, therefore, higher returns. This means that fruit growers might be willing to invest in this technique. However, it is important to keep in mind that the economic effects depend highly on farm size. Hot water treatment leads to higher costs for fruit growers, which will limit the number of growers willing to use such techniques, unless the high labour demand, energy use and costs for antagonists can be reduced. However, if it functions well, it might be interesting for organic apple growers. Mechanical thinning has some perspective. For positive economic effects, the mechanical thinning needs to result in a higher quality of the fruit.

Reached objectives / conclusions

- Development of a concept for the development of a user-friendly IT-model for simulating economical scenarios at the orchard and variety level
- Development of a first prototype
- Test and validation of the model using data from three selected European growing zones.
- Provision of a final version of the model, in four languages (English, German, French and Dutch).
- Sensitivity analyses and model verification with task 5.3.1 data and data from other work packages.
- Risk profiles based on real world data for comparing farm management factors in terms of their impact on success if changes are made.
- Productive interaction with WP 5.1, 5.2 and 4.1 for economical evaluation of ISAFRUIT innovations. Provision of comparative production cost referring to the CASA-sprayer, post harvest hot water treatment of apples and mechanical thinning of apples.
- Based on all analyses made by applying PROFITFRUIT it was concluded that additional costs, labour demand, and high yields of first quality fruit are critical success factors for innovative techniques which are meant to spare the environment.

Task 5.3.2 Part 2

Partner: Esther Bravin, Agroscope Changins-Wädenswil Research Station ACW

Activity

To evaluate the cost-effectiveness of thinning by shading we compared the costs of the following three thinning techniques:

- Thinning by shading;
- Hand thinning;
- Mechanical thinning.

We identified the additional costs of standard organic production due to material, machine, and labour for all three observed techniques. To evaluate the costs for the shading techniques, we measured the additional working and machines hours during the trials. The costs for material are the real costs for the nets (Agroflor, 2007), wire and plastic tags (Thurella, 2009) and were evaluated using praxis data. To evaluate the additional labour hours of the hand thinning techniques and the mechanical thinning, we used on-farm data and experts evaluations. To compare all three different techniques, we assumed that yield and profit (yield x price) for each technique are on the same level ("ceteris paribus"). Hourly wage rate were defined using references of the Swiss Fruit Union (2009), and the hourly rates for machines and tractors were calculated according to Gazzarin and Albisser Vögeli, (2009).

Results

Thinning by shading:

The additional annual costs (amortisation and interests) for the shading nets (incl. nets, wire and plastic tags) are 2'760 €. The specific calculated machine costs (auto-hoist and tractor) are 226 € (23 h/year auto-hoist with a specific rate of 9 €/h (ART, 2009) and 1 h/year tractor with a specific rate of 23 €/h). The annual labour costs to apply the shading net (measured 52 h/year) add up to 910 € (with hourly wage rate of 17.5 €/h).

Hand thinning:

With the evaluation of experts and on farm data, we could estimate that the additional annual hand thinning hours that growers need to achieve satisfying quality and to prevent alternate bearing by using only hand thinning are about 300 h/year. The additional costs for hand thinning add up to 5'250 € (with hourly wage rate of 17.5 €/h).

Mechanical thinning:

With the evaluation of on farm data we could estimate that the additional working and tractor hours each are 2 h/year. The additional costs are 46 € for labour (with hourly wage rate of 23 €/h for the farm manager) and the additional tractor costs are 145 € (with rate of 72.5 €/h for the thinning machines and the tractor).

	Thinning by shading	Hand thinning	Mechanical thinning
Material	2'760 €		
Machine	230 €		145 €
Labour	910 €	5'250 €	46 €
Total additional costs	**3'900 €**	**5'250 €**	**191 €**

The hand thinning has the higher total additional costs. Farmers who want to prevent alternative bearing and improve fruit quality have to invest more labour hours in the hand thinning with higher additional costs. The thinning by shading is compared to hand thinning less expensive. However, mechanical thinning is the less expensive thinning technique for organic growers, but needs additional quality thinning in later season.

To reduce the additional costs for thinning by shading, it is possible to coordinate between early and late cultivars within an orchard. Thus, material will be saved because shading nets can be used twice within an orchard. With this option, the additional costs for thinning by shading will be reduced to 2’550 € per year and will consequently be about half of the additional costs compared to the hand thinning technique.

Reached objectives / conclusions

- Productive interaction with WP 5.2 for evaluating an ISAFRUIT innovation (thinning by shading method).
- Provision of a model calculation the assessment of the comparative production cost at the orchard level for orchard managements based on technological innovations.
- Provision of comparative production cost referring to the thinning by shading method (ISAFRUIT innovation).
- The PROFITFRUIT-model has proven useful for the assessment of the economical impact of technological innovations at the production cost level.

Ad 3: Task 5.3.3 An exemplary case study for change management at the farm level for producing high quality fruit (work period: months 0-48).

Partner: François Warlop, GRAB (F)

The objectives of the task were:

- to help fruit growers to convert with limited risks;
- to provide a user-friendly tool giving relevant answers;
- to identify determining technical, social, economical attributes;
- to organize & categorize those attributes.

Activity

The complexity in organic apple production leads to high technical challenge for apple grower. Facing a lack of advisory availability, we considered, in the frame of ISAFRUIT, the possibility to propose a user-friendly decision tool, based on qualitative modeling and attributes aggregating, to help the fruit grower taking the right decision for producing good fruit quality and increase sustainability of organic and integrated fruit farms.

We used DEXi, a freeware which has been developed in different fields, including agricultural ones. DEXi organizes a complex ‘life’ problem into smaller sub-problems.

During four years of the ISAFRUIT project, the farm reality has been organized into smaller sub-problem, attributes have been identified and a first structure of the model has been achieved. With experts (agronomists, economists, advisors) workshops in a regional and national context the first structure has been organized.

A first structure of the model had been developed and is available. The decision tree like architecture of the model has been discussed in several meetings with different experts. Some lessons learned from this process are:

- It was rather difficult to work with regular meetings at the European scale, since technical, economical priorities were significantly different, and priorities were, therefore, depending on national specific bottlenecks;
- It was also difficult to organize these physical meetings without devoted funds: when writing the ISAFRUIT proposal, these meetings were unfortunately not included in the planning;
- The expert group should be formed in the very beginning with the best representation of interests, and should not change in the following steps of the process to maintain consistency in the discussion and outputs envisaged.

Results

Most advanced parts of the model were discussed and weighed thanks to decision rules and providing a hierarchy to the attributes.

Reached objectives / conclusions

- Identifying and determining technical, social, economical attributes of a sustainable fruit farm management.
- Identifying “smaller” sub-attributes and organizing them in a hierarchical way.
- A user-friendly, IT-based decision tool is available, based on attributes and sub-attributes addressing problems and sub-problems of orchard management, for supporting fruit growers to take the right decision for producing good fruit quality and increase sustainability of organic and integrated fruit farms.
- The model is pre-validated in expert discussions but requires further verification and validation. A tool is available for supporting farmers in making decisions for converting their fruit farms from the integrated to the organic system or vice versa.

Additional activity of WP 5.3 on demand of the ISAFRUIT Management Committee

PROFITFRUIT delivered further outputs. The Management Committee (MC) decided at its last meeting at Radwjik/Wageningen (NL) on 22/23 March 2010, that a study was needed for comparing fruit prices paid to farmers at the farm gate with the fruit prices to be paid by consumers at the point of sale. The MC had the impression that the efforts made by fruit farms for producing fruit in a sustainable way are not rewarded to the necessary extent by the actual pricing and marketing systems. The hypothesis was that even if farms make efforts to increase sustainability which in fact increases production cost, this effort is not rewarded by prices at the farm gate, while prices at the point of sale tend to increase.

The MC mandated work package 5.3 (PROFITFRUIT) to deliver such a study, particularly partner 2 (ACW).

The following questions were at the origin of the study:

- Which level have producer price, acquisition price and consumer price for apples in selected EU countries?
- How did prices develop in the last twenty years?
- What is the price difference between the selected countries?

The study had the following objectives:

- Collect producer price, acquisition price and consumer price for apples in selected EU countries for the last 20 years.
- Evaluate differences and development

Ever if few countries were able to deliver the necessary data for such a study, an interesting document "**Report ISAFRUIT-SURVEY apple prices Europe"** was delivered by 29 October 2010 and published by decision of the MC on the ISAFRUIT website.

Reached objectives / conclusions

- The producer price makes in Switzerland, Spain and Italy between 15% and 25% of the consumer price. In 2009 the part of producer price was lower than other years. However four years are not enough to make a significant evaluation. In Poland the producer price makes up between 49 and 57%.
- The producer prices and margins in Spain and Italy show similarities.
- The producer price for dessert apples is in Poland on a comparable level as in Italy and Spain. However the distribution and retailer margin are lower. The producer prices for dessert apples in Switzerland are higher than in the EU-countries. The proportion of producer prices on the consumer prices are the same as in Spain and Italy.

More information about producer prices in EU-countries is included in following document of the European commission:
http://ec.europa.eu/agriculture/markets/fruitveg/publi/statistics/apple_121009.pdf

Exploitable innovation and knowledge of Pillar 5

Pillar 5 delivered a high publication and presentation output (see respective listings). The nature of most of the work performed implicated that the knowledge generated is publically available and of use for practical application even if rarely commercially exploitable. However, outputs of Pillar 5 were generally screened by specialists from Pillar 7 for nevertheless identifying possible candidates for patenting. Some of the results produced could be successfully patented, others resulted to be "near-market" while they are publically available i.e. a patenting and commercialisation was not possible for these outputs due to several reasons. These outputs are herewith reported.

Exploitable knowledge and its use WP 5.1 (ECOFRUIT)

Exploitable Knowledge (description)	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use	Patents or other IPR protection	Owner & Other Partner(s) involved
Independent and individual air flow adjustment in orchard sprayer	EDAS sprayer	Crop protection in fruit growing	2008-2010	1. European Patent applic. 2. Polish Patent applic.	RIPF
Software for control of GPS navigated sprayer	EDAS sprayer	Crop protection in fruit growing	2008-2010	Polish Patent application	RIPF
Software for the estimation of nutrient uptake by apple and peach trees	Programme	Orchard management	open	Open	UNIBO and CSIC

Exploitable knowledge and its use WP 5.2 (SMARTFRUIT)

Exploitable Knowledge (description)	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use	Patents or other IPR protection	Owner & Other Partner(s) involved
Apple fruit thinning by shading	Protocol	Extension, fruit-growers	2011	-	ACW (Partner 2), UNIBO (Partner 6)
Specific primers for real-time PCR quantification of allergen transcripts	Published sequences	Research	-	-	UNIPD (Partner 33)
30k apple microarray	Microarray slides	Research	-	-	UNIPD (Partner 33)
Abscission potential markers	Nucleotide sequences	Research	-	-	UNIPD (Partner 33)

Exploitable knowledge and its use WP 5.3 (PROFITFRUIT)

Exploitable Knowledge (description)	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use	Patents or other IPR protection	Owner & Other Partner(s) involved
Excel based model to calculate returns, gross margin, marginal gross margin, fixed costs as well as labour income apple grower.	PROFITFRUIT	Fruit Farm management	2011	No	WUR-PPO
User-friendly decision tool, based on qualitative modelling and attributes aggregating, to help the fruit growers to take decision to switch to organic.	DEXI Model for apple growers to switch to organic	orchard management	2012	No	GRAB

Pillar 6 – Genetics of fruit quality and Implementation of better fruits cultivars

General objectives of Pillar 6

This Pillar aims to increase fruit quality and safety, better availability and raise consumer consciousness by improving our understanding of the genetic bases of quality, by increasing acceptability of GMOs and implementing new high quality varieties meeting the preference of consumers. This pillar is organized around 3 WPs:

WP 6.1 *GENFRUIT.* The objective is to set the genetic basis of fruit quality by mapping the major genes and QTLs involved.

WP 6.2 *TRANSFRUIT* aims to develop safer, more acceptable transformation methods.

WP 6.3 *NEWFRUIT* will evaluate how new, high quality varieties can increase fruit consumption.

Identification of genes that play a major role in fruit quality will facilitate breeding of new cultivars in the future. The project undertook basic research on cutting-edge breeding tools for improving apples, peaches and apricots, which will position Europe in the forefront of genomic research, particularly as regards on non- and low-allergenic traits.

Implementation of better fruit cultivars will also line up with higher competitiveness of the whole European fruit sector. Breeding new cultivars will not be possible within the period of this project, but **basic research on discovery of quality genes** that can be exploited in future breeding with improved methodology will increase the possibilities to meet the consumer demand. In fact, one of the current critical bottlenecks preventing from increased fruit consumption in Europe general public concern about transgenic fruits, despite the expected added value of transgenic well-known varieties with improvement of quality (organoleptic or nutritional) traits, reduction of allergenicity or increased resistance to diseases. The biotechnological sector will be strengthened by the development of fine-tuned marker-free transformation technologies. Cultivars without allergens may make it possible for the 5% of the population with fruit allergy to eat fruit, which will result in a better life and increase the consumption.

The following, non exhaustive, **Breakthroughs/Innovations** contribute to the overall thematic and ISAFRUIT objectives:

- Knowledge on the genetic basis of fruit quality, including low allergy risk;
- Molecular markers for the most critical fruit quality traits facilitating the breeding of new cultivars meeting the preferences of consumers;
- New methods to produce genetically modified fruit varieties;

Summary

Genetics of fruit quality was studied in **Pillar 6**, with special focus on quality genes in apples, apricots and peaches by finding and mapping specific quality genes for use in breeding programmes (**GENFRUIT**). Regions around specific QTLs have been saturated with markers and several candidate genes were mapped in four populations – two of peach and 2 of apricot. Genetic markers of the different components of apple fruit flesh texture were found and QTLs were identified. New functional alleles for fruit quality have been identified in apple founders not studied before. The analysis of fruit allergen in apple revealed the complexity of the allergic response but our understanding on the genetic basis of apple allergenicity was greatly improved; 24 regions show clusters of QTLs or single QTL co-localizing with known allergen

genes and regulating genes show to be important. Having more precise knowledge on the allergen composition of wider set of cultivars would give perspectives and guidance to breeders in selecting their breeding parents and seedlings, and to apple allergic citizens in tracing apples that are good to them. Concerning the ester contents of a collection of apple cultivars, a marker was found for the selection of aroma. An unstructured collection of peach cultivars was examined for the full sequence of 2 enzymes that could be involved in fruit firmness and sugar contents of the fruit. Marker-assisted Breeding (MAB) was performed on 3 apple progenies, using genetic information (SSR markers) from 7-9 genomic regions corresponding to QTLs for fruit quality and scab resistance. In summary, new genes and QTLs for fruit quality have been identified in apple, peach and apricot, and markers suitable for their selection are available for marker assisted breeding in these crops, which will facilitate the release of improved cultivars. A new generation of fruit breeders could use these new selection procedures to be more efficient in responding high level challenges as pesticide ban and climate change. It is the main aim of the new European project FruitBreedomics (FP7).

Among the different studies for avoiding the presence of antibiotic resistance genes in the final product (**TRANSFRUIT**), a promising approach was the development of a marker–free plant production system on apple and pear. This system relies on the physical removal of the antibiotic resistance gene after selection by recombination. This was achieved by activating the required recombinase activity at the desired point in time. The first marker-free pear transgenic plants were obtained using this system and the conditions for optimal production of marker-free apple were determined. Promising results were obtained in order to avoid the use of viral promoters and to control accurately transgene expression. The characterization of a new apple fruit specific promoter, which is able to regulate transgene expression in a targeted way, will be achieved and this new tool will be available for application in genetic transformation. Stability of expression of transgenes and evaluation of associated risks are the final objectives of TRANSFRUIT. A field trial of 7 transgenic apple lines, expressing the barley hordothionin gene conferring scab resistance, were planted several years ago. Both expression of the gene as well as the resistance trait can be considered stable in the field over many years. So, geneticists and breeders could develop safer, more acceptable transformation methods in the coming years.

Consumer preference mapping at European scale on the basis of consumer tests on new apple and peach/nectarine cultivars was performed (**NEWFRUIT**). For the apple tests, descriptive analysis and consumer preference mapping has been completed: 3 main axes have been retained ; interpretation of these 3 main components corresponds to ‘sweetness’ and ‘fruit odour’ for the first component, ‘acidity’ and ‘firmness’ for the second one and ‘juiciness’ and ‘crocanticity’ for the third one. The results indicate that the European consumers can be grouped in 6 clusters depending of the varieties that they prefer the most. For the peach tests, the analysis of the results stated very clearly that the group of people liking sweet peaches/nectarines is the larger in all countries. So, the breeders have to take into account specific European population segments for establishing their breeding programmes; producers have to consider more flavour besides colour and size; shippers/marketers have to separate cultivars not only by colour and size but also by major flavour classes and to communicate also flavour characteristics. Increase fruit consumption can be achieved when right cultivars are given to the right consumers.

All the tasks foreseen in the Technical Annex were completed. A total number of 108 Deliverables was produced: 38 for WP6.1, 65 for WP6.2 and 5 for WP6.3.

Pillar 6 Genetics of fruit quality and implementation of better fruit cultivars

Pillar 6 aims to increase fruit quality and safety, better availability and raise consumer consciousness by improving our understanding of the genetic bases of quality, by increasing acceptability of GMOs and implementing new high quality varieties meeting the preferences of the consumers.

Relationships between scientific, technological objectives and Pillar 6 WP's

Objectives	**Work package**	**Relationship**
A. Increase fruit quality	6.1	Knowledge on the genetic basis of fruit quality has been substantially increased: identification and mapping of candidate genes and QTL analysis of fruit quality characters in peach, apricot and apple, identification and characterization of cell wall components and QTL analysis of apple texture
	6.3	Consumer tests of new high quality apple and peach/nectarine standard varieties were performed across different European regions – also for organically grown disease and pest resistant new apple cultivars. A sensory profile description of all varieties was developed.
B. Increase fruit safety	6.1	Knowledge on genomic regions involved in allergy to apple has been substantially increased: several specific *Mal d 1*, *Mal d 2* and *Mal d 4* genes seem to be involved, Moreover, four major regulating regions are identified.
	6.2	The development of several strategies aiming at minimizing risks and increasing acceptability of transgenic fruits is under way by: 1) avoiding the presence of antibiotic resistance genes, 2) targeting the expression of the transgene, 3) reducing the presence of non plant DNA sequences in transgenic apple. Stability of expression of transgenes and evaluation of associated risks are the final objectives.
C. More convenience	-	
D. Better availability	6.1	The increased knowledge on the genetic basis of fruit quality is already applied in developing Marker Assisted Breeding on apple progenies segregating for quality and resistance attributes. A new generation of fruit breeders could use these new selection procedures to be more efficient in responding high level challenges as pesticide ban and climate change. On the longer term, the increased knowledge on allergy involved apple genes may contribute to the coming available of new allergy friendly cultivars. On the shorter term it may support apple allergic citizens in identifying the one or few specific apple cultivars they can consume without having Oral Allergy Syndrome (OAS) symptoms. which thus would allow apple consumption to otherwise apple allergic individuals. This will require interactions between apple geneticists, clinicians and dietists, a unique interaction which may require some strong incentives to become established.
	6.3	Consumer preference mapping at European scale was completed on the basis of consumer tests on new apple and peach/nectarine varieties. European consumers can be grouped in different clusters and this knowledge could help for developing desired varietal type close to the consumers.
E. Raise consciousness	6.2	All partners have been active in disseminating information about innovative gene transfer methodologies and about the research on fruit GMOs in general.
F. Lower Fruit price	-	

List of participants in Pillar 6 and their engagement in WPs and Tasks.

c	Participant name	Short name	Coun-try	WP engagement	Tasks responsibility
SCIENTIFIC PARTICIPANTS					
2	Agroscope ACW Wadenswil	ACW	CH	6.3	6.3.3
4	Institut de Recerca i Tecnologia Agroalimentŕries	IRTA	E	6.1 & 6.3	6.1.1÷6.3.1
5	Institut National de la Recherche Agronomique	INRA	F	6.1 – 6.2	6.1.1 – 6.1.2 – 6.1.3 – 6.1.6 – 6.2.2
	INRAGenHort, Angers-Nantes			6.1 & 6.2	6.1.2÷ 6.1.6 – 6.2.2
	INRA UREF, Bordeaux			6.1	6.1.3 -
	INRA Avignon			6.1	6.1.1 -
6	University of Bologna	UNIBO	I	6.1 & 6.2 6.3	6.1.3 – 6.2.4 -
8	Wageningen UR- Applied Plant Research	WUR-PPO	NL	6.3	6.3.2
9	Wageningen UR- Plant Research International	WUR-PRI	NL	6.1 & 6.2	6.1.4 – 6.1.5 – 6.2.3
10	Wageningen UR- Agricultural Economics Research Institute	WUR-LEI	NL	6.3	
11	Research Institute of Pomology and Floriculture	RIPF	PL	6.3	
14	Research institute of Organic Farming	FiBL	CH	6.3	6.3.4
16	Bundesanstalt für Züchtungsforschung an Kulturpflanzen	BAZ	D	6.1 & 6.2	6.2.1
20	Obstbau Versuchring Jork	OVR	D	6.3	
27	Centre Technique Interprofessionnel des Fruits et Légumes	Ctifl	F	6.3	
30	Istituto Sperimentale Frutticoltura	ISF-FO	I	6.3	6.3.2
31	Land-und-Forstw. Versuchszentrum Laimburg	LAIMB	I	6.3	
42	Hort Research – New Zealand – **Third country**	HR	**NZ**	6.1	
SME's PARTICIPANTS					
47	NOVADI, Tree Nursery Consortium	NOVADI	F	6.3	
49	CIV Consorzio Italiano Vivaisti	CIV	I	6.3	

Comments on participants and relationships between participants

In Pillar 6, eight research Institutions (Institutes and Universities) participated; they represented 7 different European countries. As a third country, New Zealand was associated for the knowledge of HortResearch on fruit quality studies (WP6.1). In addition, 6 experimental institutes were participating in WP6.3 and took diverse responsibilities for collecting fruits, consumer testing involving 5000 European consumers, data interpretation and dissemination of the knowledge inside their network. Also 2 SMEs (fruit nurserymen) participated to WP6.3 for their professional abilities on apple and peach varieties, on technical production, marketing and in general their knowledge of the fruit chain.

The interactions between the participants were very strong inside WP6.1 and WP6.2. WP6.3 had a specific work not really related with the 2 other WPs. During the General Assemblies, all WPs had the possibility to learn about the work of the other WPs and Pillars.

Pillar 6 was interacting mostly through 'allergenicity': through i) joined WP2.3 and WP6.1 efforts in improving understanding on the identity of the allergens involved in apple allergy by sharing clinical WP2.3 and genetic WP6.1 data; ii) through delivering content for the allergy website to WP7.4, iii) through a workshop during the 3rd General Assembly (November 2008) which was organized with contributions from WP6.1 (genetics basis of allergenicity), WP6.2 (allergenicity of GMO tissues), WP5.2 (effect of culture conditions), WP2.3 (RNAi-GMO silenced *Mal d1* fruit, influence of the chain, identification of new allergy friendly cultivars, need for standardization of Skin Prik Tests in research and clinical diagnosis), marketing cultivars as hypo-allergenic. This workshop was a good step for giving comprehensive results on allergenicity at the end of the project, during the ISAFRUIT Symposium in Lisbon (August 2010) in a special session on 'Fruits and Allergies' to which WP2, WP6, and WP7 as well as an external clinical expert.

The SPT data used to further elucidate the genetics of allergenicity were generated in WP2.3 by the Medical University of Groningen and were genetically interpreted within WP6.1, combining clinical data with molecular markers of the tested apple progenies through an association study. In the negotiation of the ISAFRUIT project, the proposal for this quite unique type of collaboration and division of tasks between Pillar 2 and Pillar 6 was understood as a way of double budgeting, and led to budget reduction for the ISAFRUIT project as a whole.

The establishment of collaborative research between plant geneticists (Pillar 6) and clinicians (Pillar 2), proved to be very fruitful. For the future, such collaborations are probably critical for making some big steps forward in the field of food allergy. As currently medical and plant research are quite separate worlds, such collaborations are something to be encouraged in forthcoming project calls.

WP6.1 Study of the genetics of fruit quality and health properties (GENFRUIT)
Leader: 4. IRTA Cabrils, (Pere Arús); other Partners: 5. INRA Angers(An), Nantes(N), Bordeaux(B), Avignon(Av); 9. WUR-PRI ; 6. UNIBO; 16. BAZ ; 42. HR. (work period: 1-57 months).

The objectives of this WP are :

- Further marker saturation of peach and apricot segregating populations and candidate gene mapping in the Prunus genome
- Development of novel techniques for fruit texture determination and application of the best techniques for QTL analysis in apple
- Determination of the main parameters of fruit quality and health in segregating populations and genetic dissection of the quantitative traits into QTLs
- Further understanding of the genes involved on allergenicity in apple and first characterization of these genes in peach
- Search for allelic diversity of candidate genes for fruit quality in apple and peach
- Use of the available genomic information in the selection of improved apples.

The overall objective of **GENFRUIT** was to increase the understanding of the inheritance of fruit quality traits in apple (*Malus x domestica*), peach (*Prunus persica*) and apricot (*Prunus* *armeniaca*), and to apply the new knowledge, as well as the pre-existing information, for launching the creation of improved varieties of these species.

The first step in the genetic analysis of fruit quality characters was to construct saturated **genetic maps** in four *Prunus* (stone fruit) segregating progenies: one of peach x peach, one of peach x *P. davidiana*, and two of apricot x apricot. At least 43 characters related with fruit quality were analyzed in each of these populations during two years. Marker-phenotype joint analysis allowed the detection of a large number (344) of genomic regions (59-121 depending on the progeny) where genes affecting fruit quality are located (quantitative-trait loci or QTLs). All these QTLs were consistent the two years assayed and approximately half of them (159 of 344) explained a high proportion of the variability for the character (more than 20% at least one of the years studied). Most QTLs were found only in one population, but 12 and 7 were common between peach and apricot progenies, respectively. Fourteen common QTLs were found in at least one population of peach and one of apricot, one of them, affecting the quinase content of the fruit, being common to all four populations. A collection of 169 candidate genes (CGs) involved in the metabolic processes related with fruit quality aspects were selected. They were mapped in the reference map (Texas x Earlygold) using a fast mapping procedure (bin-mapping); 131 of them have been mapped, using only 144 of the candidates. This is an unusually high rate of success in mapping which is attributed to the high level of polymorphism of this population. For regions chosen in the maps of these four progenies to contain interesting QTLs or QTL-CG co-locations, a total of 28 CGs were mapped in at least one of the four populations. The QTL-CG co-locations were often confirmed which identify CGs that may be directly responsible or located in the neighbourhood of genes responsible for the fruit characters measured. These results provide key information on markers (including CGs) that can be used for marker-assisted selection of fruit quality in peach and apricot breeding programs.

Fruit flesh texture. One particular fruit quality trait requiring improvement and better control concerns texture as its variability conditions fruit acceptability by consumer, post-harvest itineraries and processing ability by industries. This key character for consumer acceptance, has been analysed using three apple cultivars with contrasting texture properties; 150 seedlings of a progeny have been phenotyped and chemotyped for key characteristics of fruit quality. The genetic map and the search for texture related QTLs is completed. Some highlights of the results are:

- good correlations between instrumental and sensory texture parameters, and between instrumental texture and histological measures
- absence of correlations between cell wall chemistry and instrumental and sensory texture variations
- co-location of QTLs for instrumental, and sensory variables with histological and/or cell wall polysaccharide composition or structure
- high heritability of all the texture related variables (instrumental, sensory texture, histology, cell wall chemistry).

Analysis of the apricot and peach genotypes with selected methods of flesh texture analysis has been completed. Highlights of the results are:

- great difficulty to transpose methodologies developed to phenotype/chemotype apples to apricots and peaches, the time spent to adapt some protocols prevented deeper analyses.
- large intra-genotype variability in instrumental texture for apricot and large chemical variations in apricot cell walls which suggest that the impact of fruit ripening status is very large and difficult to asses.,

- the first evaluation of the impact of genetics on cell wall chemical variability in peaches provided promising results but needs to be validated on a larger set of fruits and genotypes.

The QTL analysis in apple confirms the location of four regions linked with instrumental and sensory texture parameters. It highlights four new instrumental texture QTLs and identified five new ones for histological parameters.

Nine QTLs for cell wall sugars and hemicelluloses were also detected; four of them co-locate with QTL of instrumental and sensory traits.

At short term, the genetic map will be completed with new markers to precise the positions of the most important QTLs. Analyses will also been carried out on date 1 (harvesting time). So, the stability of the QTLs over the different dates (harvesting and after 2 and 4 months of storage) will be studied.

Results on fruit allergens improved our understanding on the genetic base of allergenicity, delivered tools for genotyping apple cultivars, and tools for mRNA based expression studies on *Mal d 1* genes. Apple can cause allergic reactions because of the presence of four classes of allergens (Mal d 1, Mal d 2, Mal d 3, and Mal d 4), each of which represented by multiple genes. In ISAFRUIT, several new *Mal d 1*, *Mal d 2* and *Mal d 4* genes were identified through mapping approaches as through the sequencing of two BAC clones for the *Mal d 1* cluster of linkage group 16. In peach, a series of allergen genes were identified and mapped for the first time. Comparison between the positions of these genes in the peach and apple genomes substantiated a high macro-synteny conservation between apple and peach. Knowledge on the genetic diversity of *Mal d 1* was improved through assessment of the allelic constitution of *Mal d 1* genes for two low and two high allergenic reference cultivars. Protocols for qPCR based expression studies have been developed for 20 *Mal d 1* genes, as for some genes of the three other apple allergens. Application gave first insights on effects of culture conditions and genetic modification on the activity of specific *Mal d* genes: for instance, from comparisons among different tissues and maturity stages of Florina fruit we learned that only part of the *Mal d 1* genes are expressed in fruit, and that the far most of these are more strongly expressed in the skin. Application in WP6.2 showed increased constitutive expression levels of some specific *Mal d 1* genes in GMO constructs with the *Vf* gene for resistance to the major apple disease scab and the constitutively expressed S35 promoter.

To further clarify quality and quantity aspects of allergens, correlation studies were performed between on the one hand Skin Prik Test data and on the other hand i) the allelic constitution of a series of pedigreed cultivars, ii) genome wide marker profiles of two mapping populations iii) expression levels of *Mal d 1* genes in a set of cultivars. Results indicate many regions of the apple genome to be involved in allergenicity. Study participants differed greatly for the allergen genes that correlated with their SPT responses, both with regard to the allergens (Mal d 1, Mal d 2, Mal d 3), iso-allergens as variants. Also regulating genes seem to be important, one of which co-localizes with the *Ma* gene for the EU consumers desiring acidity of apple fruit, thus confounding good taste and low allergenicity in breeding. Both traits can be combined thanks to variation in linkage phase, but decreases overall breeding efficiency, as happens with the addition of any new selection criteria.

Breeding of a common allergy friendly cultivar is complicated by the high number of (iso) allergen genes and individual specificity. The increased knowledge on the wide variability in allergen composition between apple cultivars gives perspectives for matching existing apple cultivars to apple allergic citizens through for instance a “diagnostic reference set of apple cultivars”, hereby exploring individual specificity. In this respect ISAFRUIT may on the short term contribute to increased availability of apple fruit to otherwise apple allergic individuals.

To realize this potential, communication with clinicians, dieticians and house doctors seems critical.

The search for **new functional QTL alleles** was performed on two small and one large segregating apple populations that descent from a new founder, this is from a new source for disease resistance in breeding. Firmness, total sugars, total titrable acidity and starch contents have been instrumentally assessed at harvest, after 10 weeks of cold storage and after 10 weeks cold storage + 10 days at room temperature. For the latter two treatments, sensorial assessments have been made for firmness, crispness, juiciness, aroma, texture, mealiness, overall taste, funny taste and after taste. Moreover, fruit weight, harvest date and crop load was evaluated in the large population. Each population was SSR genotyped for genomic regions known to harbour QTL. Moreover, the two populations were marker-genotyped for 18 candidate genes and the third, large population was genome wide DArT genotyped with 260 informative markers. Marker-phenotype joint analysis allowed the detection of up to 76 QTL for fruit quality in a single population, 64 of which have a desired QTL from the new founder. Five QTL showed intra and two showed inter-locus interactions.

Some highlights of the results are: a) strong QTLs linked to sensory traits were confirmed including firmness, crispness, juiciness, acidity and sweetness, b) other newly identified QTLs were discovered for instrumentally assessed juice content, sensory flesh mealiness, sugar, flesh melting, harvesting date, crop load, fruit weight, after taste, starch content, c) a major QTL for firmness which was putatively identified in just one out of 26 HiDRAS mapping populations could be confirmed, d) instrumental assessment of firmness at 8MM gave the same QTL as assessments on the skin surface, however the difference between these assessments, which is considered to be an indication for the toughness of the fruit, gave two new QTL, an extremely strong QTL (LOD=16) for harvesting date is closely linked to the *Ma* gene for acidy of apple fruit, thus confounding the breeding of late maturing and good storability with the consumer desired acidity. These results on new QTLs support the initiation of Marker Assisted Breeding (MAB) on fruit quality.

Allelic variation at apple CG sequences: Apple fruit volatiles are important secondary metabolites and considerably affect the sensory quality and the economic value of an apple variety. Despite extensive research concerning the analytical and biochemical identification of hundreds of putatively aroma-related volatile compounds in apple such as esters, alcohols, aldehydes and phenylpropenes, the inheritance and molecular-genetic bases of aroma patterns and single components are widely unknown. Therefore, the research on genetic factors involved in apple fruit aroma and taste aimed at the identification, mapping and sequencing of new candidate genes using the increasing available knowledge about apple genomics and metabolomics. Ten new candidate genes were mapped in the apple genome and located on eight different apple chromosomes. For all genes, a co-localization with single QTL or QTL clusters for apple flavour compounds was found. Most interesting were some candidate genes from the LOX (lipoxygenase) pathway resulting in most important key esters such as hexyl acetate or butyl acetate. Four out of the ten candidate genes mapped in tight genetic association with volatile QTLs were selected for an assessment of allelic diversity by partial sequencing. Two additional genes of the sorbitol metabolism that might play a role in total sugar content of the apple fruit were included into the sequencing approach. Allelic diversity of the candidate genes was analyzed in at least 50 apple cultivars present in the *Malus* Gene Bank collection of JKI Dresden. The produced partial sequences of apple candidate genes putatively involved in aroma and sugar metabolism revealed the presence of a considerable allelic variation indicating that these genes have multiple different alleles and that specific allelic variants might be related in future to gene function. One selected candidate gene coding a key enzyme involved in the last step of ester biosynthesis (alcohol acyl-transferase, *MdAAT1*) was more deeply investigated in a large set of 100 apple accessions with available quantitative ester data from GC/MS-based aroma profiling experiments. *MdAAT1* was not only found to be associated with QTLs for different acetate esters with high impact on apple aroma, but also specific SNP-"haplotypes" identified by direct amplicon sequencing were strongly associated with the ester contents. The development of a first functional marker for the trait aroma seems to be possible for apple. A specific SNP marker assay is under development for Marker Assisted Breeding (MAB) which will quickly discriminate apple genotypes into "alcohol apples" or "ester apples". The functional marker developed for this key gene of volatile biosynthesis will also enable a more efficient documentation of biodiversity of aroma patterns in *Malus* gene bank collections, apple varieties and breeding material lifting the selection of suitable genotypes to a new efficiency level. These results constitute a solid basis for additional research on aroma genes, which will finally lead to a more efficient breeding for new, very tasteful apple cultivars which may on its turn increase consumption of apple.

Genotyping for CGs in a peach collection: The partial sequence analysis of thirteen genes previously mapped in the *Prunus* reference map and found to co-locate with the positions of QTLs for fruit quality was obtained in an unstructured sample of 48 peach cultivars. Sequence data identified polymorphism in only three of them. Two were selected for their potential interest as causal of the variability for flesh texture (*Pl1*) and sugar contents (*Sus1*) and were completely sequenced. The polymorphisms found (two in *Pl1* and 20 in *Sus1*) were in strong linkage disequilibrium and only two alleles (haplotypes) were found in each gene. Comparison between phenotypic data and genotypes for these two genes suggested that they were not causal to the variability studied, although given their proximity to the QTLs of interest their sequence could be the basis of new markers that will be assayed for marker assisted selection.

Marker-assisted Breeding (MAB) was performed on 3 apple progenies, using genetic information (SSR markers) from 7-9 genomic regions corresponding to QTLs for fruit quality and scab resistance. A selection index was computed based on SSR genotyping data. By ranking individuals according to their index value, Marker Assisted Selection (MAS) was performed either positively or negatively. For each apple progeny, individuals were positively selected (MAS+), and others were negatively selected (MAS-). In addition, an additional sample of individuals were chosen at random (MASr) for further MAS efficiency evaluation (comparison MAS+ *vs* MASr, and MAS+ *vs* MAS-). These results will be contrasted with field and lab evaluations when phenotyping will be possible in these populations.

A new generation of fruit breeders could use these new selection procedures to be more efficient in responding high level challenges as pesticide ban and climate change. It is the main aim of the new European project FruitBreedomics (FP7).

Redaction of several scientific papers will continue during 2010 and 2011.

WP 6.2 Development of safe and efficient transgenic techniques applicable to sustainable quality-fruit production (TransFruit) Leader: 5 INRA An (Elisabeth Chevreau); other partners : 9. WUR-PRI; 6. UNIBO-DCA; 16. BAZ. (Work period 1-57 months).

The objective of this WP was to propose innovative gene transfer methodologies based on four approaches :

- To avoid the presence of antibiotic resistance genes in transgenic fruit plants
- To control transgene expression in fruit plants with adapted plant promoters
- To explore new tools to reduce the presence of bacterial sequences in transgenic fruit plants

- To evaluate the stability of expression of a transgene along tree life and some associated risks

Apple and pear are the only temperate fruit species on which transformation of well established varieties is now feasible, therefore they were two pertinent species to include in this project ; the results obtained on one of them, will be transferable to the other.

The overall objective of **TRANSFRUIT** was to propose innovative gene transfer strategies which minimize risks and contribute to a better acceptability of transgenic fruits in Europe. One of the critical bottleneck preventing from increased fruit consumption in Europe is the absence of acceptance of transgenic fruits, despite the expected added value of transgenic well-known varieties with improvement of quality (organoleptic and nutritional) traits, reduction of allergenicity or increased resistance to diseases. Methodological work on transgenesis is necessary to provide a proof of concept of transgenic fruit varieties with added value for the consumer.

In order **to avoid the presence of antibiotic resistance genes in the final product**, one of the main objectives was to evaluate the feasibility of alternative transformation methods in apple and pear. Among the different studies, a promising approach was the development of a marker–free plant production system on apple and pear. This system relies on the physical removal of the antibiotic resistance gene after selection by recombination. This was achieved by activating the required recombinase activity at the desired point in time. The first marker-free pear transgenic plants were obtained using this system and the conditions for optimal production of marker-free apple were determined. However, a thorough molecular screening of the obtained marker-free plants appeared to be necessary because chromosomal rearrangements were found in some of the cases where multiple recombination sites were present. The main scientific achievement was the production of marker-free transgenic apple with the stacking of a second transgene, as it further demonstrates the great interest of the recombinase technique. This represents a very important step towards the establishment of a 'cisgenic' approach for these 2 species.

To avoid the use of viral promoters such as the CaMV35S promoter of the cauliflower mosaic virus, and to control accurately transgene expression, the main objective was to further characterize different promoters from apple. As a replacement for the CaMV35S promoter, the *Rubisco* gene promoter has been cloned from the apple genome and combined with the gus reporter gene. The vector has been validated for functioning of this promoter by checking expression in tobacco and apple and it was found that the apple *Rubisco* promoter and terminator could induce expression at levels similar to the CaMV35S promoter. With the aim to develop a fruit-specific promoter, the promoter of the ACC synthase gene from apple was cloned from the apple genome. Specific expression studies of two ACS-1 alleles indicated high expression of ACS-1 in fruit flesh. Several deletions of the promoter were combined with the gus reporter gene and analyzed by transient expression assays in apple fruit tissues. The promising results should be confirmed in stable transformation. In this way the characterization of this apple fruit specific promoter, which is able to regulate transgene expression in a targeted way, will be achieved and this new tool will be available for application in genetic transformation.

To reduce the presence of non plant DNA sequences in transgenic plants, the possibility of systemic acquired gene silencing in apple has been tested. First results obtained on transgenic scions expressing the *gusA* reporter gene grafted onto transgenic *gusA* silencing transmitter rootstocks were promising. The transcription of the transgene was reduced in scions as expected, but no differences were detectable at the enzymatic level. Similar results were obtained for the endogenous *MdANS* gene. The results obtained demonstrated that systemic acquired gene silencing seems to be less effective in apple and not applicable for a practical use at the current state of science.

Stability of expression of transgenes and evaluation of associated risks are the final objectives of TRANSFRUIT. A field trial of 7 transgenic apple lines, each with 40 clonally propagated individuals, of two cultivars (Elstar and Gala), expressing the barley hordothionin gene conferring scab resistance, were planted several years ago. Scab resistance data proved to be consistent for all lines in every year of the four years period and the combined results over the last four years, indicated that 4 of the 6 transgenic clones perform significantly better than the controls in showing less symptom development. Expression of the introduced hordothionin gene was checked again in the fifth year in young leaves and proved still to be there. Therefore, both expression of the gene as well as the resistance trait can be considered stable in the field over many years.

In order to **understand how scab resistance affects the expression of the major apple allergens**, which are also pathogenesis-related proteins (PR proteins), the pattern of expression of the main allergen gene families was studied on a range of ‘Gala’ transgenic (GM) clones with different levels of scab resistance in respect to ‘Gala’ and the scab-resistant cv. ‘Florina’ obtained by traditional breeding. A wide variability of expression levels was observed among and within the main allergen gene families both before and after the challenge with the pathogen *Venturia inaequalis*. The level of constitutive expression of the *Mal d* genes in GM plants is generally lower than in cv. ‘Gala’ and similar to ‘Florina, except for most of the *Mal d 1* genes, that showed a different expression level among GM genotypes. Our results show that among the *Mal d* genes, at least *Mal d 1* (all tested isoforms) and *Mal d 3.01* are strongly modulated after pathogen infection and therefore seems to be involved in the *Vf*-dependent defense response. In addition, because of the different timing and extent of modulation for the different *Mal d 1* isoforms, a diversification in the role of *Mal d 1* genes in the defense response may be postulated. These data were obtained thanks to the development within Isafruit of a new specific allergen-expression tool (WP6.1) that represents the first attempt towards the understanding of the role of *Mal d* genes in relation to scab resistance and other plant biological processes. The observed differences in the level of expression need further studies to clarify if the strongly up-regulated isoforms are also those implicated in apple allergenicity, this in order to further understand if the use of resistant plants will represent a potential risk of increased allergenicity for human health.

All partners have been active in disseminating information about their work in WP6-2, and about their research on fruit GMO in general. Four book chapters and eight scientific papers have been published or are in press, and several others are scheduled in 2011.

WP 6.3 Consumer driven implementation of new standard (non GMO) varieties across different environments (NEWFRUIT) Leader: 4, IRTA (Joan Bonany); other partners: 8. WUR-PPO; 10. WUR-LEI; 2. ACW; 27. CTIFL; 20. OVR; 31. LAIMB; 6. UNIBO-DCA; 30. ISF-FORLI; 11. RIPF; 14. FIBL; 47. NOVADI; 49. CIV. (Work period 3-36 months). The objectives were:

- To evaluate how the availability of conventional new high quality eating apple and peach/nectarine varieties can increase fruit consumption
- To develop preference map based on new apple and peach/nectarine cultivars across different European regions
- To determine the overall acceptance of organically grown new disease and pest resistant apple cultivars

The overall objective of **NEWFRUIT (WP6.3)** was to evaluate how the availability of new high quality eating apple and peach varieties can increase fruit consumption.

Final version of the common protocols that includes details of apple and peach consumer tests were produced. Consumer test of new high standard quality apple and peach/nectarine confronted with commercial varieties across different European regions were performed in January 2007 for apple and August 2007 for peach. Fruit quality analysis was carried out using instrumental analysis at harvest time, after storage (apple), just before consumption (peach).

For apple (11 varieties), a total of 4290 consumers took part in the test in 39 sessions in 20 different locations encompassing 8 countries in Europe. Data from the test was collected from the different partners and a common database for statistical analysis was created for further analysis.

For peach (12 varieties), a total of 500 consumers took part in the test in 10 sessions in 5 different locations encompassing 5 different countries in Europe.

Consumer preference mapping at European scale on the basis of consumer tests on new apple and peach/nectarine cultivars was performed.

For the apple tests, a database with results was prepared. Descriptive analysis and consumer preference mapping has been completed: 3 main axes have been retained ; interpretation of these 3 main components corresponds to 'sweetness' and 'fruit odour' for the first component, 'acidity' and 'firmness' for the second one and 'juiciness' and 'crocanticity' for the third one. The results indicate that the European consumers can be grouped in 6 clusters depending of the varieties that they prefer the most.

For the peach tests, the analysis of the results stated very clearly that the group of people liking sweet peaches/nectarines is the larger in all countries.

Consumer tests of organically grown disease and pest resistant new apple cultivars were also performed. The consumer tests to compare 3 pairs of 'organic' (scab resistant) and conventional non scab-resistant cultivars were carried out in professional sensory laboratories with panels that were chosen to be highly representative of Switzerland and Poland populations. Consumers noted that they would increase organic fruit consumption if they were better informed on organic production.

NEWFRUIT has already produced important results

A comprehensive conclusion was already addressed to the Commission in the 2009 report.

Scientific and technical papers are in progress and will be published during 2010 and 2011.

NEWFRUIT (WP6.3) was interacting with WP1.2 about consumer preferences.

Innovation ISAFRUIT WP6.1 on allergenicity

1. Primers and protocols for mRNA expression studies for *Mal d* allergen genes in apple

Responsible partners & researchers:

Partner 6: Dr. G. Pagliarani, Dr. S. Tartarini, Dr. L. Corelli, Università di Bologna, Italy

Partner 9 : Dr W.E. van de Weg and Dr P. Arens, Plant Research International, Wageningen-UR, The Netherlands; Partner 33 Dr. A. Botton, Dr. B. Ruperti and Dr. A Ramina

Expected Impact: 1) Allows identification of measurements that increase availability of low allergenic apple fruit to consumers, and 2) new directions in plant as well as clinical research, and 3) clarification on the role of the different *Mal d 1* genes on allergy.

Short description:

Mal d 1 is considered as the major allergen in apple. Mal d 1 proteins can be produced by at least 22 different but highly similar *Mal d 1* genes. To date tools lack to easily distinguish these proteins and genes, due to which the relevance of individual genes could not be clarified. Isafruit developed such a tool, namely gene specific primers for 20 *Mal d 1* genes. These primers are designed for application in expression studies through quantitative PCR, protocols for which have also been developed and validated.

This tool allows monitoring of cultivation, storage and transport effects on the expression of *Mal d 1* genes and thereby on the build up of allergen content. They can thus support the produce of allergy friendly fruit. Being able to distinguish individual genes, it also contributes to clarify the *Mal d 1* genes that are involved in allergenicity, even at a tissue specific level (skin, flesh, core). Knowledge on this will be useful for the identification of existing allergy friendly cultivars as to the directed breeding of new cultivars. Finally, this tool supports research on the effect of GM on allergen content.

This tool could be developed thanks to 1) ISAFRUIT research on the genomic organization of *Mal d 1* genes in apple (D6.1.38) which resulted in the identification of two new genes as in knowledge on their promoter regions, as to 2) Isafruit research on the allelic diversity of *Mal d 1* genes (D6.1.5, D6.1.16) which allowed the identification of the required gene specific conserved regions required, as to t3) he Isafruit initiated collaboration between the University of Bologna and Plant Research International Wageningen.

Also for Mal d 2, Mal d 3 and Mal d 4 some gene specific primers have been tested and applied (partly within WP6 an partly within WP5) which allows to study their potential contribution to allergenicity through knpwledge on their expression in apple fruit. This is especially valuable as ISAFRUIT research gave indications on Mal d 2 and Mal d 4 being more involved in allergenicity as currently thought.

Scientific impact

PhD thesis of Dr. G. Pagliarini. Several scientific papers are in preparation.

First results on application:

- Comparison of expression levels of different tissues and maturity stages of Florina fruit learned that only part of the Mal d 1 genes are expressed in fruit, and that the far most of these are more strongly expressed in the skin.
- Assessing expression studies on a few cultivars and correlating these data with previously reported Skin Prik Test data suggested an association between Skin Prik Test response and the expression level of four specific Mal d 1 genes.
- Florina apples challenged with the fungal pathogen Venturia inaequalis show enhanced expression of a specific Mal d 1 gene, indicating that this gene may be involved in an effective resistance response. This same gene was previously proposed to be associated with allergenicity, due to which resistance and low allergenicity may be conflicting items for this specific Mal d 1 gene.
- GM-Gala (enriched with a gene for resistance to scab) shows on overall enhanced constitutive expression of Mal d 1 genes, to which some specific Mal d 1 genes mostly contributed, and a reduced expression of one specific Mal d 1 gene.

- Shading, elevation, and storage significantly affected the transcription of genes for all four apple allergens, whereas water stress had only a slight influence on the expression of the studied Mal d 4 genes
- These initial findings of the University of Bologna have to be further elaborated but already demonstrate that they allow new approaches in allergy research.

Coordination with other ISAFRUIT allergy research

WP2.3 showed that RNAi silencing of *Mal d 1.02* genes is effective in reducing general level of allergenicity of a cultivar, as to make a cultivar hypo-allergenic for several individuals. Fruit tissue of this material has been sampled at stored at -80C in view of future expression studies, which could clarify which Mal d 1 genes are still expressed in fruit, still causing OAS symptoms with certain individuals.

WP2.3 also studied effects of harvest date and storage conditions on allergenicity for two allergy friendly cultivars (Santana and Elise) through oral provocations. This study can gain depth by associating the oral provocation data with expression levels of the individual *Mal d 1* genes. To enable future performance of such studies, multiple fruit samples of all treatments have and been collected and are currently stored at -80C (see D2.3.5).

Scientific impact:

- Pagliarani G, Paris R, Tartarini S, Sansavini S (2009) Cloning and expressing of the major allergen genes in apple fruit. J Hort Sci Biotech ISAFRUIT special issue 176-181.
- Pagliarani et al. Scientific paper on the full set of *Mal d 1* genes is scheduled for submission I 2011.

Through Pillar 5:

- Botton A, Lezzer P, Dorigoni A, Barcaccia G, Ruperti B, Ramina A (2008) genetic and environmental factors affecting allergen related gene expression in apple fruit (*Malus domestica* L. Borkh) Agr Food Chem 56:6707-6716
- Botton A,Lezzer P, Dorigoni P, Barcaccia G,Ruperti B, Ramina A (2009) Genetic and Environmental Factors Affecting Allergen-Related Gene Expression in Apple Fruit (Malus domestica L. Borkh) J Hort Sci Biotech ISAFRUIT special issue 182-187.

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2. Evidence that *Mal d 1* isoforms and variants differ in allergenicity.

Responsible partners & researchers:

Partner 6: Dr. G. Pagliarani, Dr. S. Tartarini, Dr. L. Corelli, Università di Bologna, Italy

Partner 9 : Dr W.E. van de Weg and Dr P. Arens, Plant Research International, Wageningen-UR, The Netherlands

Expected Impact: 1) increase in efficiency in allergy research, and 2) more frequent interactions and collaborations between plant and clinical scientists.

Short description:

In clinical research on the relation between Mal d 1 and apple allergy, to date total amount of Mal d 1 is considered. However, Isafruit results indicate that Mal d 1 proteins differ in their allergenicity, which finding indicates the need for a re-orientation on apple allergy research.

The indications are based on three Isafruit studies. Firstly, an association was found between the genetic *Mal d 1* constitution of a series of apple cultivars and their allergenicity as measured by Skin Prik Tests on participants with a mild Oral Allergy Syndrome. This association indicates that some specific iso-allergens are involved in allergy, and that also the different protein variants of such an iso-allergen differ in allergenicity. As this association also indicated allele dosage effects, also the produced amounts of these variants seems relevant.

Secondly, a study on a few cultivars in which expression levels of individual *Mal d 1* genes were correlated with SPT responses indicated a correlation for four *Mal d 1* genes whereby a moderately expressed gene seemed to have a larger impact then two highly expressed *Mal d 1* genes.

Thirdly, individuals differed for the *Mal d 1* genes for which they gave co-localizing QTL for SPT responses in association studies on two mapping populations.

Scientific impact

- Scientific publication: Gao Z, Van de Weg WE, Matos CI, Arens P, Bolhaar STHP, Knulst AC, Li Y, Hoffmann-Sommergruber K, Gilissen LJWJ (2008). Assessment of allelic diversity in intron-containing Mal d 1 genes and their association to apple allergenicity. BMC Plant Biology. http://www.biomedcentral.com/1471-2229/8/116
- Chapter in the PhD-Thesis of Dr. G Pagliarani to be elaborated to a paper for a peer reviewed journal.

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3. Evidence that Mal d 2 and Mal d 4 are important allergens in apple allergy

Responsible partners & researchers:

Partner 9 : Dr WE van de Weg and Dr P Arens, Plant Research International, Wageningen-UR, NL

Partner 35: BJ Vlieg-Boerstra and Dr AEJ Dubois, University Medical Centre Groningen, NL

Short description:

In research on apple allergy, the allergen Mal d 1 is generally considered as the major apple allergen. Association studies between SPT of 24 individuals on two segregating mapping populations (from WP2.3) and molecular marker data gave more frequently QTL that co-localize with *Mal d 2* and *Mal d 4* genes then with *Mal d 1* genes. This indicates that Mal d 2 and Mal d 4 may be more relevant in apple allergy then generally assumed and thus deserve more attention in research on (low) allergenicity. However, for firm conclusions these findings need to be validated through oral provocations and immunological studies.

Scientific impact: one scientific paper, preparation of which will start fall 2010

Innovation ISAFRUIT WP6.1 on key fruit texture determinants on apple and apricot

4. Structural determinants and QTL controlling texture on apple and apricot

Partner 5: JM Audergon, M Lahaye, F Laurens, INRA — GAFL-Avignon, BIA-Nantes, GenHort-Angers, France

Keywords:
texture, cell wall determinants, phenotyping, QTL

Short description:

One strategy to increase fruit consumption in Europe is the development of cultivars appreciated by consumers, meeting food industry specifications and with increased health benefits. Fruit textural defects are among the major quality traits orienting consumer's choice, post-harvest itineraries and processing abilities by industries. Fruit texture depends on complex multi-factorial characters implying cell wall characteristics. Within ISAFRUIT, screening procedures have been developed to assess the variability of histological features and cell wall chemical characteristics in relation with instrumental and sensory texture measurements. These new methods have made it possible to determine the genetic variability of these key texture determinants in apple and apricot and allow the identification of new genetic markers characteristic for apple texture quality traits.

The innovation of the results lies in the availability of new screening methods of key determinants of fruit texture allowing the discovery of new genetic markers in apple for use in selection strategies including texture characters.

Interest for science/industry:

The complexity of the many factors involved in fruit texture makes its control and prediction a challenging task. Thanks to the ISAFRUIT program, a new integrated screening approach of key cell wall determinants, combining histological, chemical, instrumental, sensory and genetic approaches, has been developed and applied as proof of concept on an apple progeny of 150 individuals for the identification of new texture related QTLs.

These innovative genetic results are a preliminary step before implementing molecular assisted selection (MAS) by nurserymen in their commercial breeding programmes.

Innovation ISAFRUIT WP6.1 on genetic maps for fruit quality characters

5. Maps for quality characters in *Prunus* –

Partner 4 : Pere Arús; Partner 5: Elisabeth Dirlewanger, Jean-Marc Audergon; Partner 6: Stefano Tartarini

Keywords:
Map saturation, SSRs, QTLs, Candidate genes

Short description

Using four segregating populations, two of peach and two of apricot we have saturated them using additional markers, mostly SSRs, to have maps with only a few gaps longer than 15 cM. A large number of candidate genes (CGs) for characters related to fruit quality: aroma (81), flavor (61), development (48), fruit color (49), texture (25), and regulatory genes, namely transcription factors (9 have been selected and mapped using the highly poplymorphic (almond x peach F2) *Prunus* reference population. These maps have been later used to study a set of characters: agronomical (9), organic acids, sugars and aminoacids (30) and poly-phenolocs (20). Positions of CGs and major genes or QTLs found from this analysis were compared and some interesting co-locations have been identified.

Interest for science/industry:

Finding the positions of major genes and QTLs, and markers tightly linked to them allows the use of markers at a very early stage of plant growth to identify the genotypes of interest and select them. This approach, termed Marker Assisted Selection (MAS), is an efficient breeding strategy, saving time and space when compared to classical approaches. Using CGs we can identify genes that produce the phenotypic variability in the characters of interest. These are the best possible markers for MAS, and make possible to search for molecular variability at these genes. Certain alleles or allele combinations may produce novel or more valuable phenotypes that can be characterized and selected based on their DNA variation.

Papers: One scientific paper has been published, one has been accepted, one submitted and several more are in preparation.

Innovation ISAFRUIT WP6.1 for Marker-Assisted-Selection in 3 apple progenies

6. Marker-Assisted-Selection in 3 apple progenies

Partner : Dr. C.E. Durel, Dr. F. Laurens, INRA, Angers, France

Keywords:

QTL, major gene, fruit quality, disease resistance, marker, SSR, selection index

Short description:

Genetic bases of apple fruit quality (texture, sugar content, acidity, juiciness …) have been partially elucidated thanks to QTL genetic mapping (QTL = Quantitative Trait Locus), especially in the frame of the European research project HiDRAS (Gianfranceschi and Soglio, ***Acta Hort*** 2004). Major resistance genes and resistance QTL against scab (*Venturia inaequalis*) have also been mapped. Microsatellite (SSR) markers closely linked to QTL were identified, which makes it possible to implement practical marker-assisted selection (MAS).

About 2,800 seedlings deriving from 3 controlled crosses were genotyped with these SSR markers. The controlled crosses were chosen according to the knowledge available on the linkage phases between linked SSR and target QTL for the parents. In the progenies, the probability of presence of the favorable allele presence for each QTL (PPFA) was inferred according to the available marker data. A selection index was computed for each seedling by weighting each QTL PPFA with a coefficient fixed according to the breeding importance of the trait and R^2 value of the target QTL. The global selection index was thus a linear combination of weighted PPFA. By ranking seedlings according to their respective selection index value, MAS was performed either positively (higher selection indexes) or negatively (lower selection indexes). To evaluate MAS efficiency, ~100 positively selected (MAS+) seedlings, ~50 negatively selected (MAS-) seedlings, and ~50 randomly chosen (MASr) seedlings were retained per progeny to be evaluated in the field (Figure 1).

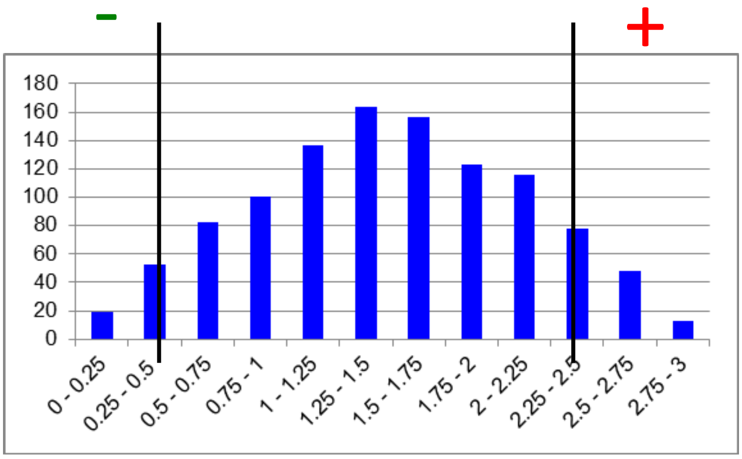

Figure 1. Distribution of the computed selection indexes for the 1082 individuals of the second progeny studied. On the right, the 100 positively selected seedlings (MAS+) exhibit a selection index value higher than 2.35 (average value ~2.57). On the left, the 50 negatively selected seedlings (MAS-) exhibit a selection index value smaller than 0.41 (average value ~0.28).

Interest for science/industry:

The concept of PPFA is useful in marker-assisted selection to deliver a probability value for each individual and each QTL even when some data are missing. Thus it allows to compute a selection index for each individual, and then to perform selection by ranking.

On a practical point of view, this experiment is, to our knowledge, the first one developed on marker-assisted selection in apple by combining both scab resistance and fruit quality genetic factors.

Dissemination:

Durel C.E., Mathis F., Denancé C., Lasserre P., Laurens F. 2010. Marker assisted selection for fruit quality and disease resistance in apple. ISHS 28th International Horticultural Congress, Lisboa, 22-27 August 2010.

Innovation ISAFRUIT WP6.2

7. pMF-vectors for marker-free technology

Partner 9: Dr. F.A. Krens, Dr. J.G. Schaart, Wageningen UR Plant Breeding, Plant Research International, Wageningen, The Netherlands

Keywords:
Genetic modification, marker-free, cisgenesis, gene pyramiding

Short description:
pMF is an optimized plant transformation vector produced by Plant Research International, Wageningen. Use of the pMF vectors in genetic modification enables effective removal of undesired DNA sequences, like antibiotic resistance genes, from the obtained transgenic plants or plant tissues. Removal of selectable marker genes may lead to genetically modified crops with increased consumer acceptance, e.g. cisgenesis. In addition, marker removal enables pyramiding of transgenes by recurrent transformations. The innovation lies with the optimization of the conditions for marker removal in apple, extension of the applicability of the system to another fruit crop, pear, and with the demonstration of successful accumulation of multiple genes-of-interest in an apple still being free of selectable marker genes. Genome stability could be affected using this approach; however, the desired end-products were obtained.

Interest for science/industry:
The concept of cisgenesis or intragenesis has been widely embraced by fruit breeding institutes around the world. Central is the use of genes from the fruit crops themselves for crop improvement by genetic modification and the absence of antibiotic resistance genes or other undesired gene sequences in the end-products. Research institutes and commercial fruit breeders and growers hope to produce in this way new varieties with improved quality traits that will be acceptable to the consumer and might be exempted from strict regulatory measures. For this, a marker-removal system is a prerequisite and the pMF-vectors are tested globally in several crops, a.o. fruit crops. Gene pyramiding has been identified as another advantage of these systems, but so far, no evidence for success has been presented. Within ISAFRUIT this has been done for the first time, demonstrating the potential of the system but cautioning for genome instability that was observed in some cases. This information will be part of a scientific paper.

Innovations WP 6.3

8. Apple and peach preference map for European consumers
Coordinated by Partner 4: Joan Bonany
Keywords: apple, peach, preference map, consumer acceptance

Short description
Preference map for apple and peach have been constructed from a wide European consumer test carried out during 2007/2008 season. The consumer test for apple consisted of testing acceptance of 11 varieties (some standard ones and some newly developed) and 10 peach varieties (including for two of them, two harvest dates). Near 5000 consumer took part in the tests. Results of the preference map segment the population regarding the acceptance of different varieties and the quality characteristics of these varieties for each population group. Interestingly enough the relevance of gender and age in shaping these segments was of no practical significance. Regarding country, although there were statistically significant differences regarding the composition of the groups in each country, the magnitude of these differences were not as big as initially thought. Overall, the results of these consumer tests have been able to gain a better knowledge regarding acceptance of new and standard varieties of

apple and peach by the European consumer and showed that increased fruit consumption can be achieved when the appropriate variety is given to each consumer or group of consumers.

Interest for industry
Results obtained can be of use by the different parts of the fruit value chain. Breeders can use the results to tailor the breeding programs to maximize the acceptance of consumers. Growers and shippers could make use of results to better adapt the varieties that they grow and ship to the markets that they are targeting. Distributors and retailers, likewise, could also better adapt their offer to their customers.

Pillar 7. Dissemination

Summary

Pillar 7 was set up as a horizontal technology dissemination (TD) Pillar with the charge of disseminating and transferring the knowledge innovation produced by the RTD Pillars to end-users that might be placed in different point of the fruit chain. It was expected that more traditional forms of dissemination (such as publications in scientific and industry journals, participation to scientific meetings, etc.) would be undertaken on their own by the Project's participants, therefore dissemination via these media was not placed as a major goal of Pillar 7, although it was not ruled out. Instead, Pillar 7 identified 3 types of knowledge which might be difficult to profile, or which might lend themselves to innovative dissemination forms. These were: i) *technical information* that might be transferred to end-users via training sessions; ii) *scientific knowledge*, as could be presented at meetings, symposia, via regular publications etc., for which amplification was seeked via internet-based, as well as more traditional, resources; iii) *Intellectual Property*, as a gap in awareness was detected in general among fruit-chain scientists, which were not paying sufficient attention to preserving their rights for IP protection because of their means of presenting and disseminating the knowledge derived from their research work. An additional workpackage was formed, tasked with the creation and maintenance of the Project website, which has acted as a one-stop point of access to ISAFRUIT and its growing number of internet-based resources. Because of its mission, it was anticipated from the start that the activity of Pillar 7 would keep increasing with the Project's unfurling.

Dissemination has taken many forms, with a special effort to access innovative communication platforms. For example, the decision to produce podcasts was made even before the official start of the project, a time when the YouTube website was much in its infancy, and yet already showing the potential to reach out to a very broad audience. Although from that early decision to the first video placed on line some time had to elapse (understandably), this proved to be a right decision, as more than 7,400 hits collectively have been gathered by the 29 videos placed on the ISAFRUIT channel. This number could have been increased if more attempts wold have been made at building a network of cross-referencing sites. In fact, just linking the CASA sprayer podcast to a Polish site greatly increased the number of hits on the video. A further improvement of the production of videos would have been achieved by alerting the media about the existence of filmed material available for download, that might be featured in TV shows, newscasts, etc. This was confirmed by the number of hits that were recorded on one Italian online daily fruit-dedicated newsletter, where 9 articles appeared dealing with ISAFRUIT, that totalled 3200 views.

Dissemination in traditional forms (conference proceedings, articles, posters, presentations, etc.) was mostly achieved by Partners on their own. However, some significant results were obtained by Pillar 7, by producing two Scripta Horticulturae volumes of the ISHS in addition to a Special Issue of the Journal of Horticultural Science and Biotechnology, which are and will be available on the Internet for free download. A form of interaction that was attempted but which proved difficult to put in place on a regular basis was alerting the media about innovative scientific papers immediately before their publication. It has been the experience of Pillar 7 that industry media, both “traditional” as the monthly Eurofruit, or the Fresh Produce Journal, or online ones, such as FreshPlaza, were quite interested in this information, because they welcome previews of innovative research about to appear, to evaluate whether or not to include it in their publications. This was difficult to organize, probably because authors feared that the pre-view might reduce, rather than increase, the impact of their results.

More effort should be devoted to properly communicate this possibility to the authors and to purse it.

The high degree of collaboration in Pillar 7 is demonstrated by the integration of resources from the various WPs in the Pillar, which widely collaborated in the attempt to achieve a high-impact profiling of the Project. For example, as the skills and capacity for filming were developed particularly by WP 7.2, it became usual for this WP to be involved in the events staged by WP 7.1, for filming and interviewing the participants, and later produce the podcasts. The 14 events organized by WP 7.1, or the 16 counted by 7.2, were in fact made possible also by contributions from the other 2 WP and viceversa, such as was the case for the two participations to FruitLogistica and the S11 Symposium at the IHC in Lisbon 2010, organized by WP 7.4.

Events were targeted at different stakeholders: growers, professionals along the chain, policy makers, peer scientists, the entire chain, at national and international venues, such as FruitLogistica. The European tour of the CASA sprayer was a multi-site display of innovation that touched 9 countries in 2009.

This Work required knowledge of the Project, knowledge of which partners could execute a dissemination event, and also knowledge on the part of the partners about Pillar 7, so that they would come to Pillar 7 proposing (rather than being asked for) dissemination opportunities. This two-way process was facilitated after the Pillar held an extensive meeting with the RTD Pillar Coordinators, during which the strategy for dissemination was explained, and a clear demand for contribution was placed to the Pillars.

The impact of the dissemination events was only recorded after the Project reviewers raised the issue and demanded to assess it. Therefore, since the number of hits or attendance figures have been recorded only from a certain point in time, the effectiveness of the Project in reaching out to the fruit chain has been greater than reported here and will keep increasing, as the online resources will largely be maintained for a long time yet. It can be added that, although the Project has ended, there have already been 3 occasions to profile it thanks to invitations to some or all of the Pillar Coordinators to speak at international and national audiences. It can reasonably be expected that more of these occasions may rise in the next few years.

The innovation reported included all the areas in the Project, during whose course several events were staged for each Pillar. However, more often several Pillars were called to contribute to a specific event. That has been for example the case at the FruitLogistica events (2007 and 2009), the Health Forum in Brussels (2008), the Professional Day in Angers (2009), the ISAFRUIT Symposium in Lisbon (2010), among others.

General objectives of Pillar 7 as defined in Annex 1

To identify all advances in knowledge arising from, or prompted by the six RTD Pillars of ISAFRUIT, and to provide appropriate paths of dissemination of such knowledge.

WP 7.1 *FRUITSKILLS.* The objective is to organise and carry out activities aimed at maximising the dissemination of “hands-on” knowledge related to tools produced by ISAFRUIT.

WP 7.2 *FRUITSCIENCE.* The objective is to make available to the relevant stakeholders scientific knowledge arising from the Project.

WP 7.3 *FRUITIMPACT* will research the body of knowledge arising from within ISAFRUIT with the goal of increasing the impact of ISAFRUIT through identifying options for exploitation including bundling knowledge together and identifying potential exploitation partners and end-users.

WP 7.4 ***FRUITWEB*** aims to make the knowledge from within ISAFRUIT as accessible to the relevant stakeholders as possible.

Basic information, contractors, work performed, end results

Relationships between scientific, technological objectives and Pillar 7 WP's

For many of the Objectives spelled out in the Annex I of ISAFRUIT, Pillar 7 has indirectly contributed by organizing events, producing press releases, podcasts, maintaining internet-based dissemination media (YouTube channel; GoFruit Blog; the Fruit Allergy website; the Vasco da Gama website; the ISAFRUIT website), providing publication opportunities (via the ISAFRUIT Special Issue of the J. of Horticultural Science & Biotechnology; via 2 ISHS Scripta). All the WPs in Pillar 7 have always collaborated in a tight form, as witnessed by the numerous initiatives that were reported in the subsequent Pillar's annual reports. Because of this, a standard reply is given to several of the objectives listed in the table below, which aims to highlight the fact that the Pillar collaborated on these. Only for the Objectives more tightly connected to the Pillar's mission is a detailed indication given.

Objectives	Work package	Relationship
A. Increase fruit quality	7.1	Events have been held to highlight the results of pre- and post-harvest research that has allowed improvement of fruit quality.
	7.2	The dissemination of scientific knowledge was augmented by state-of-the-art communication via the Internet.
	7.3	Assistance was provided in the attempt of economic exploitation of the Decision-support-systems developed in WPs 4.2 and 5.3.
B. Increase fruit safety	7.1	In addition to events devoted to improved management techniques (including crop load control), this WP supported events on control of post-harvest rots via innovative methods. A signal event was the “European tour of the CASA Sprayer” developed by WP 5.1, which was shown at growers days across many European countries. This Sprayer reduces up to 50% amounts of Active Ingredient applied.
	7.2	Working in synergy with WP 7.1, this WP has filmed many events, from which it has produced the videos visible from the YouTube site.
C. More convenience	All	The 4 WP in Pillar 7 have joined forces towards the goal of providing visibility to the Partners that contributed to this Objective. Typically, events of training or scientific dissemination relevant to this objective were planned and organized under the leadership and economic contribution of WP 7.1 or 7.2, which provided media exposure prior to and after each event, including filming and producing videos to be placed on the Internet. WP 7.3 was involved with consulting on IP-related issues, and WP 7.4 provided ISAFRUIT website backing to all activities.
D. Better availability	All	The 4 WP in Pillar 7 have joined forces towards the goal of providing visibility to the Partners that contributed to this Objective. Typically, events of training or scientific dissemination relevant to this objective were planned and organized under the leadership and economic contribution of WP 7.1 or 7.2, which provided media exposure prior to and after each event, including filming and producing videos to be placed on the Internet. WP 7.3 was involved with consulting on IP-related issues, and WP 7.4 provided ISAFRUIT website backing to all activities.
E. Raise consciousness	7.1	The training dissemination was carried out by first identifying Partners whose research had potential for training dissemination, making the necessary contacts, developing a program, providing financial support and media information to help promote the event and reach the targeted public. A total of 14 events has been executed. Many were filmed by WP 7.2 and podcasts have been produced and placed on YouTube. This WP has provided financial support for the final ISAFRUIT events in Lisbon.
	7.2	Responsible for the scientific dissemination, this WP has organized events (in parallel, and often in collaboration, with WPs 7.1 and 7.4); provided traditional publishing opportunities to ISAFRUIT members (via the ISAFRUIT Special Issue of the JHS&B and two ISHS Scripta, for which it provided financial support as well as editorial and coordination work); produced advertising materials; profiled the Project on many innovative media. These include: press releases, newsletters, podcast production and placement on the Internet, a Blog, a Twitter channel, two websites (on fruit allergy and on the Vasco da Gama Process). In addition, it has been involved in the Vasco da Gama Process and has presented posters at all the ISAFRUIT General Assemblies.
	7.3	This WP prepared and published on the ISAFRUIT website the ISAFRUIT Knowledge Map, a searchable tool that described the layout and the contents of the entire Project. This has been followed by work focussing on IP protection consulting for the Project members, resulting in some patent applications from Project partners; an internal newsletter that has been circulated in cooperation with WP 7.4; broad cooperation with the other WPs in the Pillar on many endeavours; presentation of posters at all the ISAFRUIT General Assemblies..

	7.4	The set-up and maintenance of the ISAFRUIT website; the execution of ISAFRUIT profiling events at FruitLogistica (2), the convening of the S11 Symposium at the Lisbon IHC; the participation and facilitation of the Vasco da Gama Workshop (W6) at the Lisbon IHC; press releases; participation in the Vasco da Gama Process, including the editorial coordination of the resulting Scripta; presentation of posters at all the ISAFRUIT General Assemblies.
F. Lower Fruit price	-	

List of participants in Pillar 7 and their engagement in WPs and Tasks.

#	Participant name	Short name	Country	WP engagement	Tasks responsibility
4	Institut de Recerca i Tecnologia Agroalimentàries	IRTA	E	7.3	7.3.1÷7.3.3
6	University of Bologna	UNIBO	I	7.4	7.4.1÷7.4.4
12	University of Warwick	UW	UK	7.2	7.2.1÷7.2.4
27	Centre Technique Interprofessionnel des Fruits et Légumes	Ctifl	F	7.1	7.1.1÷7.1.3
42	Hort Research – New Zealand – **Third country**	HR	**NZ**	7.3	7.3.1÷7.3.3
49	CSO Ferrara – **Small/Medium Enterprise**	CSOF	**I**	7.4	7.4.4

Comments on participants and relationships between participants

Six Institutions (universities, research/technical institutions, one small/medium enterprise) from 5 countries, including a third country (New Zealand) participated in Pillar 7. The Partners brought to the Pillar expertise in the organization of training events, in the dissemination of scientific information, including via innovative technology media, in-house press resources, deep knowledge on Intellectual Property protection and exploitation. In addition to these, a variable number of ISAFRUIT Partners have been included, over time, in the roster of Pillar 7 members, as this was needed from a financial point of view: these Partners received the funds necessary for their dissemination activities from the budgets of WPs 7.1 and 7.2, disbursed directly by the Project Management.

Because of its charge, Pillar 7 has interacted widely with all the other Pillars. In addition to this, however, there was a very strong collaboration within Pillar 7, whose members contributed to many aspects of the collective work to be done. Since the very start of the Project, when a discovery survey was launched across the ISAFRUIT constituents, this collaboration was evident: all the WPs contributed to its preparation, administration and interpretation of the results, as the survey contained questions whose answers were particularly relevant to each workpackage. As already stated, 7.1 and 7.2 often collaborated in the organization of events, contributing different skills/capacities. WP 7.3 had a more definite charge within the Pillar, but was also the leader in the preparation of the ISAFRUIT internal newsletter, although it was circulated (and the replies collected) by WP 7.4. The latter WP was not only active in the upkeep of the ISAFRUIT website, but it took a very active lead in the preparation and execution of some events, and in the Vasco da Gama Process, in addition to providing talks about ISAFRUIT at several events organized by WPs 7.1 and 7.2.

Description of achievements within each work package during the course of the project

WP 7.1 Skill Dissemination (FRUITSKILLS) Leader: 27 CTIFL (Catherine Lagrue – Work period 1-57 months). The objectives of this activity were:

- To develop and implement an effective plan to collect and disseminate technological skills deriving from the research carried out in each Pillar of ISAFRUIT;
- To develop appropriate high quality and innovative technology transfer events and materials aimed to stakeholders placed along the entire chain.

This Workpackage was assigned the mission to provide the technological transfer to fruit-chain stakeholders of knowledge arising from the Project. The leadership of this WP was assigned to Catherine Lagrue of CTIFL, which brought to the Project this organization's broad and long-lasting experience in technology development and transfer to French stakeholders. This required identification of the offer of such knowledge, making contacts with the researchers that might be involved in the technology transfer, planning, coordinating and executing events featuring such technology transfer. Apart from the type of knowledge involved, this WP had an entirely parallel mission to that of WP 7.2, which was tasked with the dissemination of scientific results. The WP assisted the owners of the knowledge by preparing press materials, organizing the program and making all necessary contacts. This was complemented by providing the supporting funds for the travel of the speakers involved, and for other expenses related to the event.

The discovery of the offer of knowledge was made difficult by the size of the Project and the variety of fields of research which were involved. The search for items/Partners for dissemination events started with the administration of the online ISAFRUIT survey that Pillar 7 collectively prepared and carried out through the website. The seven-part survey focused on: the previous experience and skills of ISAFRUIT partners at research dissemination; the facilities available to them that could be used for dissemination events; a brief description of the results expected from their work that could be amenable to dissemination; the potential for exploitation perceived for their results (in the form of patents, or otherwise); the capacity of the respondents to perform dissemination in countries other than their own. A subsequent plan was devised and put in place to start preparing the technology dissemination activities. With time, the process of finding partners for events was reverted, i.e. there was more than one instance when the WP was approached with offers of events that might be staged.

The first events were held in 2007 and their number has increased over the years. At the end of the Project, 14 events have been organized and executed, in cooperation with researchers from all RTD Pillars. These events have mostly been organized for specific stakeholders, such as technical consultants, but in a few instances, for the entire chain, such as was the case for the Professional Day in Angers, at the end of the 4th year of the Project. The total number of attendees at these events easily surpasses 3,000 (in the first 2 years of the Project, no data were collected on attendance to the events). Some of the events have been quite unique, such as the **European CASA Sprayer Tour**, which has been featured in Farm Days in 7 European Countries in 2009. The logistics and coordination for such an event were quite demanding. More than 1,200 people have attended these demonstrations, while the podcast produced and placed on YouTube has had more than 3,500 views. The WP has provided support to more than 110 scientists in their participation to the ISAFRUIT Symposium in Lisbon, August 2010. This event was the last dissemination event executed by the WP, in cooperation with

WPs 7.4 (which convened the Symposium) and 7.2 (which provided a press release and Twittered live from the Symposium). The ISAFRUITers who contributed to the program, either in oral or poster form were financially supported by the WP.

The budget of this WP was under the responsibility of and controlled by the WP Coordinator, but it was organized so that most of it was held in reserve by the Project Management, which with each installment of funds received disbursed the proper amounts (communicated by WP 7.1) to the Partners involved in the planned events. This helped keep the budget under firm control, but also demanded that events be planned fairly in advance, at least enough to be included in an 18-month forward planning report, in order for the Project Management to be able to disburse the funds appropriately. Initially, this procedure created some difficulty, but subsequently the process became sufficiently streamlined and events were well planned and executed, although the requirement to foresee them by at least 1 year existed throughout the Project's duration.

The Workpackage collaborated with the rest of the Pillar, in particular WP 7.2 and 7.4. The collaboration with WP 7.2 often revolved about the fact that while one WP was providing organizational support, the other was profiling the event on the innovative media that have been addressed by the ISAFRUIT dissemination program. Many of the technology dissemination events, for example, lent themselves quite aptly to filming, for the realization of podcasts. As already stated, the number one podcast for number of hits is the one describing the CASA sprayer.

The CASA sprayer at work during some of the demonstrations.

Through the events it organized this WP has reached a very significant number of stake-holders, since these events were attended by technical personnel, growers, specialized media. Most of the events were unique, in that they provided one-day (in most cases) programs featuring scientists that presented the results of their work in a context of technological trans-fer. After most of the events, the audience was requested to fill out a questionnaire, and in general the majority of participants considered the events quite worthwhile and well-executed.

Often the event was planned back-to-back with a WP or Pillar meeting, in order to save on travel expenses for the speakers.

WP 7.2 Science Dissemination and Transfer (FRUITSCIENCE) Leader: 12 University of Warwick (initially Michael Wilson but, from September 2006, Sharon Hall – Work period 1-57 months). The objectives of this activity were:

- To develop and implement an effective plan to collect and disseminate all relevant scientific knowledge emerging from each Pillar of ISAFRUIT;
- To develop appropriate high quality and innovative materials and continuing education events to transfer new knowledge to international, national and regional researchers, Government and industry leaders, the media, consumer groups and the wider public.

The mission of WP 7.2 was accomplished much in parallel to that of WP 7.1, since their charge was quite similar. The discovery of contributors to the program of scientific dissemination began with the initial ISAFRUIT survey, which served also to establish contacts within the Project. The WP started profiling the Project in its first year, with press releases, short articles for trade publications and an editorial by Ole Callesen in the Journal of Horticultural Science & Biotechnology. The WP was also deeply involved in the preparation and execution of the ISAFRUIT stand at Fruit-Logistica 2007. From this event, the WP started also gathering contacts of an external network that became the target of the external ISAFRUIT newsletter. The mailing list for this network eventually approached the 2,000 entry mark. The WP has been quite active in promoting-requesting cooperation with the other WPs in the Pillar which, as already stated, has been a trademark of Pillar 7. The extent of this cooperation has covered the span from preparing exhibition material, to writing press releases or other ISAFRUIT descriptive copy, to assisting in publishing in traditional forms, to providing content for scientific publications, the planning and execution of events.

The forms of dissemination have included scientific dissemination events (16); facilitating the publication of scientific papers (ISAFRUIT Special Issue of the J.Hort.Sci.&B. + 2 ISHS Scripta); setting up, populating and maintaining innovative Internet-based resources aiming at the broadest possible circulation of the ISAFRUIT brand and scientific output, and even the production of children-aimed dissemination materials dealing with fruit. Typically, the events were planned, organized and executed, in addition to being supported financially by the WP budget, part of which, in analogy to that of WP 7.1, was held in reserve by the Project Management, and disbursed to the Partners indicated by the WP that were involved in the events. Among the events organized was the Health Forum, which showcased to EU officers in Brussels ISAFRUIT state-of-the-art research results about health-related properties of fruit and the increasing relevance of fruit-allergy among European consumers. The latter has been addressed specifically by setting up a dedicated fruit allergy website, in English and Spanish, to ensure the widest possible outreach for ISAFRUIT results on this topic. The WP had the know-how and in-house facilities for the production of podcasts, and the set-up of other Internet resources, such as a blog, a twitter, and an electronic newsletter distributed to a wide network of stakeholders outside ISAFRUIT. The WP has also contributed to the Vasco da Gama process in several ways: (i) providing financial support to the publication of an ISHS Scripta that is due for publication early in 2011; (ii) contributing to the development of the scientific concept; (iii) setting up a dedicated website and producing a booklet illustrating the Process (which was distributed at the VdG Workshop held within the IHC in Lisbon, 2010). WP 7.2 has also produced press-releases and other profiling materials for ISAFRUIT.

The cover of the ISAFRUIT Health Forum, Scripta N. 8.

The innovation in ISAFRUIT communications. WP 7.2 did not have as a mandate to stimulate publishing in scientific journals, because that was left to the individual researchers but, as indicated above, it still facilitated the publication of three separate printed documents, which are available for free download from the internet (the second Scripta after its publication in 2011). In addition to these and to the events executed, the activity of WP 7.2 was augmented by the fact that it turned out to be quite well positioned to carry out a strategy (that was decided right at the start of the Project), to profile ISAFRUIT in innovative dissemination media. This was achieved bythe creation of a Blog, a Twitter account, a YouTube channel, an electronic newsletter, two dedicated websites: one specifically aimed at allergy sufferers and one for the Vasco da Gama Process. As a whole, these resources complemented the main ISAFRUIT website, which distributed the articles/deliverables produced by the Project that could be released in the public domain, in addition to official documentation and event announcements. The production of these resources, in particular the podcasts, meant that WP 7.2 was involved in as many of the events of ISAFRUIT dissemination as possible, with the charge of filming, editing, post-producing and finally acquiring permission to place the films online from the ISAFRUIT researchers featured in the videos before placing them on the internet. The blog and the Twitter channels represented two other forms of communication to the social networks about ISAFRUIT, distinctly separated in their charge. The blog served as a dissemination of fruit-related facts that also included ISAFRUIT-generated information whenever available. The Twitter was used to generate a group of followers, which included several journalists, generally in international trade publications, such as EuroFruit, Fresh Produce Journal, FruchtHaendel. This group received live feed from the final ISAFRUIT events at the 2010 IHC in Lisbon, as WP 7.2 was twittering from the lecture room. Finally, the external newsletter was sent electronically to an audience of about 2,000 addressees, which included national government and EU agriculture-related officers. A further communication vehicle was identified in an illustrated book for young children (first-graders), featuring an apple-related story, a PowerPoint presentation on the development of an apple fruit from a mixed-bud and a kit for simple experiences about fruit. This initiative proved quite popular in the UK, where 1,000 copies of the book+kit were distributed amongst several schools, with a positive feedback from all of them.

The impact. As for WP 7.1, the number of attendees was not recorded in the first few events, so any number that could be compiled would be an approximation by defect. However, some data can easily be reported: 29 podcasts, for a total of several hours of video, were placed on the YouTube channel, which generated over 7400 views at the time of writing the present report. Numbers of this kind for the published materials are difficult to gauge because not only have printed copies been produced and disseminated (600+ subscribing institutions received the ISAFRUIT Special Issue), but these documents are freely available for download from servers maintained by the ISHS and the JHS&B (and the number of downloads from these servers was not available). However, it is probable that these numbers also add up in the range of some thousands. The impact of the blog and the twitter are much less, but perhaps of great impact because journalists are among those connected to these communication instruments. For example, the Italian online daily newsletter FreshPlaza has been following ISAFRUIT mainly through these channels, and as a result, the articles written have generated 3200 hits, mostly in Italy. The effectiveness of the social network channels would have probably been increased if cross-links between sites/pages had been more frequent. The linking of the YouTube channel to a Polish website dedicated to agriculture greatly increased the number of hits to the CASA sprayer video, which alone received about half of the hits. The WP was cross-linking with other sites, for example several five-a-day programs in several countries, outside as well as inside Europe. With the end of the Project, these sites and pages will not be updated, but will be maintained, as will the official ISAFRUIT website.

WP 7.3 Increasing the Impact of ISAFRUIT Innovation (FRUITIMPACT). Leader: Partner 4 IRTA (Agustì Fonts). Additional member: 42 HortRes. (Jill Stanley) (Work period 1-57 months). This WP had the following objectives:

- To research the body of knowledge arising from the project, looking for opportunities to increase the innovative impact of its results and, therefore, increasing the competitive advantage of each of their members.
- To make the project partners aware of the suitability of their research results (as such, or as part of a bundle) to commercial exploitation. Any decision on such exploitation, however, will be left to pursue according to the terms spelled out in the ISAFRUIT Consortium Agreement.

This Workpackage had the mission to provide consulting relative to Intellectual Property Right (IPR) issues to the partners of the project. The coordinator of this WP had 10 years employment at the European Patent Office previous to his present position (deputy Director of IRTA, partner 4) and brought his in depth experience to this endeavour. Initially the WP was involved in the discovery of the "offer for potentially protectable items of research" from the Project, which was carried out jointly by Pillar 7 via the online ISAFRUIT survey. As a result of this discovery, the **Knowledge Map of ISAFRUIT** was prepared, using an innovative, open-source software, C-Map, which offers a depiction of the Project easy to surf, but which can also be used to explore in depth the Project, its Partners, the publications, the reporting documents, etc. This tool was periodically updated by the WP, with inclusion of further deliverables, articles or other dissemination items. Within the WP, the map served the purpose of placing in a general context the different parts of ISAFRUIT, and particularly those potentially amenable to some form of exploitation.

Information in The Knowledge Map of ISAFRUIT can be explored in three ways: the Fruit Chain, Partners and Project. The next figure corresponding to Pillar 4 is as an example of showing information through the Project path

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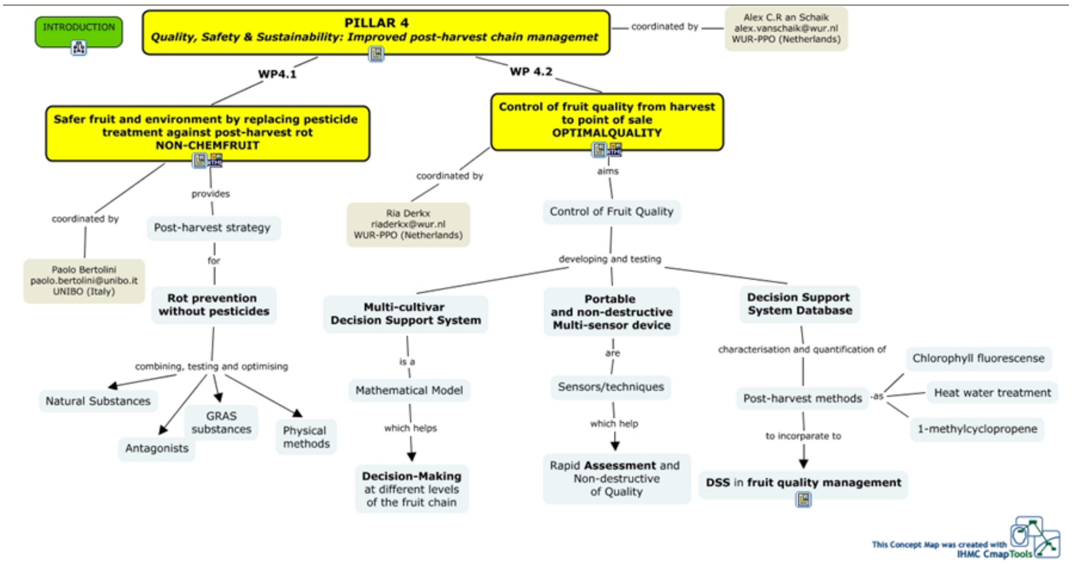


Pillar 4 in the Knowledge Map

The protection of IP has proven to be difficult among the scientists involved in ISAFRUIT, many of which are used to present their data in public meetings, or via publications in the open domain. This can be explained with the fact that most work for public Institutions, whose mission used to be to make knowledge available for the public. Therefore, this realization indicates the need to help change this situation, as the current reality of funding, and the need to make research results profitable for those who obtain them, is shifting the paradigm towards a more careful behaviour in disclosing one's finding, so as not to thwart the potential for exploitation. To this end, the WP has helped a few Project partners in formulating descriptions of results that would be informative without breaching their patentability and/or exploitation potential and in choosing their model of exploitation via a dedicated seminar with experts in the field. Unfortunately, the WP had to realize that in most instances of potentially exploitable know-how, this was really not the case since the knowledge was no longer protectable due to improper early communication. A paper in the Special Issue of the J. Hort. Sci. & Biotech. by this WP illustrates the process of ISAFRUIT innovation and IP achievements and obstacles detected during the project. At the same time, the WP has organized two events in support of ISAFRUIT partners and the patentability of some of their research results:

- *Partnering meeting: presentation of the ISAFRUIT decision support tools (DST) to industry, Girona, 26th November 2008*. During this meeting both DST of the project (Peaple developed by WP 4.2 and Profitfruit by WP 5.3) were presented to industry, such disclosure was done without breaching of confidentiality. A representative of the European Patent Office gave also a presentation during the partnering meeting, in the attempt to raise the awareness of ISAFRUIT scientists about the proper ways to protect their work.
- *Technology transfer meeting, 27th September 2010, Barcelona*, which was aimed to develop an exploitation plan, including IPR, of those innovations obtained during the project. The

objective was also showing the need for scientists to approach the exploitation of their results with the support of technology transfer officers (TTO) of their institutions and to provide tools to transfer ISAFRUIT outputs to industry. It was also found necessary to involve the Technology Transfer Officers in this process to assure the knowledge transfer once the projects finishes.

The following table shows the exploitation model and innovation stage of the ISAFRUIT innovations:

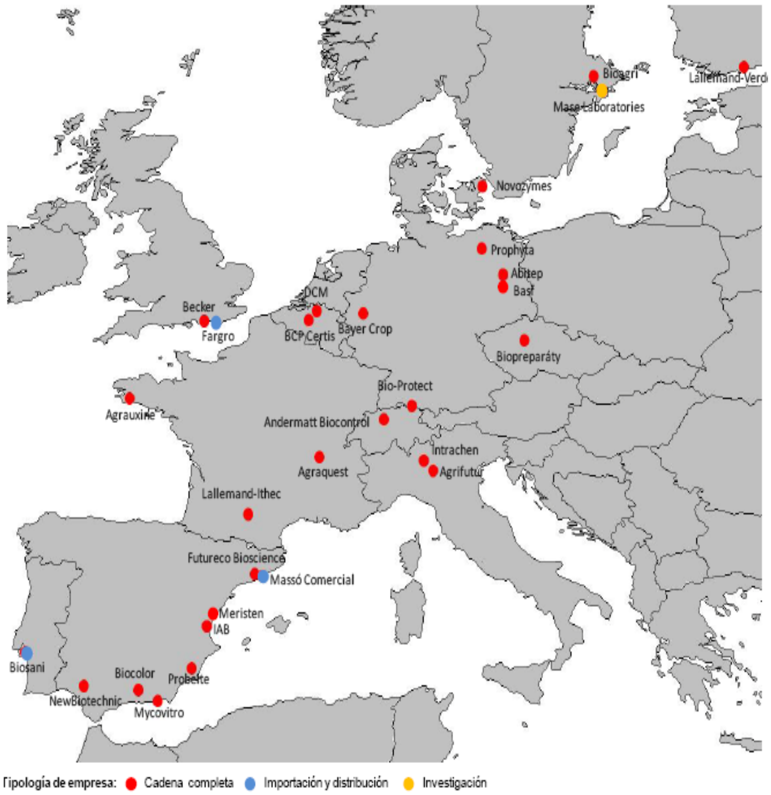
ISAFRUIT technology	Exploitation model	Innovation Stage
New fruit products with added nutraceuticals using freeze-chill and vacuum processing	IV	4
New fruit products using microwave processing	IV	2
New fruit products using high pressure processing	IV	2
Alternative techniques to reduce food-borne pathogens	II	3
Prototype of decision- support system for fruit quality management	IV	3
Portable and non-destructive equipment for fruit quality assessment	IV	2
Fruit components used to lower cholesterol levels	IV	1
Production of dried fruits by optimised combined processes (osmotic dehydration and drying or freeze-drying)	IV	4
Crop adapted spray application system (CASA)	II	4
Decision support tool for economical decisions in farm management	IV	4
Cloudy juice	IV	3

a) Exploitation model: trade secret (I); patent (II); copyright (III); publication/free access (IV).

b) Innovation phase: basic research (1); proof of concept (2); early-stage technology development (3); product development (4); production/marketing (5).

Three patents have been applied in 2010 (2 in Pillar 5 and one in Pillar 3) with the assistance of WP 7.3, which has provided the scientists of WP 3.1 with a state of the art analysis to verify whether patents existed already, which could attack the novelty requirement. This analysis concluded that the use of this microorganism can be patented.

Other activities aiming to increase the impact of the project. Different studies have been also carried out to explore the market and to identify possible end-users of some ISAFRUIT innovations. The following figure is shown as an example of the sort of analysis this WP has provided in these studies: *Innovation-promoting companies in Europe in the biocontrol sector*



Distribution of European entities working in the biocontrol sector

The WP finally has prepared and disseminated (in coopertation to WP 7.4) the internal **ISAFRUIT Newsletter**, a tool aimed at two-way communication within the Project, as it provided information to Partners, while it demanded a feed-back, for example relative to any information that a Partner might have which would be amenable to dissemination within the Project, or outside it.

WP 7.4 ISAFRUIT promotion (FRUITWEB)

Leader: Partner 6 UNIBO (Luca Corelli Grappadelli) (Work period 1-57 months). Other Participant: Partner 49 CSOF. The objectives of this WP were:

- Provide the Consortium with a web site responding to the Project's needs for visibility, for dissemination of knowledge, for the protection of novel knowledge, and for the operation of the Project.
- Design and prepare, in cooperation with WPs 7.1, 7.2 and 7.3, ISAFRUIT advertising materials, (including, but not limited to, a display stand) to be showcased at fruit-related events worldwide.

The WP provided the Project with a highly performing website, comprised of two sections, one for the general public and one for the Partners of ISAFRUIT, which were allowed various degrees of interactivity depending on their ISAFRUIT role and status: Pillar Coordinators and Work Package leaders had permission to operate (file transfer, creation of news, events, alerts) at Pillar/WP level, respectively. The public area of the site has provided information about the Project and its activities, including all publishable products released by project partners, including articles or other deliverables. The links to the ISAFRUIT Special Issue of

JHS&B and to the Scripta on Health and the upcoming on the Vasco da Gama workshop are and will be placed on the site even after the Project's end. The links to the other Internet-based resources of ISAFRUIT are also placed on the home page. The site has provided a **communication platform** to the Project partners, who have used it in varying degrees. Some, Pillar 1 for example, have been quite proactive in adopting it, with the production of press releases that were placed almost from the beginning on the server. In other instances, the exploitation of the facility has been less quick. In several cases, the site could have ensured easier and more secure transfer of large files, for example, but most members often preferred the e-mail attachment form of delivery of a document, with attendant problems of size, file integrity, etc. The restricted area of the site has served as repository of all internal ISAFRUIT documentation, and in particular of that which the Partners haven't released for the public domain. At the end of the 57th month, 721 items have been uploaded in the reserved area as follows: 409 reports (internal reports and communications); 252 publications (articles, abstracts and poster presentations) and 60 news and events. The number of hits on the site has reached, over the years, many hundreds of thousands. Such a large number is difficult to put in a perspective, since it may be the consequence of spiders, robots, search engines, etc. visiting the site, and may not mean necessarily a purposeful visit. In addition, in comparison to general public sites, even several hundred thousand hits does add to a significant number, even when millions users are on the social networks at any one time. The nature of a science oriented site in fact, is such that a number of hits comparable to those of social networks should not be expected.

<u>The WP has led initiatives to profile ISAFRUIT</u> in tradeshows such as FruitLogistica (Berlin) and Interpoma (Bolzano, Italy), which were both attended twice. It is difficult to gauge the impact of these activities, as there are no direct means to measure it. It can be said that, in all cases, there have been articles in international publications such as Freshfruit, FPJ, the Italian online daily newletter FreshPlaza. This WP has also often contributed to the scientific program of events across Europe, providing descriptions of the entire Project, or in representation of specific ISAFRUIT Pillars/WPs.

Photo: a moment of the ISAFRUIT Round Table at FruitLogistica, Berlin, February 2009.

The WP has also convened the final ISAFRUIT Symposium at the ISHS International Horticultural Congress in Lisbon, 22-27 August 2010. The venue and occasion had been singled out since the beginning of ISAFRUIT as the proper opportunity to showcase the Project results, whose scientific part was supposed to have been concluded by the end of 2009 (48^{th} month). The decision to extend the duration of the Project to 57 months was justified by the need to allow this extension in order for the ISAFRUITers to be able to participate to this event. The final program featured 4 days of oral and poster communication and almost 180 contributions, most of which from ISAFRUIT.

The Vasco da Gama Process. WP 7.4 has been involved since the beginning with the VdG Process, which was led by Pillars 1 and 5, with the participation of Pillars 4 and 7. in this endeavour, the WP has been involved in all aspects, from the scientific to the organizational ones, to the cordination and editing of the ISHS Scripta which is expected to be published in early 2011.

Pillar 8 Project management and co-ordination.

The management during the project period is in detail described in the management report section 1. For information about the management activities we refer to section 1 of the final reports.

II. DISSEMINATION AND USE (ALL PUBLISHABLE RESULTS)

ISAFRUIT–All publishable results 2006-2010

A. Scientific publications in anonymus peer-reviewed international journals

Pillar 1

2010
Verdouw, C.N., Beulens, A.J.M., Trienekens, J.H. & Wolfert, J. 2010. Process modeling in demand-driven supply chains: A reference model for the fruit industry. *Computers and Electronics in Agriculture,* 73,(2), 174-187.

2009
Bertschinger, L., Corelli-Grappadelli, L., Derkx, M.P.M., Hall, S., Kockerols, K., Sijtsema, S., Steiner, S., Van der Lans, I.A., Van Schaik, A.C.R., & Zimmermann, K.L. 2009. A systematic method to bridge between pre-harvest, post-harvest and consumer research aimed at increasing fruit consumption: The "Vasco da Gama" process. *Journal of Horticultural Science and Biotechnology,* 84, ISAFRUIT Special Issue, 2-6.

Hennen, W.H.J.G., Benninga, J. 2009. Application of Trend Impact Analysis for predicting future fruit consumption. Paper in press for the special Isafruit Issue of *Journal of Horticultural Science and Biotechnology,* 84, ISAFRUIT Special Issue, 18-21.

Kraszewska, M., Zając, J., Van der Lans, I.A., Jasiulewicz, A., Van den Berg, I. & Bolek, A. 2009. Role of product characteristics for adoption of fruit and fruit product innovations. *Journal of Horticultural Science and Biotechnology,* 84, ISAFRUIT Special Issue, 28-33.

Lemanowicz, M. & Krukowski, A. 2009. Comparisons of qualitative and quantitative issues in the fruit supply industries in The Netherlands, Poland, Greece, and Spain. *Journal of Horticultural Science and Biotechnology*, 84, ISAFRUIT Special Issue, 13-17.

Zając, J. & Van der Lans, I.A. 2009. An inventory of recent innovations in fruit and fruit products. *Journal of Horticultural Science and Biotechnology*, 84, ISAFRUIT Special Issue, 22-27.

Zimmermann, K.L. & Van der Lans, I.A. 2009. The paradigm of consumer-driven and responsive supply chains: An integrated project approach. *Journal of Horticultural Science and Biotechnology*, 84, ISAFRUIT Special Issue, 7-12.

2008
Trienekens, J., Van Uffelen, R., Debaire, J. & Omta, O. 2008. Assessment of innovation and performance in the fruit chain: the innovation performance matrix. *British Food Journal,* 110 (1), 98-127.

201x

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Pillar 7

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ISAFRUIT videos available on YouTube. 7 shorter videos (highlights from events, interviews with researchers, quiz) 14 full lengths presentations from ISAFRUIT forums and conferences. Total length: 6.3 hours. Total views (5 Aug. 2009-14 Jun 2010): 5122.

Gofruit blog (<http://www.gofruit.org>www.gofruit.org)
The blog contains short news stories on fruit from ISAFRUIT and beyond, ISAFRUIT videos, fruit recipes, photos, teaching materials and a quiz.
3630 views since launch in March 2009.

Twitter (ISAFRUIT)
Twitter is used to constantly update the subscribers on the latest information flow from ISAFRUIT such as blog stories, newsletters and project websites. We currently have 38 followers including Americafruit, eurofruit, freshproduce Malaysia, fructhandel magazine.

Fruit and allergy website
Information portal developed for fruit allergy sufferers. The website has had 1696 hits since the launch on 19 January 2010. There have been 324 submissions of a questionnaire from which fruit allergy sufferers can find their type of allergy. A Spanish version of the Fruit and Allergy website was launched on 10 June to broaden the audience.

The Great Greedy Gogmagog
1000 copies of a children's book about apples have been distributed by WP7.2 to 26 institutions and organisations around the UK, primarily to schools. To promote the book and evaluate how it is used one of the authors has visited 6 schools and will take part in a celebration day of a 'growing with schools' project in which 100 school children are expected to participate. WP 7.2 has been represented on 4 school visits (2 with and 2 without the author) and is carrying out an evaluation of the impact of this communication tool. We are attempting to arrange one additional visit.

The Vasco da Gama process (web site)
A website was designed by WP7.2 to inform both ISAFRUIT researchers and those external to the project about the Vasco da Gama process and the ISAFRUIT House of quality. This was launched on 26 January. There have been a total of 475 page views since the launch.

WP7.2 newsletters (available on the ISAFRUIT website (www.isafruit.eu)
There have been 10 ISAFRUIT newsletters published by WP7.2 These are distributed to industry stakeholders and the academic community, whose contacts are held on the ISAFRUIT database created by Pillar 7.

2007
Hall, S., Schmidt-Andersen, M.B. 2007. Which fruit allergy character are you? Dialog based web initiative to promote fruit consumption. 2nd ISAFRUIT General Assembly, Bologna, Italy 20-22 June, 2007 (poster),

Pillar 8

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Annex A : List of tasks and deliverable numbers

Pillar 1

Task 1.1.1: State of the art of European Fruit Consumption (D1.1.1, D1.1.2, D1.1.3)
Task 1.1.2: Scenarios for increasing European Fruit Consumption. (D1.1.4, D1.1.5, D.1.1.6, D.1.1.7))
Task 1.2.1: Theoretical framework consumer preference formation.(D1.2.1,D1.2.2, D1.2.3, D1.2.4, D1.2.5)
Task 1.2.2: Consumer Segments. (D1.2.6, D1.2.7)
Task 1.3.1 Consumer acceptance and choice of fruit innovations. (D.1.3.1, D.1.3.3, D.1.3.4, D.1.3.5, D.1.3.8)
Task 1.3.2: Fruit product innovations (D.1.3.2, D.1.3.6, D.1.3.7, D.1.3.9, D.1.3.10)
Task 1.4.1: Model for Chain management and organizational innovativeness. (D1.4.1, D.1.4.2, D.1.4.3)
Task 1.4.2: Case-study European Fruit Industries. (D.1.4.4, D1.4.5, D1.4.6, D1.4.7)
Task1.5.1: Transition and innovation strategies from research results of ISAFRUIT (D.1.5.1)
Task 1.5.2: Transition strategies for European Fruit Chains. (D.1.5.2, D.1.5.3)

Pillar 2

WP 2.1 Health effects of whole and processed fruit (fruit effects)

Task 2.1.1. Short-term animal studies on fruit nutrigenomics (D 2.1.1, D 2.1.2, D 2.1.7, D 2.1.8, D 2.1.9, D 2.1.13, D 2.1.18, D 2.1.20).

Task 2.1.2. Medium-term studies (D 2.1.3, D 2.1.6, D 2.1.8, D 2.1.22, D 2.1.25, D 2.1.29, D 2.1.32)

Task 2.1.3 Fruit nutrigenomics in healthy volunteers (D 2.1.9, D 2.1.10, D 2.1.15, D 2.1.18, D 2.1.19, D 2.1.20).

Task 2.1.4. Fruit nutrigenomics in patients with metabolic syndrome (D 2.1.4, D 2.1.7, D 2.1.11, D 2.1.16, D 2.1.19, D 2.1.24, D 2.1.26, D 2.1.28, D 2.1.31, D 2.1.32).

Task 2.1.5. Fruit and processed fruit in cancer prevention (D 2.1.5, D 2.1.12, D 2.1.14, D 2.1.17, D 2.1.20, D 2.1.21, D 2.1.27, D 2.1.32).

Task 2.1.6 Metabolomics analyses of fruit and health (D 2.1.16, D 2.1.21, D 2.1.23, D 2.1.27, D 2.1.29, D 2.1.30, D 2.1.32).

WP2.2 Fruit in the prevention of overweight (fruitslim)

Task 2.2.1 Pilot study on fruit intake and weight control (D 2.2.1, D 2.2.2, D 2.2.4., D 2.2.6).

Task 2.2.2 Full cohort study on fruit intake and weight control (D 2.2.3, D 2.2.4, D 2.2.5, D 2.2.6, D 2.2.7, D 2.2.8, D 2.2.9, D 2.2.10, D 2.2.11, D 2.2.12).

WP2.3 Non-allergy and low-allergy fruit (fruitsafe)

WP 2.3.1 Formation and testing of Mald1-silenced apples (D 2.3.6, D 2.3.7, D 2.3.11, D 2.3.14).

WP 2.3.2 Identification of low-allergy apple varieties (D 2.3.1, D 2.3.3, D 2.3.4, D 2.3.8, D 2.3.9, D 2.3.10, D 2.3.11, D 2.3.12).

WP 2.3.3 Low-allergenic chains (D 2.3.2, D 2.3.5, D 2.3.5A, D 2.3.11, D 2.3.13, D 2.3.15).

WP2.4 The safety of novel pre- and postharvest procedures (fruitclean)

Task 2.4.1 Efficacy of new post-harvest rot treatments (D 2.4.1, D 2.4.2, D 2.4.3, D 2.4.4, D 2.4.6).

Task 2.4.2 Critical steps for chemical contaminants in the chain from harvest to consumer (D 2.4.1, D 2.4.3, D 2.4.5).

Task 2.4.3 A comparison trial of new spraying techniques (D 2.4.1, D 2.4.2, D 2.4.3, D 2.4.4, D 2.4.5, D 2.4.7).

Task 2.4.4 The effect of production methodology on mycotoxin contamination (D 2.4.1, D 2.4.2, D 2.4.3, D 2.4.4).

Pillar 3

WP 3.1

T 3.1.1. Procurement of raw material (D 3.1.1; D 3.1.21; D 3.1.22).

T 3.1.2. Freeze-chill and *sous vide* processes (D 3.1.2; D 3.1.3; D 3.1.13; D 3.1.14; D 3.1.23; D 3.1.24; D 3.1.25; D 3.1.42; D 3.1.44; D 3.1.45).

T 3.1.3. Microwave processing of fruit, fruit preparations, salads, desserts to preserve quality and enhance microbial stability of these products (D 3.1.4; D 3.1.15; D 3.1.27).

T 3.1.4. Alternative techniques to reduce FBPs on minimally processed foods (D 3.1.5; D 3.1.7; D 3.1.16; D 3.1.28; D 3.1.29; D 3.1.30; D 3.1.46).

T 3.1.5. Food safety and microbial stability of novel products (D 3.1.5; D 3.1.6; D 3.1.7; D 3.1.15; D 3.1.16; D 3.1.17; D 3.1.18; D 3.1.26; D 3.1.31; D 3.1.32).

T 3.1.6. Researching the commercialization of production of chilled salads and fruit desserts to preserve and enhance microbial stability of these products (D 3.1.8;.D 3.1.18; D 3.1.20; D 3.1.33; D 3.1.34; D 3.1.35).

T 3.1.7. High Pressure processing of fruit products (13÷57 mo.) (D 3.1.17; D 3.1.36; D 3.1.37; D 3.1.38; D 3.1.39).

T 3.1.8. Developing a marketing plan for work package results/outcomes (D 3.1.9).

T 3.1.9. Developing fruit salads with nutraceutical and other bioactive agents (D 3.1.2; D 3.1.3; D 3.1.10; D 3.1.11; D 3.1.12; D 3.1.40; D 3.1.41; D 3.1.42; D 3.1.43).

T 3.1.10. Management of WP 3.1.

WP 3.2

T 3.2.1. Characterisation of raw materials, production and thorough analysis of processed apple products (D 3.2.1; D 3.2.2;.D 3.2.9; D 3.2.10; D 3.2.21; D 3.2.22; D 3.2.28).

T 3.2.2. Optimisation of technology and selection of raw material for production of direct, preferably cloudy juices (D 3.2.3; D 3.2.4; D 3.2.5;. D 3.2.12; D 3.2.16; D 3.2.17; D 3.2.23; D 3.2.26; D 3.2.27; D 3.2.32; D 3.2.36).

T 3.2.3. Selection of high acidity scab resistant apples, suitable for juice/concentrate production (D 3.2.3; D 3.2.5; D 3.2.6; D 3.2.11; D 3.2.13; D 3.2.14; D 3.2.15; D 3.2.24; D 3.2.35).

T 3.2.4. Transfer of specific compounds from mash to juice (D 3.2.7; D 3.2.8; D 3.2.18; D 3.2.25; D 3.2.31; D 3.2.34).

T 3.2.5. Collection of analytical data for investigated cultivars of appl;es, sour cherries, plums and black currants juices (D 3.2.8; D 3.2.19; D 3.2.29; D 3.2.37).

T 3.2.6. Consumer tests of juices (13÷57 mo) (D 3.2.20; D 3.2.33).

T 3.2.7. Management of WP 3.2.

WP 3.3

T 3.3.1. Optimization of processing of fruits by osmotic dehydration combined with drying or freeze drying (D 3.3.1; D 3.3.2; D 3.3.9; D 3.3.12; D 3.3.16; D 3.3.19; D 3.3.22; D 3.3.23; D 3.3.25.; D 3.3.33; D 3.3.34; D 3.3.36).
T 3.3.2. Evaluation of quality of investigated products in relation to raw material, processing and shelf-life conditions (D 3.3.1; D 3.3.3; D 3.3.4; D 3.3.11; D 3.3.13; D 3.3.14; D 3.3.26; D 3.3.27; D 3.3.28; D 3.3.31).
T 3.3.3. Design of new fruit based products and/or products enriched with dried fruit (D 3.3.5; D 3.3.6; D 3.3.10; D 3.3.15; D 3.3.17; D 3.3.18; D 3.3.21; D 3.3.24; D 3.3.32; D 3.3.35; D 3.3.37).
T 3.3.4. Evaluation of consumers’ response on novel products promoted as healthy snacks (D 3.3.7; D 3.3.8; D 3.3.20; D 3.3.29; D 3.3.30; D 3.3.38).
T 3.3.5. Management of the WP.

WP 3.4

T 3.4.1. Assessment of pomace as source of phytocomponents (D 3.4.1;.D 3.4.13; D 3.4.24; D 3.4.25; D 3.4.35).
T 3.4.2. Extraction and purification of polyphenols and dietary fibre from pomace (D 3.4.2; D 3.4.3; D 3.4.10; D 3.4.11; D 3.4.12; D 3.4.14; D 3.4.15; D 3.4.20; D 3.4.21; D 3.4.26; D 3.4.29; D 3.4.31; D 3.4.36).
T 3.4.3. Characteristion of polyphenol extracts and dietary fiber from pomace from apple and red fruit (D 3.4.4; D 3.4.7; D 3.4.8; D 3.4.9; D 3.4.11; D 3.4.12; D 3.4.16; D 3.4.18; D 3.4.22; D 3.4.23; D 3.4.28; D 3.4.30; D 3.4.32; D 3.4.33).
T 3.4.4. Interaction between polyphenols and cell walls: understanding formation of the pomace (D 3.4.5; D 3.4.19; D 3.4.27).
T 3.4.5. Stabilisation of red fruit cloudy juices by admixture of apple phytocomponents (D 3.4.6; D 3.4.17; D 3.4.34).
T 3.4.6. Management of WP 3.4.

Remark: Some tasks were introduced during the project and are not listed in Annex 1. These were: T 3.1.7; T 3.2.6)

Pillar 4

WP 4.1

T 4.1.1 Integrating the most effective methods (D 4.1.1; D 4.1.2; D 4.1.3; D 4.1.4; D 4.1.6; D 4.1.7; D 4.1.8; D 4.1.9; D 4.1.10, D 4.1.11)

WP 4.2

T 4.2.1 Prototype of a multi-cultivar DSS
    D 4.2.1; D 4.2.2; D 4.2.3; D 4.2.9; D 4.2.10; D4.2.11; D4.2.13; D4.2.21; D4.2.22; D 4.2.24.
T 4.2.2 Portable and non-destructive equipment for fruit quality assessment
    D 4.2.4; D4.2.12; D 4.2.18; D 4.2.19; D 4.2.20
T 4.2.3 Additional DSS databases for decision making in fruit quality management
    D 4.2.5; D 4.2.6; D 4.2.7; D 4.2.14; D 4.2.15; D 4.2.16
T 4.2.4 Testing and validation of the concept DSS
D 4.2.8; D 4.2.17; D 4.2.23

Pillar 5

WP 5.1

T 5.1.1 Development of a Crop Identification System (CIS) to adjust spray application to the target characteristics. (D 5.1.1; D 5.1.2; D 5.1.3; D 5.1.5; D 5.1.15; D 5.1.16; D 5.1.17; D 5.1.5; D 5.1.19; D 5.1.21; D 5.1.22; D 5.1.24; D 5.1.34; D 5.1.40; D 5.1.41; D 5.1.43)

T 5.1.2 Development of a Crop Health Sensor (CHS) for identification of tree health status determining pesticide application. (D 5.1.1; D 5.1.2; D 5.1.4; D 5.1.5; D 5.1.7; D 5.1.20; D 5.1.19; D 5.1.25; D 5.1.26; D 5.1.27; D 5.1.28; D 5.1.29; D 5.1.30; D 5.1.31; D 5.1.32; D 5.1.43)

T 5.1.3 Development of Environmentally Dependent Application System (EDAS) for adjusting the spraying equipment according to the environmental circumstances. (D 5.1.1; D 5.1.2; D 5.1.3; D 5.1.5; D 5.1.19; D 5.1.23; D 5.1.35; D 5.1.36; D 5.1.37; D 5.1.41; D 5.1.43)

T 5.1.4 Evaluation of the effects of the crop adapted spray applications techniques. (D. 5.1.6: D 5.1.7; D 5.1.20; D 5.1.21; D 5.1.31; D 5.1.38; D 5.1.39; D 5.1.41; D 5.1.42; D 5.1.43)

T 5.1.5 Development of a model for the prediction of nutrient demand rates. (D 5.1.8; D 5.1.10; 5.1.11; D 5.1.15; D 5.1.22)

T 5.1.6 Development of site-specific fertilizer application methods for minimized nutrient loss. (D 5.1.9; D 5.1.10; D 5.1.12; D 5.1.16; D 5.1.23)

T 5.1.7 Optimizing tree nutrition in organic fruit production to achieve top quality fruit while increasing the efficient use of nutrients and improving tree performance. (D 5.1.13; D 5.1.14; D 5.1.24)

WP 5.2

T 5.2.1 Non-alternating top quality fruit orchard cropping patterns. (D 5 2.1; D 5.2.2; D 5.2.3; D 5.2.4)

T 5.2.2 Efficient use of naturally occurring limited resources. (D 5 2.1; D 5.2.2; D 5.2.4; D 5.2.5; D 5.2.8)

T 5.2.3 Tools for an optimal exploitation of the quality potential of peach and apple. (D 5 2.1; D 5.2.4; D 5.2.6; D 5.2.7; D 5.2.9)

WP 5.3

T 5.3.1 Transdisciplinary data collection and analyses and mutual learning. (D 5.3.1; D 5.3.5; D 5.3.6)

T 5.3.2 Decision support tool for fruit growers. (D 5.3.2; D 5.3.3; D 5.3.6; D 5.3.7)

T 5.3.3 An exemplary case study for change management at the farm level for producing high quality fruit. (D 5.3.4)

Pillar 6

WP6.1

T6.1.1.Identification and mapping of candidate genes (CGs) for fruit quality in saturated maps of peach and apricot. (D6.1.1; D6.1.2; D6.1.12; D6.1.23; D6.1.33; D6.1.34)

T6.1.2. Identification and characterisation of the most relevant cell wall components of apple texture: application for QTL analysis in apple and validation of methodologies on apricot and peach. (D6.1.3; D6.1.24; D6.1.25; D6.1.26a&b; D6.1.27a&b)

T6.1.3. QTL analysis of fruit quality (D6.1.4; D6.1.13; D6.1.14; D6.1.15;

T6.1.4. Analysis of allergenicity genes in apple and peach (D6.1.5; D6.1.6; D6.1.7; D6.1.9; D6.1.16; D6.1.20; D6.1.28; D6.1.38)

T6.1.5 Allelic diversity of Candidate Genes and QTLs for fruit quality (D6.1.8; D6.1.10; D6.1.11; D6.1.17; D6.1.21; D6.1.22; D6.1.29; D6.1.30; D6.1.35; D6.1.36)

T6.1.6. Marker-assisted breeding (MAB) in apple. (D6.1.18; D6.1.19; D6.1.31; D6.1.32; D6.1.37)

WP6.2

T6.2.1. Alternative transformation methods, without antibiotic resistance genes. (D6.2.2, D6.2.3, D6.2.8, D6.2.9, D6.2.10, D6.2.17, D6.2.19, D6.2.20, D6.2.27, D6.2.31, D6.2.32, D6.2.37, D6.2.38, D6.2.39, D6.2.40, D6.2.46, D6.2.47, D6.2.48, D6.2.59, D6.2.60, D6.2.61, D6.2.62)

T6.2.2. Plant promoters that can regulate transgene expression in a constitutive or targeted way**.** (D6.2.4, D6.2.5, D6.2.6, D6.2.11, D6.2.12, D6.2.13, D6.2.14, D6.2.21, D6.2.22, D6.2.23, D6.2.28, D6.2.33, D6.2.34, D6.2.41, D6.2.42, D6.2.49, D6.2.50, D6.2.51, D6.2.52, D6.2.53, D6.2.63)

T6.2.3. Tools for “clean” gene integration or silencing technologies (D6.2.1, D6.2.15, D6.2.25, D6.2.35, D6.2.36, D6.2.43, D6.2.44, D6.2.54, D6.2.57, D6.2.58, D6.2.64)

T6.2.4. Stability of expression of the transgene along the tree life and associated risks (D6.2.7, D6.2.29, D6.2.30, D6.2.45, D6.2.55, D6.2.56, D6.2.65).

WP6.3

T6.3.1. Development of a set of common protocols within the field of sensory analysis and market research related to the objectives established and selection of new apple and peach/nectarine cultivars to be used in Tasks 6.3.2, Task 6.3.3 and 6.3.4. (D6.3.1)

T6.3.2. Consumer test of new high quality apple and peach nectarine standard varieties confronted to commercial varieties across different European regions. (D6.3.2; D6.3.3)

T6.3.3. Consumer preference mapping at European scale on the basis of consumer tests on new apple and peach/nectarine cultivars. (D6.3.4; D6.3.5; D6.3.7)

T6.3.4. Consumer tests of organically grown disease and pest resistant new apple cultivars (D6.3.6)

Pillar 7

Most of the tasks assigned to each WP in Pillar 7 were of a continuing nature. Therefore, even though a single deliverable per task is often listed, these were produced repeatedly during the life of the project, at due dates that were part of a rolling sequence. Some of the Tasks that had a 0-57 month lifetime, for example, have generated deliverables at intermediate times, that have been consolidated in the final one, provided at month 57. To reflect this, in some instances this section contains more than one reference to a single deliverable, with a progressive time of delivery. A more precise detail of the timesequence of these deliverables can be derived from the tables in Annex B of this report.

WP 7.1

Task 7.1.1: Identification of offer of training opportunities – months 0-12 (D 7.1.1).

Task 7.1.2 Identification and formation of a network of stakeholders – months 9-18 (D 7.1.2).

Task 7.1.3 Report to Project and Planning of training schemes – months 0-57 (D 7.1.3 @ months 25, 26, 48).

Task 7.1.4 Scheme of training – months 0-57 (D 7.1.4).

WP 7.2

Task 7.2.1 Internal ISAFRUIT information gathering. Survey of all relevant research within ISAFRUIT amenable to dissemination and promotion – months 0-15 (D 7.2.1).

Task 7.2.2 Establish an external stakeholder/customer database. Establish network and database of external stakeholders for two-way knowledge dissemination – months 9-57 (D 7.2.2).

Task 7.2.3 Promote innovative scientific dissemination activities. Develop and implement first stage of a plan of courses, workshops, and seminars to disseminate the knowledge of ISAFRUIT – months 0-57 (D7.2.3).

Task 7.2.4 Prepare 12-month Report and an 18-month Forward Plan for the ISAFRUIT MC. Prepare Work Package Report including future plans and actions – months 9-57 (D 7.2.4).

WP 7.3

Task 7.3.1 Knowledge and information platform defined and developed – months 0-57 (D 7.3.1 @ months 12, 30 & 42; D 7.3.2 @ month 30).

Task 7.3.2 Establish database of potentially protectable knowledge and potential end-users and identify ways to increase IP impact – months 0-57 (D 7.3.2 @ month 18 & 42; D 7.3.3 @ month 42).

Task 7.3.3 Prepare report to ISAFRUIT Core Group – months 0-57 (D 7.3.3 @ month 18 & 57; D 7.3.4 @ month 57)

WP 7.4

Task 7.4.1: Design and update of the web site – months 0-57 (D 7.4.1).

Task 7.4.2: Maintenance of the web site – months 0-57 (D 7.4.2).

Task 7.4.3: Organisation of Isafruit events – months 0-57 (D 7.4.3 @ months 24, 48, 57).

Task 7.4.4: Press releases – months 0-57 (D 7.4.4 @months 24, 48, 57).

Annex B: List of deliverables worked out

Pillar 1

Delive-rable No	Deliverable title		Delivery date	Actueal Delivery date	Nature	Dissemi-nation level
D 1.1.1	Quick scan of European fruit consumption, scientific report (2007)			2007		
D 1.1.2	Report on quick scan for European countries, on website (8.PPO, 10.LEI)	8. PPO, 10 LEI	+ 8 months	+11 months	R	Pu
D 1.1.3	Report on more detailed consumption behaviour for selected countries on website	8.PPO, 10.LEI	+ 14 months	+ 16 months	R	Pu
D 1.1.4	List of trends and developments for the selected cases	8.PP, 10.LEI	+ 16 months	+16 months	O	Pu
D 1.1.5	Trends in fruit consumption – summaries of expert interviews in Greece, the Netherlands, Poland and Spain, scientific report (2008)	8.PPO, 10.LEI	+ 16 months	+24 months	R	Pu
D 1.1.6	Trends and impacts for future fruit consumption, scientific report (2008)	8. PPO, 10. LEI	+ 24 months	+ 24 months	R	Pu
D 1.1.7	Final report	8.PPO, 10 LEI)	+ 24 months	+ 24 months	R	Pu
D 1.2.1	Theoretical Framework of Consumer Preference Formation with Respect to Fruit, scientific report (2007)			2007		
D 1.2.2.	Report on focus group results, scientific report (2007)	24.UPM, 10.LEI	+ 13 months	+20 months	R	Pu
D 1.2.3	List of motives and barriers for fruit consumption (formerly List of reasons for not eating fruit)	24.UPM10. LEI	+ 15 months	+24 months	O	Pu
D 1.2.4	List of measurement instruments, scientific report	24.UPM10. LEI	+ 16 months	+ 24 months	O	Re
D 1.2.5	Validation of the measurement instruments, scientific report (2008)	10. WUR-LEI		18 /36.	R	PP

Delive-rable No	Deliverable title		Delivery date	Actueal Delivery date	Nature	Dissemi-nation level
D 1.2.6	Report on consumer behaviour with respect to fruit (2009), scientific report	10. WUR-LEI		37/41	R	PP
D 1.2.7.	Reinders, M.J., M.C. Onwezen, S.J. Sijtsema, K.L. Zimmermann, I. van den Berg, A. Jasiulewicz, M.D. Guardia, and L. Guerrero (2010). *Deliverable 1.2.7: Cross-cultural benefit segmentation of consumers.* Scientific Report. Project no.: 016279, ISAFRUIT.	10. WUR-LEI		37/51	R	PU
D 1.3.1	Theoretical framework on consumer innovativeness for fruit, scientific report (2007)	38.WAU,10. LEI	+ 16 month	+24 months	O	Pu
D 1.3.2	List of selected fruit innovations	38.WAU. 10.LEI	+ 16 month	+24 months	O	PP
D 1.3.3.	D.1.3.3 Report on consumer focus groups discussions (month 33)	38 WAU		18/33	R	PP
D 1.3.4	Development of a questionnaire for a survey into consumer innovativeness in the context of novel fruits and fruit products (2009)	38. WAU		36 /42	R	PU
D 1.3.5	Consumer acceptance of novel fruits and fruit products, scientific report (2010)	38	WAU	M48 53	R	PU
D 1.3.5	Onwezen, M.C., Bartels, Kraszewska, M., Papoutsi, G. & Briz, T (2010). *Deliverable 1.3.5 Consumer acceptance of novel fruits and fruit products.* Scientific Report. Project no.: 016279, ISAFRUIT.	10.WUR-LEI		36 /52	R	PU

Delive-rable No	**Deliverable title**		**Delivery date**	**Actueal Delivery date**	**Nature**	**Dissemi-nation level**
D 1.3.6.	Van ’t Riet, J, Onwezen, M.C., Bartels, J. Kraszewska, M. & Briz, T. (2010). *Deliverable 1.3.6 Investigating the effects of marketing claims on the adoption of innovative fruits and fruit products: A choice experiment.* Scientific Report. Project no.: 016279, ISAFRUIT.	10. WUR-LEI		36/52	R	PU
D 1.3.7.	Bakker, T., Benninga, J., Rakowska, J. & Bartels, J (2010). *Deliverable 1.3.7 Report on case studies of fruit innovations.* Scientific Report. Project no.: 016279, ISAFRUIT.	10. WUR-LEI		36/53	R	PU
D 1.3.8.	Onwezen, M.C., Bartels, J. Kraszewska, M., Papoutsi, G. & Briz, T (2010). *Deliverable 1.3.8 Cross-Cultural Consumer Segmentation on the Relevance of Product Characteristics for Fruit-Innovation Adoption.* Scientific Report. Project no.: 016279, ISAFRUIT.	10.WUR-LEI		36/54	R	PU
D 1.3.9.	Bakker, T., Benninga, J., Rakowska, J. & Bartels, J (2010). *Deliverable 1.3.9 List of characteristics for future fruit innovations.* Scientific Report. Project no.: 016279, ISAFRUIT.	10.WUR-LEI		36/55	R	PU
D 1.3.10	Bartels, J., Onwezen, & Kraszewska, M. (2010). *Deliverable 1.3.10. Guidelines for stimulating consumer innovative behaviour.* Scientific Report. Project no.: 016279, ISAFRUIT.	10.WUR-LEI		36/56	R	PU
D 1.4.1	Literature study on supply chain performance	10.WUR-LEI	+ 18 months	+24 months	O	Pu
D 1.4.2	Conceptual framework of supply chain performance	10.WUR-LEI	+18 months	+24 months	R	Pu
D 1.4.3.	Qualitative and quantitative description of the fruit supply industry in the Netherlands, Poland, Greece and Spain, scientific report (2009)	10 WUR-LEI		33/36	R	PP

Delive-rable No	**Deliverable title**		**Delivery date**	**Actueal Delivery date**	**Nature**	**Dissemi-nation level**
D 1.4.4.	Case studies on consumer driven innovative supply chains, scientific report (2009)	10. WUR-LEI		36/43	R	PU
D 1.4.5.	Critical Success Factors of consumer driven innovative fruit supply chains, scientific report (2009)	10. WUR-LEI		36/43	R	PU
D 1.4.6.	Guidelines for stimulating SME innovativeness and for chain organisation and management, scientific report (2009)	10.WUR-LEI		36/43	R	PU
D 1.4.7.	Final Report ISAFruit WP 1.4 Innochain, scientific report (2009)	10.WUR-LEI		36/43	R	PU
D 1.5.1	Review of the strategy-related results of all Pillars and identification of successful innovation strategies, scientific report (2009)	29 AUA		44/46	R	PU
D 1.5.2	Iliopoulos, C., Theodorakopoulou, I., and Lazaridis, T. (2010), *Deliverable 1.5.2: Results of workshop/group interviews concerning the implementation of the identified strategies*. Scientific Report, ISAFRUIT Project, 6th Framework Program, Agricultural University of Athens, Athens, Greece, available at: http://www.isafruit.org/Portal/publications.php.	29 AUA		48/55	R	PU
D 1.5.3	Theodorakopoulou, I., Iliopoulos, C., and Lazaridis, T. (2010), *Deliverable 1.5.3: Roadmap for the implementation of innovation implementation and transition strategies*. Scientific Report, ISAFRUIT Project, 6th Framework Program, Agricultural University of Athens, Athens, Greece, available at: http://www.isafruit.org/Portal/publications.php	29 AUA		48/55	R	PU

Pillar 2

Deliverable No[6]	Deliverable title	Lead participant	Delivery date/actual date [7]	Nature [8]	Dissemination level[9]
D 2.1.1	Ethical approval of fruit intervention study in healthy volunteers	KVL	01-10-2006/ 01-06-2006		
D 2.1.2	Progress report on cholesterol lowering and related health effects from fruit and processed fruit fractions in animals and in humans. Guidance to fruit processing	21 KVL	18/24	R	PP
D 2.1.3	Plan for medium-term rat study	3 DFVF	21/21	O	PP
D 2.1.4	Draft plan for intervention study with fruit fractions and fruit fibre in patients with bowel disorders	21 DFVF	21/21	O	PP
D 2.1.5	Draft plan for biobank-based cohort study: selection of first set of biomarkers	15 DCS	18/18	O	PP
D 2.1.6	Manuscript for first rat study submitted for publication to an international scientific journal	3 DFVF	24/24	O	CO
D 2.1.7	Application for ethical approval of next human dietary study	21 KVL	30/35	O	CO
D 2.1.8	First manuscript based on mouse studies submitted	25 OU	30/40	O	CO
D 2.1.9	Draft manuscript for first human study distributed to co-authors	21 KVL	30/36	O	CO
D 2.1.10	Manuscript for popular scientific paper on apples and health submitted	21 KVL	30/36	O	PU
D 2.1.11	Plan for IBD (next human dietary) study accepted by ethics committee	21 KVL	36/38	O	PP
D 2.1.12	Draft manuscript for cohort based study on fruit intake and ACS colorectal cancer	15 DCS	30/29	O	CO

[6] Deliverable numbers in order of delivery dates: D1 – Dn
[7] Month in which the deliverables will be available. Month 0 marking the start of the project, and all delivery dates being relative to this start date.
[8] Please indicate the nature of the deliverable using one of the following codes:
- **R** = Report
- **P** = Prototype
- **D** = Demonstrator
- **O** = Other

[9] Please indicate the dissemination level using one of the following codes:
- **PU** = Public
- **PP** = Restricted to other programme participants (including the Commission Services).
- **RE** = Restricted to a group specified by the consortium (including the Commission Services).
- **CO** = Confidential, only for members of the consortium (including the Commission Services).

D 2.1.13	An overview paper based on task 2.1.1 submitted	3 DFVF	36/35	O	PP
D 2.1.14	Draft manuscript for human questionnaire based on study on apple/fruit and colorectal cancer ACS	15 DCS	36/36	O	CO
D 2.1.15	The second draft manuscript for the first human study distributed to co-authors	21 KVL	52/cancelled	O	CO
D 2.1.16	Overview of metabolomics data from rat studies	21 KVL	45/48	O	CO
D 2.1.17	Plan for metabolomics and chemometrics analysis of biobank samples	15 DCS	42/42	O	CO
D 2.1.18	One manuscript for each of the the short-term rat studies submitted	3 DFVF	45/48	O	CO
D 2.1.19	All patients for second human study have given informed consent	21 KVL	45/48	O	CO
D 2.1.20	Public presentation on fruit and health offered at an international fruit fair	21 KVL	42/42	O	CO
D 2.1.21	Results overview for chemometric analysis of human biobank based metabolomics study for colon cancer	15 DCS	45/48	O	CO
D 2.1.22	Draft manuscript for transgenic atherosclerosis mouse study submitted	3 DFVF	52/52	O	CO
D 2.1.23	Overview of metabolomics data from first human intervention study	21 KVL	52/52	O	CO
D 2.1.24	Report on main results overview for second human study	21 KVL	52/52	O	PP
D 2.1.25	Overview paper submitted on animal studies and health effects of fruit	3 DFVF	48/54	O	CO
D 2.1.26	At least one presentation at international Food Science meetings in collaboration with pillar 3 and/or 7	21 KVL	48	O	PU
D 2.1.27	Draft manuscript for human biobank based metabolomics study on colon cancer	15 DCS	54/56	O	PP
D 2.1.28	Status on manuscript based on second human study	21 KVL	54/54	O	PP
D 2.1.29	Manuscript on comparative metabolomics across species and study types	21 KVL	54/54	O	PP
D 2.1.30	At least one presentation at international Food Science meetings in collaboration with pillar 3 and/or 7	21 KVL	54/59	O	PU
D 2.1.31	Results overview for chemometric analysis of human biobank based study for a 600 sample subset of CVD cases and controls	KVL			
D 2.1.32	Presentation of all major results at the IHCS conference in Lisbon Aug. 2010 <u>On schedule</u>. All major results were presented at the IHCS.				

D 2.2.1	Ethical approval of pilot study	DFVF	01-05-2006		
D 2.2.2	A progress report detailing a power analysis for the performance of a main trial on fruit intake and inhibition of weight gain	3 DFVF	18/18	R	PP
D 2.2.3	Project plan for main study, if feasible	3 DFVF	27/27	O	PP
D 2.2.4	Manuscript submitted to scientific journal based on first intervention study	3 DFVF	30/30	O	CO
D 2.2.5	Written consent from participating European companies	3 DFVF	33/36	O	PP
D 2.2.6	A report detailing the baseline results of the intervention study	3 DFVF	36/40	O	PP
D 2.2.7	A short popular overview paper submitted on fruit interventions and overweight	3 DFVF	36/36	O	PU
D 2.2.8	An internal report on the intermediary measurements on fruit intake and snack patterns during the first 3 months of the fruit intervention	3 DFVF	42/45	R	CO
D 2.2.9	Manuscript on urinary flavonoids as biomarker for fruit intake submitted	3 DFVF	44/49	O	CO
D.2.2.10	Manuscript on fruit intake as s part of the snacking patterns at the workplace submitted	3 DFVF	45	O	CO
D 2.2.11	Manuscript on a cross-sectional study on fruit intake and bodyweight in Europe submitted	3 DFVF	52/54	O	PP
D 2.2.12	Manuscript on the results from the fruit intervention study submitted	3 DFVF	52/54	O	PP
D 2.3.1	A list of cultivars with low allergenicity	9 WUR-PRI	18/18	R	CO
D 2.3.2	A draft plan for sampling and analysis of low-allergenic apples along the chain	9 WUR-PRI	18/27	R	PP
D 2.3.3	Report on the allergenicity of 120-180 Breeding accessions as assessed by SPT	35 UMCG	36/36	O	CO
D 2.3.4	Report on the allergenicity of apple cultivars according to oral challenges	35 UMCG	36/36	R	PU
D 2.3.5	A master plan for sampling and analysis of low-allergenic apples along the chain	9 WUR-PRI	33/42	R	PP
D 2.3.5A	Review paper submitted on allergenicity to stone fruit in Europe: Preparation for a home page information site for fruit allergy sufferers (collaboration with pillar 7).	21 KVL	45/45	O	PP
D 2.3.6	Report or draft paper detailing grafting experiments with transgenic apples	3 DFVF	30/36	R	PP

D 2.3.7	Report on draft paper on expression analyses in transgenic apple plant tissues	3 DFVF	36/36	R	PP
D 2.3.8	Report on the allergenicity of apple cultivars at the European level	35 UMCG	48	R	PU
D 2.3.9	Paper submitted on SPT and oral tests of 60 cultivars	35 UMCG	48/52	O	CO
D 2.3.10	Report on the allergenicity of apple cultivars at the European level	9 WUR-PRI	54/54	R	CO
D 2.3.11	Report submitted on pricking location	35 UMCG	48/51	R	PU
D 2.3.12	Paper submitted on SPT of 180 breeding accessories	9 WUR-PRI	54	O	CO
D 2.3.13	Low allergenic chains	9 WUR-PRI	56/61	R	PP
D 2.3.14	Formation and testing of Mald1-silenced apples	35 UMCG	54/54	R	PP
D 2.3.15	Report presented at international horticulture conference in Lisbon on allergy along the chain	9 WUR-PRI	57	R	PU
D 2.4.1	Sampling schemes and plans for distribution to the analytical laboratory for fruit collected from experiments under WP 4.1, 4.2, and 5.1	3 DFVF	18/18	R	PP
D 2.4.2	Analytical programme for experiments to be conducted during second harvest	3 DFVF	18/18	R	PP
D 2.4.3	Draft manuscript on analytical method for pesticides and patulin	3 DFVF	30/30	O	PP
D 2.4.4	Submitted manuscript on result of pesticide residues analysed from pilot spraying experiment	3 DFVF	48/52	O	PU
D 2.4.5	Submitted manuscript results of pesticide residues and patulin in samples from experiments concerning the chain from harvest to consumer	3 DFVF	45/55	O	PU
D 2.4.6	Submitted manuscript on the susceptibility of different apple cultivars used in organic horticulture naturally and artificially infected with penicillium expansum	3 DFVF	48/52	R	PP
D 2.4.7	Submitted report on result of pesticides residues analysed from spraying experiment in 2010 (together with WP 5.1)	3 DFVF	57/57	R	PP

Pillar 3

Work Package 3.1 – Leader Dr Ronan Gormley (till retirement), then Dr Nigel Brunton

(AFRC, Partner No 34)

Delive-rable No	Deliverable title	Lead participant	Delivery date	Nature[1]	Dissemi-nation level[2]
D 3.1.1	Delivery of optimised raw material to the partners on an ongoing basis.	34 AFRC	M18	O	PP
D 3.1.2	Protocols for the application of freeze-chill and *sous vide* processes delivered and new generation fruit products (phase 1 products) developed.	34 AFRC	M18	R+P	PP+CO (industrial aspects)
D 3.1.3	New generation of fruit salad products (phase 1 products) prepared and tested.	34 AFRC	M18	P	CO
D 3.1.4	Progress report on the effect of microwave treatment on sensorial acceptance of the novel products and on their physical properties (colour and texture).	4 IRTA	M18	R	PP
D 3.1.5	Progress report on effect of microwave treatment on FBPs determined.	4 IRTA	M18	R	PP
D 3.1.6	Knowledge of population dynamics of FBPs on fresh cut fruits.	4 IRTA	M18	R	PU
D 3.1.7	Progress report on efficacy of biocontrol agents in preventing FBPs development on minimally processed fresh fruits.	4 IRTA	M18	R	PP
D 3.1.8	Progress report on researching on commercialisation of chilled fresh fruit salads production.	56 NBL	M18	O	PP
D 3.1.9	Blueprint on the marketing plan for the dissemination/technology transfer of work package.	34 AFRC	M18	R	PP
D 3.1.10	Probiotic apple slices.	34 AFRC	M30	R	PP
D 3.1.11	Suitable apple cultivars for fruit salad applications.	34 AFRC	M30	R	PP
D 3.1.12	Quality status of supermarket apple samples.	34 AFRC	M24	R	PP
D 3.1.13	Second generation freeze chilled and sous vide apple products.	34 AFRC	M30	R	PP
D 3.1.14	Partner apple cvs. (first tranche) for process applications identified.	34 AFRC	M30	R	PP
D 3.1.15	Progress report on the effect of electromagnetic (microwave and radio frequency) treatment on sensorial acceptance of the novel products and on their physical properties (colour and texture).	4 IRTA	M30	R	PP
D 3.1.16	Progress report on the efficacy of bacteriocins in preventing FBPs development on minimally processed fresh fruits.	4 IRTA	M30	R	PP
D 3.1.17	Initial report on effect of HPP treatment at 900 MPa of apple products.	4 IRTA	M30	R	PP
D 3.1.18	Progress report on the microbial stability of novel products treated by industrial microwaves.	23 UdL	M30	R	PP
D 3.1.19	Suitability of 'Sampion' apples for fresh fruit salads.	56 NBL	M24	O	CO
D 3.1.20	Establishment of acceptable benchmarks for retailing.	56 NBL	M30	O	CO
D 3.1.21	Delivery of optimized raw material to the partners on an ongoing basis (Phase 2).	4 IRTA; 34 AFRC	M30	O	PP
D 3.1.22	Delivery of optimized raw material to the partners on an ongoing basis (Phase 3)	4 IRTA; 34 AFRC	M42	O	PP
D 3.1.23	Third generation chilled, freeze - chilled and sous vide apple products.	34 AFRC	M42	R	PP
D 3.1.24	Partner apple CVs (second tranche) for process applications identified.	34 AFRC	M42	R	PP
D 3.1.25	Product process applications to other permanent fruits (phase 1).	34 AFRC	M54	R	PP
D 3.1.26	Report of population dynamics of FBPs on fresh-cut melon and pineapple.	23 UdL	M30	R	PU
D 3.1.27	Design of new fruit products for electromagnetic treatment.	4 IRTA	M42	R	PP

Delive-rable No	Deliverable title	Lead participant	Delivery date	Nature[1]	Dissemi-nation level[2]
D 3.1.28	Progress report on efficacy of biocontrol agents in preventing FBPs development on minimally processed fresh fruits (apple and peach) (Phase 2).	4 IRTA	M42	R	PP
D 3.1.29	Progress report on efficacy of bacteriocins in preventing FBPs development on minimally processed fresh fruits (*In vivo* studies, Phase 2). Dependent on D.3.1.16 outcome.	4 IRTA; 56 NBL	M42	R	PP
D 3.1.30	Progress report on efficacy of GRAS agents in preventing FBPs development on minimally processed fresh-cut fruits.	4 IRTA; 56 NBL	M42	R	PP
D 3.1.31	Effect of HPP treatment on indigenous microbiota on fruit products and their shelf life.	23 UdL; 4 IRTA	M48	R	PP
D 3.1.32	Report of population dynamics of FBPs on fresh-cut fruit packaged in MAP.	23 UdL	M36	R	PU
D 3.1.33	Progress report on experiences with a range of trays/films combinations for fruit salad.	56 NBL	M42	O	CO
D 3.1.34	Progress report on chlorine and alternatives (Citrox and Catallix) as pre-wash for fresh cut fruits.	56 NBL	M42	O	CO
D 3.1.35	Suitability of Shampion apple for fresh fruit salads (phase 2 test)	56 NBL	M42	O	PP
D 3.1.36	Final report on effect of HPP treatment at 400, 600 and 900 MPa of fruit products.	4, IRTA	M48	R	PP
D 3.1.37	Design of a HPP treatment for fruits products.	4 IRTA	M48	R	PP
D 3.1.38	Inter-comparison of HPP systems.	4 IRTA; 34 AFRC	M42	R	PP
D 3.1.39	Optimization of HPP treatments for novel fresh fruit products.	4 IRTA; 56 NBL	M54	O	CO
D 3.1.40	Apple slices with nutraceuticals and/or edible coatings.	34AFRC; 56 NBL	M42	R	PP
D 3.1.41	Suitable apple cultivars for fruit salad applications (tranche 2 samples).	34 AFRC; 56 NBL	M42	R	PP
D 3.1.42	Product/process applications.	34 AFRC, 56 NBL	M54	R	CO
D 3.1.43	Probiotic apple slices.	34 AFRC 56 NBL	M48	R	CO
D 3.1.44	Shelf life of process products.	34 AFRC	M54	O	PP
D 3.1.45	Oligosaccharide stability in heat processed apple purée system.	34 AFRC	M54	O	PP
D 3.1.46	Final report on efficiency of bacteriocin or GRAS combined with novel technologies in preventing grows of microorganism.	4 IRTA; 23 UdL	M53	R	PP

[1]The nature of the deliverable using one of the following codes:

- **R** = Report
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- **D** = Demonstrator
- **O** = Other

[2]Please indicate the dissemination level using one of the following codes:

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Work Package 3.2 – Leader Prof. Dr Witold Plocharski (RIPF, Partner No 11)

Deliverable No	Deliverable title	Lead participant	Delivery date	Nature	Dissemination level
D 3.2.1	Production of processed apple products needed for medical studies (purees, clear and cloudy juices.	11 RIPF	M6	R	PP
D 3.2.2	Characterisation of raw material and processed products prepared for medical studies.	11 RIPF	M6	R	PP
D 3.2.3	Method of analysis of polyphenols in plums elaborated.	5 INRA	M22	R	PP
D 3.2.4	Visit of Ph.D. student for analyses of DF and phenols in red fruits and juices.	5 INRA 11 RIPF	M8	R	PP
D 3.2.5	Progress method on optimised technology of direct juice/nectar production.	11 RIPF	M14	R	PP
D 3.2.6	Progress method on selection of scab resistant apple cultivars suitable for juice industry.	11 RIPF	M11	R	PP
D 3.2.7	Progress report on conditions of better extraction of procyanidins to apple and polyphenols to plum juice.	5 INRA	M18	R	PP
D 3.2.8	Analytical Data for the COP from the first season.	11 RIPF	M18	R	PP
D 3.2.9	Production of processed apple product needed for medical studies (clear and cloudy juices) - harvest 2006.	11 RIPF	M17	R	PP
D 3.2.10	Characterisation of raw material and processed products prepared for medical studies - harvest 2006.	37 TU Lodz	M17	R	PP
D 3.2.11	Report on juice sensory analysis, expert panel tests.	65 ESA	M 18	R	PP
D 3.2.12	Study visit of Ph.D. student to analyse phenols in red fruits juices and pomaces.	5 INRA-URC	M18	R	PU
D 3.2.13	Juice production on a laboratory scale.	11 RIPF	M24	R	PP
D 3.2.14	Production of apple juices for consumer testing.	11 RIPF	M24	R	PP
D 3.2.15	Juice chemical composition analysis.	11 RIPF	M24	R	PP
D 3.2.16	Report on progress in optimising technology of direct juice/nectar production – harvest 2007.	11 RIPF	M28	R	PP
D 3.2.17	Report on cell wall and dietary fibre content in red fruit juices.	5 INRA-URC	M33	R	PP
D 3.2.18	Report on transfer of polyphenols from fruit and fruit skin to juice.	5 INRA-URC	M41	R	PP
D 3.2.19	Analytical Data for the COP from the second season.	11 RIPF	M30	R	PP
D 3.2.20	Report on consumer testing of juices produced by RIPF and INRA-URC.	65 ESA	M30	R	PP
D 3.2.21	Characterisation of chemical composition of raw materials (apples, blackcurrant and sour cherries) provided to 2.1 – season 2007.	37 TU-Lodz	M31	R	PP
D 3.2.22	Fruit supply, production and characterisation of ingredients from pomace for studies in WP 3.1 – season 2008.	37 TU-Lodz	M35	R	PP
D 3.2.23	Report on sensory evaluation of red fruit juices by experts.	65 ESA	M36`	R	PU
D 3.2.24	Production of juices at the laboratory scale and report on technology and on composition.	11 RIPF	M38	R	PP
D 3.2.25	Report on transfer of procyanidins from plum to plum juice.	5 INRA-URC	M54	R	PU
D 3.2.26	Report on progress in optimising technology of direct juice/nectar production – harvest 2008.	11 RIPF	M40	R	PP
D 3.2.27	Report on cloud composition in cloudy juices.	37 TU-Lodz	M40	R	PP
D 3.2.28	Report on composition of raw material provided to W.P. 2.1. from harvest 2008.	37 TU Lodz	M41	R	PU
D 3.2.29	Analytical data for COP.	11 RIPF	M42	R	PU
D 3.2.30	Production of red fruit juices for consumer tests.	11 RIPF	M43	R	PP
D 3.2.31	Report on impact of PEF on polyphenol extractability from apple skin and mash.	5 INRA-URC	M54	R	PP
D 3.2.32	Report on chemical composition of red fruit juices for consumer tests.	11 RIPF, 37 TUL	M48	R	PP
D 3.2.33	Consumer tests on cloudy red fruit juices.	65 ESA	M48	R	PP
D 3.2.34	Report on transfer of procyanidins and other polyphenols from plum to plum juice.	5 INRA	M50	R	PP

Delive-rable No	Deliverable title	Lead participant	Delivery date	Nature	Dissemi-nation level
D 3.2.35	Report on chemical composition of juices from selected French and Polish apple cultivars.	5 INRA-U, 11 RIPF, 37 TU-Lodz	M51	R	PP
D 3.2.36	Industrial trials with cloudy red fruit juices (conditionally depending on processing plant availability).	11 RIPF, 54 Alpex	M52	R	PP
D 3.2.37	Analytical data for COP – third season.	11 RIPF	M52	R	PU

Work Package 3.3 – Leader Dr Catherine Bonazzi (INRA – Genial, Partner No 5)

Delive-rable No	Deliverable title	Lead participant	Delivery date	Nature	Dissemi-nation level
D 3.3.1	Kinetics of osmotic dehydration versus operating conditions.	5 INRA-G.	M15	R	PP
D 3.3.2	Kinetics of transformation of fruits related to osmotic dehydration parameters.	5 INRA-G.	M14	R	PP
D 3.3.3	Report on nutritional value of dried products.	37 TU-Lodz	M18	R	PP
D 3.3.4	Sorption isotherms prepared for selected products and technologies.	37 TU-Lodz	M18	O	PP
D 3.3.5	Experimental batches of dried materials.	11 RIPF	M17	O	PP
D 3.3.6	Batches of dried material for testing in WP 2.1.	55 CELIKO	M 15	O	PP
D 3.3.7	Questionnaire on consumers’ expectations towards dried fruits	11 RIPF	M10		
D 3.3.8	A database on consumers' expectation.	11 RIPF	M18	O	PP
D 3.3.9	SHS pilot dryer.	5 INRA-G.	M19	P	PP
D 3.3.10	Specifications for new dried products.	55 CELIKO	M21	R	PP
D 3.3.11	Report on nutritional value of raw material 2006 and 2007	37 TU-Lodz	M24	R	PP
D 3.3.12	Optimized conditions on sensorial criteria.	11 RIPF	M25	R	PP
D 3.3.13	Stability of FOS in dried products.	37 TU-Lodz	M25	R	PP
D 3.3.14	Sorption isotherms on dried fruits.	37 TU-Lodz 5 INRA-G.	M52	O	PP
D 3.3.15	New processing line.	55 CELIKO	M26	P	PP
D 3.3.16	Progress report on impact of soaking solution on osmo-convective dehydrated or freeze dried fruits.	5 INRA-G.	M27	R	PP
D 3.3.17	Recipe and technological concepts for the production of new products (confid.).	55 CELIKO	M27	O	CO
D 3.3.18	Processing line for pilot production of osmo-convective dried products.	55 CELIKO	M28	O	PP
D 3.3.19	Impact of syrup management and drying parameters on antioxidants in red fruits.	11 RIPF	M30	R	PP
D 3.3.20	Report on the analysis of collected data.	11 RIPF	M30	R	PU
D 3.3.21	Proposal of a range of new products.	55 CELIKO	M32	O	CO
D 3.3.22	Feasibility of SHS drying for fruits.	5 INRA-G	M54	R	PU
D 3.3.23	Influence of DII process and variety on polyphenols in apples.	5 INRA-G	M33		PU
D 3.3.24	Products for consumers tests in T3.3.4.	55 CELIKO	M33	D	PU
D 3.3.25	Impact of osmo-dehydration parameters on nutrients in osmo-dried apples.	5 INRA-G	M37	R	PU
D 3.3.26	Nutritional value of raw materials from 2008.	37 TU-Lodz	M37	R	PU
D 3.3.27	Sorption isotherms for final products.	37 TU-Lodz	M38	R	PU
D 3.3.28	Distribution of selected nutrients between dried fruits and hypertonic solution.	37 TU-Lodz	M39	R	PU
D 3.3.29	Acceptability of tested fruit products.	65 ESA	M40	R	PP
D 3.3.30	Report on consistency of consumers’ intentional behaviour with real behaviour.	11 RIPF	M40	R	PU
D 3.3.31	Influence of 12-months storage on properties of dried products.	37 TU-Lodz	M42	R	PU
D 3.3.32	Optimized parameters for osmotic treatments.	55 CELIKO	M42	R	PP

Delive-rable No	Deliverable title	Lead participant	Delivery date	Nature	Dissemi-nation level
D 3.3.33	Optimization of osmo convective drying of red fruits (black currant, sour cherry) for taste and antioxidants (scientific publication).	11 RIPF	M52	R	PU
D 3.3.34	Optimisation of osmo convective drying of apples (scientific publication).	5 INRA	M45	R	PU
D 3.3.35	Optimised description of the pocessing line.	55 CELIKO	M45	R	CO
D 3.3.36	Modelling of antioxidant degradation vs dehydration parameters	5 INRA	M55	R	PU
D 3.3.37	Technical documentation of new products (confidential).	55 CELIKO	M47	R	CO
D 3.3.38	Influence of nutritional and health information on consumers' acceptability of dried products.	11 RIPF	M48	R	PU

Work Package 3.4 – Leader Dr Catherine Bonazzi (INRA – Avignon, Partner No 5)

Delive-rable No	Deliverable title	Lead participant	Delivery date[10]	Nature	Dissemi-nation level
D 3.4.1	Report on pomace compositions and variability.	5 INRA-URC	M18	R	PP
D 3.4.2	Progress report on extractability and proposal of method for larger scale extraction.	37 TU-Lodz	M18	R	PP
D 3.4.3	Apple extracts: chlorogenic acid-rich polyphenol extract, procyanidins with varying sizes and high viscosity soluble fibre.	48 VDV	M8	R	PP
D 3.4.4	Progress report on composition and properties of pomace extracts.	5 INRA-URC	M22	R	PP
D 3.4.5	Progress report on mode of retention of polyphenols in pomace and their extractability; proposal for optimization of extraction.	5 INRA-URC	M21	R	PP
D 3.4.6	Report on impact of apple extracts on stability of red fruit juices.	11 RIPF	M21	R	PP
D 3.4.7	Report on influence of enzymes used in juice technology on fruit glycosides.	37 TU-Lodz	M21	R	PP
D 3.4.8	Data on properties of black currant industrial pomace collected.	37 TU-Lodz	M21	R	PP
D 3.4.9	Report on MS characterization of isolated phenolics.	5 INRA-URC	M24	R	PP
D 3.4.10	Polyphenol-enriched extracts from black currant.	48 VDV	M26	R	PP
D 3.4.11	Report on DF composition ad properties in pomaces.	37 TU-Lodz	M27	R	PP
D 3.4.12	Samples of dried, ground apple pomaces sent for further studies to other partners.	37 TU-Lodz	M28	R	PP
D 3.4.13	Report on characterization of pomace composition.	37 TU-Lodz	M30	R	PP
D 3.4.14	Production of polyphenol concentrates and post-extraction DF.	37 TU-Lodz	M30	R	PP
D 3.4.15	Report on characterization of polyphenol concentrates.	37 TU Lodz	M30	R	PP
D 3.4.16	Report on composition and properties of DF in nectars.	5 INRA- AV	M33	R	PP
D 3.4.17	Report on chemical composition and cloud stability in red fruit nectars.	11 RIPF	M30	R	PP
D 3.4.18	Study visit of PhD student from TU Lodz to INRA-URC to analyse by HPLC-MS the components of red fruits extracts.	37 TU-Lodz	M38	R	PP
D 3.4.19	Report on enzymatic digestibility of model apple pomace.	5 INRA-URC	M54	R	PU
D 3.4.20	Report on impact of drying and use of ultrasound for polyphenol extraction from apples.	5 INRA-AVI	M34	R	PP
D 3.4.21	Large scale production of BC extracts from industrial dried BC pomace (phenolics and DF).	48 Val-de-Vire	M34	R	PP
D 3.4.22	Report on influence of enzymes used in sour cherry juice technology on phenolic glycosides.	37 TU-Lodz	M35	R	PU
D 3.4.23	Report on properties of polyphenol concentrates and post-extraction dietary fibre from selected scab-resistant apples pomaces.	37 TU-Lodz	M39	R	PU

[10] Month in which the deliverables will be available. Month 0 marking the start of the project, and all delivery dates being relative to this start date.

Delive-rable No	Deliverable title	Lead participant	Delivery date[10]	Nature	Dissemi-nation level
D 3.4.24	Report on characterization of pomace from industrial experimental drier.	37 TU-Lodz	M40	R	PP
D 3.4.25	Report on dietary fibre in plums.	37 TU-Lodz	M41	R	PU
D 3.4.26	Report on polyphenol concentrates and post extraction dietary fibre preparations from blackcurrant and cherry.	37 TU-Lodz	M42	R	PU
D 3.4.27	Report on impact of mash oxidation on polyphenols extractability of apple pomace.	5 INRA-AVI	M52	R	PU
D 3.4.28	Proanthocyanidins from cherry and plum fruit and pomace.	5 INRA-URC	M43	R	PP
D 3.4.29	Report on composition of extract from red fruits for enrichment studies.	37 TU-Lodz	M46	R	PP
D 3.4.30	Report on dietary fibre in plums – synthesis.	37TU-Lodz	M47	R	PP
D 3.4.31	Report on use of innovative technologies for extraction of apple polyphenols.	5 INRA-URC	M48	R	PP
D 3.4.32	Report on MS characterization of phenolics from red fruit extracts and isolates.	37 TU Lodz	M49	R	PP
D 3.4.33	Summary on the influence of enzymes on fruit polyphenols.	37 TU-Lodz	M50	R	PP
D 3.4.34	Effect of phytocomponents on colour and turbidity of red fruit nectars.	11 RIPF	M52	R	PP
D 3.4.35	Rapport on pomace composition – synthesis.	37 TU-Lodz	M51	R	PU
D 3.4.36	Characterization and extractability of pomace fractions resulted from industrial fractionation of wet pomaces.	37 TU-Lodz	M51	R	PP

Pillar 4 – List of deliverables worked out

Work Package 4.1 – Leader Prof. Paolo Bertolini

Deliverable No	Deliverable title	Lead participant	Delivery date	Nature	Dissemina-tion level
D 4.1.1	Collecting and growing of the available (registered for use or in the pipe) biological control agents	6 UNI.BO 4 IRTA 13 UGEM	9	R	PP
D 4.1.2	Aassessment of the tolerance to hot water treatment of the main varieties of peaches and nectarines, particularly of the new ones, and of a few new apple varieties.	6 UNI.BO 4 IRTA	9 (18 for apple)	R	PP
D 4.1.3	Collecting or extracting the natural substances for GRAS treatments	6 UNI.BO	9	R	PP
D 4.1.4	First results of the efficacy of the combined treatments (physical, biological and GRAS)	6 UNI.BO 4 IRTA 8WUR PPO 41 EMR	18	R	PP
D 4.1.6	Separate and combined effects of Hot Water Treatment and BCA on post- harvest rot in different apple cultivars	6 UNI.BO	30	R	PP
D 4.1.7	Separate and combined effects of Hot Water Treatment and GRAS treatments on post harvest rot in different apple cultivars	8 WUR. PPO	30	R	PP
D 4.1.8	Separate and combined effects of Hot Water Treatment, BCA andor GRAS on post harvest rot in peaches and nectarines	4 IRTA -UNI.BO	36	R	PP
D 4.1.9	Effect of combined treatments (BCA and HW) on postharvest rot during commercial storage on apples	8 WUR PPO	46	R	PP
D 4.1.10	Provide a protocol for the control of postharvest rots of peaches and nectarines by hot water treatment and by GRAS treatment	6 UNI-BO	48	R	PP
D 4.1.11	Application of Hot Water Treatment on plums: first evaluation	6 UNI-BO	50	R	PP

Work Package 4.2 – Leader Dr Ria Derkx (WUR-PPO, Partner 8)

Deliverable No	Deliverable title	Lead Participant	Delivery date	Nature	Dissemination level
D 4.2.1	DSS design and first evaluation of data	11 RIPF	8	R	PP
D 4.2.2	First evaluation calculation rules underlying DSS decision making	11 RIPF	15	R	PP
D 4.2.3	First evaluation of programmed concept DSS	11 RIPF	18	R	PP
D 4.2.4	Preliminary experiments and first evaluation of concept ND	4 IRTA	18	R	PP
D 4.2.5	First evaluation of 1-MCP effects on fruit quality and characteristics of DSS decision making	17 KOB	18	R	PP
D 4.2.6	First evaluation of hot water treatment effects on DSS decision making	8 WUR-PPO	18	R	PP
D 4.2.7	First evaluation of Chlorophyll Fluorescence storage control on DSS decision making	31 LAIMB	18	R	PP
D 4.2.8	First concept DSS ready for testing in existing apple and peach chains	11 RIPF	27	R	PP
D 4.2.9	First version of firmness model of peaches integrated in DSS system	11 RIPF	28	R	PP
D 4.2.10	First version of the DSS model for apples and peaches including helpsystem	11 RIPF	30	R	PP

Deliverable No	Deliverable title	Lead Participant	Delivery date	Nature	Dissemination level
D 4.2.11	Second evaluation data and calculation rules underlying DSS decision making	11 RIPF	32	R	PP
D 4.2.12	Second evaluation of concept non-destructive equipment	4 IRTA	32	R	PP
D 4.2.13	Second evaluation of programmed concept DSS	11 RIPF	34	R	PP
D 4.2.14	Second evaluation of 1-MCP effects on fruit quality and characteristics of DSS decision making	17 KOB	34	R	PP
D 4.2.15	Second evaluation of hot water treatment effects on fruit quality and characteristics of DSS decision making	8 WUR-PPO	34	R	PP
D 4.2.16	Second evaluation of Chlorophyll Fluorescence storage control on fruit quality and characteristics of DSS decision making	31 LAIMB	34	R	PP
D 4.2.17	Second concept DSS ready for testing in existing apple and peach chains	11 RIPF	45	R	PP
D 4.2.18	Evaluation of the use of DA meter in peach in order to define the best storage strategy, consumer acceptance in relation to ripening and optimal timing of 1-MCP application.	6 UNIBO	46	R	PP
D 4.2.19	Data processing to improve peach quality determination by non destructive techniques.	4 IRTA	48	R	PP
D 4.2.20	Evaluation of the use of DA meter in apple in order to define the best storage strategy consumer acceptance in relation to ripening and optimal timing of 1-MCP application.	6 UNIBO	54	R	PP

Deliverable No	Deliverable title	Lead Participant	Delivery date	Nature	Dissemination level
D 4.2.21	The importance of aroma volatile biosynthesis for overall quality of peach fruit	23 UdL	54	R	PP
D 4.2.22	Changes in cell wall composition underlying firmness loss in peach and nectarine fruit	23 UdL	54	R	PP
D 4.2.23	Testing and validation of concept DSS in existing and simulated apple and peach chains	8 WUR-PPO	57	R	PP
D 4.2.24	Final version of the prototype DSS model for apples and peaches, including help-system	11 RIPF	57	R	PP

Pillar 5

Work Package 5.1 – Leader Dr Gergorzy Doruchowski (RIPF Partner No 11)

Deliverable No	Deliverable title	Lead participant	Delivery date	Nature	Dissemi-nation level
D 5.1.1	The principals of crop adapted spray application methods elaborated (Tasks 1-3)	11.RIPF, 32.DEIAFA 9.WUR-PRI 8.WUR-PPO	M6	R	CO
D 5.1.2	Sensors for crop adapted spray application developed (Tasks 1-3)	32.DEIAFA	M13	P	CO
D 5.1.3	Software for crop adapted spray application developed (Tasks 3,5)	11.RIPF	M13	P	CO
D 5.1.4	Results of laboratory test of crop health sensor obtained (Task 2)		was transformed into 5.1.7.	R	PU
D 5.1.5	Crop adapted spray application systems developed (Tasks 1-3)	11.RIPF	M18	P	CO
D 5.1.6	Protocols for evaluation of crop adapted spray application techniques delivered (Task 4)	8.WUR-PPO	M24	R	RE
D 5.1.7	Results of preliminary biological test for crop heath sensor obtained and disseminated (Task 4)	8.WUR-PPO	M15	R	PU
D 5.1.8	Model of nutrient demand rates delivered (Task 5)	6.UNIBO	M12	P	CO
D 5.1.9	Protocol for samples collection and analysis of nutrient loss delivered (Task 6)	26.CSIC	M3	R	RE
D 5.1.10	Set-up of experimental sites for nutrient application defined (Tasks 5,6)	6.UNIBO 26.CSIC	M6	R	RE
D 5.1. 11	Preliminary data on nutrient uptake delivered (Task 5)	6.UNIBO	M36	R	PU
D 5.1.12	Samples and meteorological data collected, data on nutrient (nitrogen, iron) leaching delivered (Task 6). New title: Data on leached N and Fe assessed	26.CSIC	M36	R	PE
D 5.1.13	Progress report and dissemination of the results of the first 12 months on organic fertilizer testing trials, organic fertigation systems and the comparison of various tree nutrition concepts in organic apple production (Task 7)	14.FiBL	M13	R	PP
D 5.1.14	Working plan for activities of months 12-24 based on preliminary data of first year developed (Task 7)	14.FiBL	M11	R	PP
D 5.1.15	Results of preliminary spray depostion field tests with DIS system obtained and disseminated (Task 1)	32.DEIAFA	M24	R	PP/PU
D 5.1.16	CIS software updated on the basis of experimental results (Task 1)	32.DEIAFA	M36	P	CO
D 5.1.17	CIS equipped sprayer ready for biological tests (Task 1)	32.DEIAFA	M36	P	RE
D 5.1.18	Results of field test of EDAS system obtained (Task 3)	11.RIPF	M36	R	PU
D 5.1.19	CIS, CHS and EDAS integrated on a prototype model (Task 3)	11.RIPF	M48	P	CO
D 5.1.20	Results of spectral measurements of infected leaves reported (Task 2,4)	9.WUR-PRI	M48	R	PP
D 5.1.21	Results of pesticide residue in fruit sprayed with CASA sprayer disseminated (Task 1,2,4)	8.WUR-PPO	M57	R	PP
D 5.1.22	Data on nutrient uptake on apple and peach delivered (Task 5)	6.UNIBO	M48	R	RE
D 5.1.23	Fertilization strategies for reducing leaching assessed (Task 6)	26.CSIC	M36	R	RE
D 5.1.24	Final reports on experimental and dissemination activities accomplished (Task 7)	14.FiBL	M36	R	PP/PU
D 5.1.26	Results of CHS verification and spectral measurements of different nutrient uptakes (Task 2)	9.WUR-PRI	M48	R	PP
D 5.1.28	Results of CHS verification and spectral measurements on early disease detection and leaf disease development in time (Task 2)	9.WUR-PRI	M48	R	PP
D 5.1.29	First prototype of CHS system (Task 2)	9.WUR-PRI	M57	P	CO

Deliverable No	Deliverable title	Lead participant	Delivery date	Nature	Dissemi-nation level
D 5.1.30	Development of algorithm relating crop health sensor signal and spraying a plant protection product(Task 2)	9.WUR-PRI	M57	P/RE	CO
D 5.1.31	Results of first tests of CHS system prototype in orchard (Task 2)	9.WUR-PRI	M57	R	PU
D 5.1.32	Integration of first protoype of CHS system on sprayer (Task 2)	9.WUR-PRI	M57	P	CO
D 5.1.33	Biological tests of CIS sprayer	32.DEIAFA	M36	P	CO
D 5.1.34	Results of biological tests with CIS sprayer disseminated (Task 1)	32.DEIAFA	M48	R	PP/PU
D 5.1.35	Software for EDAS integrated	11.RIPF	M36	R	PP
D 5.1.36	Results of field measurements (spray deposit and drift) with integrated EDAS system obtained (Task 3)	11. RIPF	M48	R	PP
D 5.1.37	EDAS software modified based on the results of field measurements (Task 3)	11. RIPF	M48	R	PP
D 5.1.38	Analysis and report of deposition and biological efficacy trials with CIS (Italy), EDAS (Poland) and reference sprayers delivered	8.WUR-PPO	M48	R	PP
D 5.1.39	Report on estimated effect of technology developed (crop adapted spray application) on fruit production costs delivered (interaction with T5.3.1., Esther Bravin	8.WUR-PPO	M48	R	PP
D 5.1.40	Results of spray distribution tests with CIS sprayer disseminated (Task 1)	32.DEIAFA	M48	R	PP
D 5.1.41	Report on Trans Europe Demo Tour of CASA integrated sprayer	8.WUR-PPO	M48	R	PU
D 5.1.42	Results of spray drift measurements delivered	8.WUR-PPO	M48	R	PP
D 5.1.43	Papers and presentations at Int. Workshop SuProFruit (Wageninden, September 2009) delivered	8.WUR-PPO 9.WUR-PRI 11. RIPF 32.DEIAFA	M48	R	PU
D 5.1.44	Results of fruit quality evaluation as affected by Fe application	11. RIPF	M57	R	PP
D 5.1.45	Progress Report on the Interaction Plan (Pillar 5)	2. ACW 6. UNIBO 11. RIPF	M57	R	PP

Work Package 5.2 – Leader Prof Guglielmo Costa (UNIBO, Partner No 6)

Deliverable No	Deliverable title	Lead participant	Delivery date	Nature	Dissemi-nation level
D 5.2.1	Protocol (crop load adjustment study – Task 1) Protocol (for shading studies – Task 2) Protocol (to irrigate trees under nets – Task 2) Protocol (for biometric determination – Task 3)	6.UNIBO 5.INRA IRTA	M12 M24 M36 M48	R	PP
D 5.2.2	Records (of natural crop load etc. – Task 1) Records (bio data and climate – Task 2) Records (of return bloom – Task 2)	6.UNIBO	M12 M24 M36 M48	R	PP
D 5.2.3	Preparation of molecular tools to assess fruit allergenicity (Task 1)	33.UNIPD 6.UNIBO	M24 M36	D	PP
D 5.2.4	Statistical analyses (Tasks 1-3)	6.UNIBO 5.INRA	M24 M36	R	PP
D 5.2.5	Feasibility study for commercial orchards (Task 2)	2. ACW	M24 M36	R	PP
D 5.2.6	Isolation of abscission-related genes (Task 3)	6.UNIBO 33.UNIPD	M24 M36	R	PP
D 5.2.7	Prediction of the apple fruit weight two months before harvest (Task 3)	6.UNIBO 5.INRA	M12 M24 M36	R	PP
D 5.2.8	Methodology for determination of lenght of shading for thinning	6. UNIBO	M48	R	PP
D 5.2.9	Implementation of the abscission related genes collection	33.UNIPD	M48	R	PP
D 5.2.10	Progress Report on the Interaction Plan (Pillar 5)	6. UNIBO	M57	R	PP
D 5.2.11	Forecasting the June drop for apple	2.ACW	M57	R	PU

Work Package 5.3 – Leader Esther Bravin (ACW, Partner No 2)

Deliverable No	Deliverable title	Lead participant	Delivery date	Nature	Dissemi-nation level
D 5.3.1	Data set documented (Task 1)	6. UNIBO 2.ACW	M12 M24	O	RE
D 5.3.2	Potential for an IT-model clarified (evaluation of key factors, including sensitivity analysis and model testing) (Task 2)	8.WUR-PPO	M24	D	RE
D 5.3.3	Data standardisation procedure developed (Task 2)	8.WUR-PPO	M24	R	PU
D 5.3.4	Case study identified and conceptualised (Task 3)	28. GRAB	M24	R	PU / RE
D 5.3.5	Comparison of collected data for each country (Task 1)	2.ACW	M24	R	RE
D 5.3.6	Identify success factor (Task 1) (Former: Draft with scenarios)	2.ACW	M36	R	PP
D 5.3.7	Evaluate risk profile (Task 2)	8.WUR-PPO	M48	R	PP
D 5.3.8	Progress Report on the Interaction Plan (Pillar 5)	2. ACW	M48 M57	R	PP

Pillar 6

Work Package 6.1– Leader Pere Arus (IRTA, Partner No 4)

Deliv. No	Deliverable name	Lead participant	Delivery date	Nature	Dissemi-nation level
D 6.1.1	Saturated maps of the 4 Prunus populations obtained	P5 (Avignon)	18	O	PU
D 6.1.2	A collection of candidate genes for *Prunus*	P4 IRTA	12	O	PU
D 6.1.3	Methodologies allowing to distinguish texture contrasted fruits on physical and chemical grounds identified	P5 (Nantes)	24	O	PU
D 6.1.4	First year evaluation of 2 *Prunus* progenies analysed for all characters	P5 (Bordeaux)	18	R	PU
D 6.1.5	Allelic constitution of 18 *Mal d 1* loci of two high and two low allergenic cultivars	P9 WUR-PRI	36	R	PU
D 6.1.6	Identification of sequences of new *Mal d 4* loci .	P9 WUR-PRI	25	O	PU
D 6.1.7	Number and position of lipid transfer protein (LTP) genes in *Prunus* determined	P4 IRTA	18	R	PU
D 6.1.8	Allelic variation of two apple candidate genes	P16 BAZ	48	R	PU
D 6.1.9	First season phenotypic data of the offspring of the two apple new founders	P9 WUR-PRI	25	R	PU
D 6.1.10	One progeny of 600 seedlings, combining favorable fruit quality and disease resistance alleles	P5 (Angers)	18	O	PU
D 6.1.11	Defined set of CG markers and at least 2 SSRs flanking the targeted apple QTLs	P5 (Angers)	25	R	RE
D 6.1.12	Bin mapping of candidate genes in the TxE Prunus population	P4 IRTA	24	R	PU
D 6.1.13	Field data for populations JxF, BC2, GxM and LxB completed	P5 (Bordeaux)	24	R	PU
D 6.1.14	MNR and polyphenolics measurements of fruit extarcts for all *Prunus* populations	P5 (Bordeaux)	32	R	PU
D 6.1.15	QTL analysis for the peach (JxF, BC2) and apricot (GxM, LxB) populations	P5 (Bordeaux)	34	R	PU
D 6.1.16	Allele-specific molecular markers for high low allergenicity	P9 WUR-PRI	36	R	PU
D 6.1.17	Selection of a collection of 50 peach genotypes adequate for association studies	P4 IRTA	24	R	PU
D 6.1.18	Complete Marker-Assisted Breeding performed on the first apple population (P1)	P5 (Angers)	31	R	RE
D 6.1.19	Second apple progeny ready for MAB	P5 (Angers)	30	R	PU
D 6.1.20	Recombinantly produced protein of a putatively low and a putatively high allergenic Mal d 1 variant	P9 WUR-PRI	48	R	PP
D 6.1.21	Second season phenotypic data of the offspring of the two apple new founders	P9 WUR-PRI	30	R	PU
D 6.1.22	Allelic variation of the remaining apple CG sequences	P16 BAZ	55	R	PU
D 6.1.23	Bin mapping of candidate genes in TxE Prunus reference map (in excess of 75)	P4 IRTA	31	R	PU

Deliv. No	Deliverable name	Lead participant	Delivery date	Nature	Dissemi-nation level
D 6.1.24	Supply of stone fruit materials for instrumental analysis of texture	P5 (Avignon)	47	R	PU
D 6.1.25	Phenotyping of apple progenies	P5 (Nantes)	45	R	PU
D 6.1.26a	First step towards QTL mapping of apple components of texture	P5 (Angers)	49	R	PU
D 6.1.26b	First step towards QTL mapping of apple components of texture	P5 (Angers)	57	R	CO
D 6.1.27a	Phenotyping of apricot for cell wall texture components	P5 (Nantes)	49	R	PU
D 6.1.27b	Phenotyping of peaches for cell wall texture components	P5 (Nantes)	51	R	PU
D 6.1.28	Indications on the relevance of regulatory genes in apple allergenicity.	P9 WUR-PRI	58	R	PU
D 6.1.29	Assessment of allelic diversity in one validated CG in 100 apple accessions	P16 BAZ	52	R	PU
D 6.1.30	Validation of new CGs in apple segregating progenies	P16 BAZ	48	R	PU
D 6.1.31	Complete Marker-Assisted Breeding performed on the second apple population	P5 (Angers)	43	R	RE
D 6.1.32	Third apple progeny ready for MAB.	P5 (Angers)	43	R	PU
D 6.1.33	Marker saturation of 3-4 QTL regions of interest on the four populations	P4 IRTA	48	R	PU
D 6.1.34	Map 5-10 CGs in each of the four peach populations	P4 IRTA	56	R	PU
D 6.1.35	Genotyping of peach collection for 5 CGs	P4 IRTA	46	R	PU
D 6.1.36	Allelic variation for 2 CGs in peach collection	P4 IRTA	48	R	PU
D 6.1.37	Complete MAB of the third apple population	P5 (Angers)	48	R	RE
D 6.1.38	Assembled sequence of two apple BAC clones containing Mal d 1 clusters in LG16	P6 UNIBO	48	R	PP

Work Package 6.2 – Leader Elisabeth Chevreau (INRA, Partner N° 5)

Delive-rable No	Deliverable title	Lead participant	Delivery date[11]	Nature	Dissemi-nation level
D 6.2.1	Action 6-2-3-2 Binary vectors with the gene construct (pHELLSGATE) for gus silencing	16 – BAZ	12	P	RE
D 6.2.2	Action 6-2-1-2 Preparation of plant material of the selected apple genotypes for gene-transfer transformation with recombinase strategy	9 – WUR-PRI	12	R	PP
D 6.2.3	Action 6-2-1-3 Binary vector with PMI and uidA genes	5 – INRA-An	12	R	PU
D 6.2.4	Action 6-2-2-1 Genomic fragments with putative Rubisco apple sequence	9 – WUR-PRI	12	R	PU
D 6.2.5	Action 6-2-2-3 Binary vector with the constructs Ypr10a-gus	16 – BAZ	12	P	RE
D 6.2.6	Action 6-2-2-4 Explants inoculated with Agrobacterium tumefaciens carrying harpin N constructs	5 – INRA-An	12	R	PU
D 6.2.7	Action 6-2-4-2 Primers for specific amplification of the main apple allergens	6– UNIBO-DC	12	R	PU

[11] Month in which the deliverables will be available. Month 0 marking the start of the project, and all delivery dates being relative to this start date.

Delive-rable No	Deliverable title	Lead participant	Delivery date[11]	Nature	Dissemi-nation level
D 6.2.8	Action 6-2-1-1 D-amino acid concentration to inhibit regeneration from leaf explants	16 – BAZ	12	R	PU
D 6.2.9	Action 6-2-1-2 Explants inoculated with Agrobacterium tumefaciens	9 – WUR-PRI	12	R	CO
D 6.2.10	Action 6-2-1-3 Explants inoculated with Agrobacterium tumefaciens	5 – INRA-An	19	R	PU
D 6.2.11	Action 6-2-2-1 Putative 5'upstream, untranslated sequences of the apple rubisco gene cloned in a GUS construct	9 – WUR-PRI	12	R	PU
D 6.2.12	Action 6-2-2-2 Md-ACS1 putative promoter sequence	6 – UNIBO-DC	15	R	PU
D 6.2.13	Action 6-2-2-4 Shoots, from transformation with harpin constructs, growing on kanamycin containing medium	5 – INRA-An	12	R	PU
D 6.2.14	Action 6-2-2-5 Explants inoculated with Agrobacterium tumefaciens carrying ferritin constructs	5 – INRA-An	12	R	PU
D 6.2.15	Action 6-2-3-1 Characterized P-DNA sequences from apple genome	16 – BAZ	12	R	PP
D 6.2.16	Action 6-2-4-1 Year one scab incidence data from field trial	9 – WUR-PRI	12	R	PU
D 6.2.17	Action 6-2-1-2 Shoots growing on kanamycin containing medium	9 – WUR-PRI	15	R	CO
D 6.2.18	Action 6-2-3-2 Transgenic plants containing the silencing construct	16 – BAZ	18	R	RE
D 6.2.19	Action 6-2-1-1 Transformation protocols with D-amino acid selection	16 – BAZ	18	R	PP
D 6.2.20	Action 6-2-1-2 Leaves treated with Dex, growing on FC medium	9 – WUR-PRI	18	R	CO
D 6.2.21	Action 6-2-2-1 Confirmed apple rubisco promoter, from expression data on transgenic tobacco	9 – WUR-PRI	18	P	PU
D 6.2.22	Action 6-2-2-3 Transgenic plants containing nptII and gus genes	16 – BAZ	18	R	RE
D 6.2.23	Action 6-2-2-4 str246C pathogen inducible promoter – Choice of characterized diploid transgenic lines	5 – INRA-An	24	R	PU
D 6.2.24	*Action 6-2-2-5 Intermediate deliverable, replaced by deliverable D6.2.28*	*5 – INRA-An*			
D 6.2.25	*Action 6.2.3.1 Postponed and transformed into deliverable D6.2.43*	*16 – BAZ*			
D 6.2.26	Action 6-2-4-2 Semi-quantitative RT-PCR procedure set-up for apple allergens	6 – UNIBO-DC	19	R	PU
D 6.2.27	Action 6-2-1-4 Recombinase system – Explants inoculated with Agrobacterium tumefaciens	5 – INRA-An	24	R	PU
D 6.2.28	Action 6-2-2-5 sgd24 pathogen inducible promoter – Quantitative evaluation of ferritine expression at RNA level	5 – INRA-An	24	R	PU
D 6.2.29	Action 6-2-4-1 Year two scab incidence data from field trial	9 – WUR-PRI	24	R	PU
D 6.2.30	Action 6-2-4-2 Pattern of expression in leaves and fruits of Gala and a Vf-carrying cultivar in field**)**	6 – UNIBO-DC	24	R	PU
D 6.2.31	Action 6-2-1-1 Molecular characterization of putative transformants obtained from kanamycin and D-serine selection	16 – BAZ	26	R	PU
D 6.2.32	Action 6-2-1-2 Evaluation of optimal conditions for production of marker-free apple plants	9 – WUR-PRI	36	R	PU
D 6.2.33	Action 6-2-2-1 The rubisco-hth gene cassette incorporated in the pMF binary vectors	9 – WUR-PRI	30	P	CO
D 6.2.34	Action 6-2-2-2 Deletion constructs of Md-ACS1 promoter regions	6 – UNIBO-DC	30	R	PU
D 6.2.35	*Action 6-2-3- Replaced by deliverable D6.2.36*	*16 – BAZ*			
D 6.2.36	Action 6-2-3-2 Transgenic plants with silencing constructs grafted in greenhouse	16 – BAZ	28	R	PU
D 6.2.37	Action 6-2-1-1 Assessment of efficiency of D-valine and D-isoleucine to eliminate *dao1* transgenic plants	16 – BAZ	30	R	PU
D 6.2.38	Action 6-2-1-1 Explants inoculated with *A. tumefaciens* and placed under D-alanine selection	16 – BAZ	30	R	PU
D 6.2.39	Action 6-2-1-3 Mannose selection – Explants inoculated with Agrobacterium tumefaciens and placed under mannose selection	5 – INRA-An	30	R	PU
D 6.2.40	Action 6-2-1-4 Recombinase system – Molecular characterization of putative transformants from the first transformation experiment with pRCNG construct	5 – INRA-An	30	R	PU
D 6.2.41	Action 6-2-2-3 Molecular characterization of regenerated plants from transformation with Ypr10-gus	16 – BAZ	30	R	PP
D 6.2.42	Action 6-2-2-4 str246C pathogen inducible promoter – First greenhouse assessment of fire blight susceptibility of transgenic clones expressing the harpin gene	5 – INRA-An	30	R	PU
D 6.2.43	*Action 6.2.3.1 Postponed and transformed into deliverable D6.2.57*	*16 – BAZ*			
D 6.2.44	Action 6-2-3-2 in vitro grafting of gus and gus-silencing transgenic plants	16 – BAZ	30	R	PU
D 6.2.45	Action 6-2-4-1 Evaluation of the long-term stability in the field of GM-trait (scab resistance)	9 – WUR-PRI	30	R	PU

Delive-rable No	Deliverable title	Lead participant	Delivery date[11]	Nature	Dissemi-nation level
D 6.2.46	Action 6-2-1-1 Assessment of efficiency of D-amino acid selection system for apple	16 – BAZ	36	R	PU
D 6.2.47	Action 6-2-1-2 Obtention of regenerants form transgenic leaves treated with Dex, then subject to 5FC selection	9 – WUR-PRI	36	R	CO
D 6.2.48	*Action 6-2-1-4 Intermediate deliverable, replaced by deliverable D6.2.62*	*5 – INRA-An*			
D 6.2.49	Action 6-2-2-3 Gus expression pattern in apple under the control of Ypr10 promoter	16 – BAZ	36	R	PP
D 6.2.50	Action 6-2-2-4 str246C pathogen inducible promoter – Second greenhouse assessment of fire blight susceptibility of transgenic clones expressing the harpin gene	5 – INRA-An	36	R	PU
D 6.2.51	Action 6-2-2-4 str246C pathogen inducible promoter – Harpin N expression pattern in apple under the control of the str246C promoter	5 – INRA-An	36	R	PU
D 6.2.52	Action 6-2-2-5 sgd24 pathogen inducible promoter – Second greenhouse assessment of fire blight susceptibility of transgenic clones expressing the ferritin gene	5 – INRA-An	36	R	PU
D 6.2.53	Action 6-2-2-5 sgd24 pathogen inducible promoter – Ferritin expression pattern under the control of the sgd24 promoter	5 – INRA-An	36	R	PU
D 6.2.54	*Action 6-2-3-3 Intermediate deliverable, replaced by deliverable D6.2.64*	*9 – WUR-PRI*			
D 6.2.55	Action 6-2-4-1 Assessment of the degree of correlation between hordothionin expression level and scab resistance among transgenic lines in field trial (9 – WUR-PRI)	9 – WUR-PRI	50	R	PU
D 6.2.56	Action 6-2-4-2 Pattern of expression in leaves of Gal and Vf-carrying cultivar, after scab inoculation in greenhouse	6 – UNIBO-DC	36	R	PU
D 6.2.57	Action 6-2-3-1 Final conclusion about the search of P-DNA sequences in the apple genome	16-BAZ	48	R	PU
D 6.2.58	Action 6-2-3-2 Development of a systemic acquired silencing (SAS) system for apple	16-BAZ	48	R	PU
D 6.2.59	Action 6-2-1-3 Mannose selection – First assessment of efficiency of mannose selection system for pear	5 – INRA-An	42	R	PU
D 6.2.60	Action 6-2-1-4 Recombinase system – Molecular characterization of regenerants from the second transformation experiment	5 – INRA-An	42	R	PU
D 6.2.61	Action 6-2-1-3 Mannose selection – Final assessment of efficiency of mannose selection system for pear	5 – INRA-An	54	R	PU
D 6.2.62	Action 6-2-1-4 Recombinase system –Assessment of the efficiency of the recombinase strategy on pear.	5 – INRA-An	54	R	PU
D 6.2.63	Action 6.2.2.2 Assessment of the expression pattern of the ACS-promoter through transient expression analyses	6-UNIBO	54	R	PU
D 6.2 64	Action 6.2.3.3 Assessment of efficiency of gene stacking through marker-free transformation technique	9 – WUR-PRI	55	R	PU
D 6.2.65	Action 6.2.4.2 Final evaluation of allergen expression in control and transgenic apples.	6-UNIBO	48	R	PU

Work Package 6.3– Leader Joan Bonany (IRTA, Partner No 4)

D 6.3.1	Common procedures for sensory analysis regarding consumer tests and preference mapping.	P4 - IRTA	12	R	PU
D 6.3.2	Progress report on consolidated results of consumer test of new high quality apple cultivars across different European regions	P4 - IRTA	24	R	PP
D 6.3.3	Progress report on consolidated results of consumer test of new high quality peach/nectarine cultivars across different European regions	P30 – ISF Forli	36	R	PP
D 6.3.4	Progress report on consumer preference mapping for apple fruits across different European regions	P4 - IRTA	24	R	PU
D 6.3.5	Progress report on consumer preference mapping for peach/nectarine fruits across different European regions	P4 - IRTA	36	R	PP
D 6.3.6	Progress report on consumer preference of apple cultivars suited for organic production	P14 - FiBL	24	R	PP
D 6.3.7	Identification of Sensory Characteristics in terms of Appearance (Colour/Size..) & Texture driving Consumption of fresh, unprocessed Apples	P4 - IRTA	57	R	PU

Pillar 7

Work Package 7.1 – Leader Dr Catherine Lagrue (CTIFL, Partner No 27)

Delive-rable No	Deliverable title	Lead participant	Delivery date	Nature[1]	Dissemi-nation level[2]
D 7.1.1	Survey of research within ISAFRUIT amenable to exploitation	27 CTIFL	M12	R	CO
D 7.1.2	Establish an external stakeholder/customer database	12 WU	M26	O	CO
D 7.1.3	Report to Project and Planning of future work	27 CTIFL	M25 M36 M48	R	PU
D 7.1.4	Future training sessions or technical dissemination	27 CTIFL	M30	R	PU

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- **P** = Prototype
- **D** = Demonstrator
- **O** = Other

[2]Please indicate the dissemination level using one of the following codes:
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Work Package 7.2 – Leader Dr. Michael Wilson, subsequently Dr. Sharon Hall (UW, Partner No 12)

Delive-rable No	Deliverable title	Lead participant	Delivery date	Nature	Dissemi-nation level
D 7.2.1	Internal ISAFRUIT information gathering – Survey of all relevant research within ISAFRUIT amenable to dissemination and promotion.	12 UW	M15	R	CO
D 7.2.2	Establish an external stakeholder/customer database – Establish network and database of external stakeholders for two-way knowledge dissemination.	12 UW	M57	O	PU
D 7.2.3	Promote innovative scientific dissemination activities – Develop and implement first stage of a plan of courses, workshops, and seminars to disseminate the knowledge of ISAFRUIT.	12 UW	M57	O	PU
D 7.2.3	Fruitlogistica-Stand and exhbition materials	12 UW	M14	O	PU
D 7.2.3	Campden and Chorleywood Food Research association Group open day	12 UW	M18	O	PU
D 7.2.3	Publication in JHSB-Ole Callesen editorial on ISAFRUIT	12 UW	M19	O	PU
D 7.2.3	ISAFRUIT newsletters	12 UW	M57	O	PU
D 7.2.3	Press releases	12 UW	M57	O	PU
D 7.2.3	ISAFRUIT representation at Re:Fresh 08	2 ACW	M29	O	PU
D 7.2.3	Portable stand-ISAFRUIT Total Chain approach	12 UW	M29	O	PU
D 7.2.3	ISAFRUIT Health forum:Brussels	12 UW	M34	O	PU
D 7.2.3	ISAFRUIT Health Forum Scripta ISHS	12 UW	M34	O	PU
D 7.2.3	Web-based tool for fruit allergy sufferers	12 UW	M34	O	PU
D 7.2.3	GoFruit Blog	12 UW	M35	O	PU
D 7.2.3	Apple and Pear Growers event: East Malling , UK	41 EMRS	M39	O	PU
D 7.2.3	Fruit processing workshop	11 RIPF	M41	O	PU
D 7.2.3	ISAFRUIT YouTube Channel	12 UW	M44	O	PU
D 7.2.3	Vasco da Gama website	12 UW	M49	O	PU
D 7.2.3	ISAFRUIT Twitter account	12 UW	M49	O	PU
D 7.2.3	Fruit Forum conference, Ashtown Food Research Centre, Dublin	34 NFC	M53	O	PU
D 7.2.3	The Great, Greedy Gomagog, book for children (including ISAFRUIT related activities promoting fruit consumption).	12 UW	M52	O	PU
D 7.2.3	School visits (UK)	12 UW	M57	O	PU
D 7.2.3	Spanish version of the Fruit Allergy website	12 UW	M54	O	PU
D 7.2.3	ISAFRUIT scripta-based on the Vasco da Gama process	6 UNIBO	To be completed	O	PU
D 7.2.4	Prepare 12-month Report and an 18-month Forward Plan for the ISAFRUIT MC - Prepare Work Package Report including future plans and actions	12 UW	M42	R	CO

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Work Package 7.3 – Leader Dr Agustí Fonts (IRTA, Partner No 4)

Delive-rable No	Deliverable title	Lead participant	Delivery date	Nature	Dissemi-nation level
D 7.3.1	Definition and development of the structure of the knowledge & information platform.	4 IRTA	M12	O	CO
D 7.3.2	Platform with potentially protectable knowledge,initial potential end-users and list of virtual marketplaces available in participants-only section of the web site.	4 IRTA	M18	O	CO
D 7.3.3	Work Package Report prepared	4 IRTA	M18	R	PU & CO
D 7.3.1	Establish a database with potentially protectable knowledge, initial potential end-users and list of virtual marketplaces available in participants-only section of the web site (by month 18). After month 18 the database will be continuously updated	4 IRTA	M30	O	CO
D 7.3.2	Relevant information from the database published in the knowledge map by month 24. After month 24 the knowledge map will be continuously updated	4 IRTA	M30	O	PU & RE
D 7.3.1	A report identifying existing products and technologies which are relevant to the objectives of the ISAFRUIT research and European companies that may have potential for utilizing new innovations.	4 IRTA	M42	R	CO
D 7.3.2	Newsletters distributed to ISAFRUIT partners which provide relevant information related to IP and exploitation of ISAFRUIT results and encouraging the use of the knowledge map.	4 IRTA	M42	O	PU & RE
D 7.3.3	A list of technology bundles which may include a combination of ISAFRUIT innovations or may also include external sources.	4 IRTA	M42	R	CO
D 7.3.2	Newsletters distributed to ISAFRUIT partners which provide relevant information related to IP and exploitation of ISAFRUIT results and encouraging the use of the knowledge map.	4 IRTA	M57	O	PU & RE
D 7.3.3	Prepare a report with financial strategies to develop ISAFRUIT prototypes into marketable products	4 IRTA	M57	R	CO
D 7 3.4	A report addressed to the Management Committee (achievements and obstacles detected during the project)	4 IRTA	M57	R	CO

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Work Package 7.4 – Leader Prof. Luca Corelli Grappadelli (UNIBO, Partner No 6)

Delive-rable No	**Deliverable title**	**Lead participant**	**Delivery date**	**Nature**	**Dissemi-nation level**
D 7.4.1	Launch of Web Site.	6 UNIBO	M6	O	PP
D 7.4.2	Maintenance of the web site .	6 UNIBO	M6-57	O	PP
D 7.4.3	Organisation of Isafruit events as: FruitLogistica 2007, 2009, S11 and W6 at ISHS IHC 2010	6 UNIBO	M14 M 38 M56	R	PP
D 7.4.4	Press releases	6 UNIBO	M0-57	R	PP

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Annex C

Annex D: A list of PhD thesis prepared and defended (or to be defended) within ISAFRUIT project

Pillar 2

A List of PhD Theses prepared and defended (or to be defended) within ISAFRUIT project

Krogholm, Kirstine Suszkiewicz: Flavonoids as fruit and vegetable intake biomarkers – Development, validation and application of flavonoid biomarkers in nutritional research. The National Food Institute, Technical University of Denmark / Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen – Thesis defended on March 18, 2011.

Kristensen, Mette: Nutri-metabolomics. Effects and exposure markers of apple and pectin intake. Department of Food Science, Faculty of Life Sciences, University of Copenhagen - Defence in August 2011.

Hansen, Louise: PhD Project Metabolomics profiling and risk of breast cancer, colon cancer and coronar syndrome – The Danish Cancer Society / Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen (Studies from February 1, 2008 – January 31, 2011 – prolonged until December 31, 2011 due to maternity leave).

Rago, Daniela: PhD Project: Biomarker Identification in Metabolomics of Dietary Studies on Nordic Foods (Studies at Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen, from May 1, 2010 – April 30, 2013).

Rentsch, Rikke: PhD Project: Changes in the rat transcriptome after feeding with apples and apple fractions – The National Food Institute, Technical University of Denmark (Studies initiated 2007 – prolonged until October 31st, 2012 due to several leaves).

Alinia, Sevil: PhD Project: Effect of Fruit-Intervention in Workspaces on Bodyweight Management. The National Food Institute, Technical University of Denmark (Studies initiated August 2007 – prolonged until August 24, 2011 due to several leaves).

Pillar 3

Alegre, Isabel. Nuevos métodos de control de patógenos de transmisión alimentaria en fruta mínimamente procesada [New methods for controlling foodborne pathogens in minimally-processed fruit]. Food Technology Department (University of Lleida, Spain). Student grant financed by Spanish government. However, most of the research was carried out in the frame of Isafruit project. To be defended in May 2011.

Devic Emily 2010: Impact of dehydration on mass transfer and mechanism of degradation of ascorbic acid and phenolic compounds. Application to six different table and cider apple cultivars (in French). Thesis defended on 6th of July, 2010 at Food Process Engineering, AgroParisTech, Massy, France

Jesionkowska Katarzyna, 2010: Use of osmotic dehydration technology as an alternative method for post-harvest utilisation of sour cherries and black currant fruit (in Polish). Thesis under review – defence planned on 10th December 2010 at the Research Institute of Pomology and Floriculture, Skierniewice, Poland.

Keenan, D. A new generation of novel processed fruit purees containing added functional ingredients. In preparation.

Kosmala Monika, 2009: Composition and properties of dietary fibre preparations obtained from selected fruit pomaces (in Polish). Thesis partially financed within Isafruit project and defended on 20th of October 2009 at the Faculty of Biotechnology and Food Sciences Technical University of Lodz, Poland.

Mieszczakowska-Frąc Monika, 2010: Influence of raw material and technology of black currant nectars production on their quality and composition of phenolic compounds (in Polish). Thesis after positive review – defence planned on 3rd December 2010 at the Research Institute of Pomology and Floriculture, Skierniewice, Poland.

Rößle, C. A new generation of minimally processed fresh-cut apple products containing added functional ingredients. Thesis under review – defence planned on the 18th of November at University College Dublin, Dublin, Ireland.

Sójka Michał, 2010: Bioactive substances of black currant pomace (in Polish). Thesis defended on 12th of October, 2010 at the Faculty of Biotechnology and Food Sciences Technical University of Lodz, Poland.

Uczciwek Małgorzata, 2010: Production and properties of stone and berry fruit dehydrated osmotically in fructooligosaccharides (in Polish). Thesis defended on 12th of October, 2010 at the Faculty of Biotechnology and Food Sciences, Technical University of Lodz, Poland.

Pillar 4

PhD thesis prepared and defended (or to be defended) within ISAFRUIT project

Alice Spadoni 2009: Induced resistance by hot water in apple: role of defence protein. Thesis defended on 18th July 2009 at the Faculty of Agriculture, University of Bologna. Italy

Pillar 5

El Jendoubi, Hamdi. No title yet. University of Lleida. To be defended within 2011.

Orera, Irene. Development and application of new analytical methodologies for the study of ferric fertilizers. Faculty of Sciences (University of Zaragoza). Defended July 11, 2010 "Sobresaliente *cum laude*" by unanimity.

Ravaglia, Daniela. Control of peach phenolic compounds content. Dipartimento Colture Arboree (University of Bologna, Italy). Defended April 19, 2010.

Rellán, Rubén. Long distance transport and metabolism changes in iron deficient plants. Faculty of Sciences (Autonomous University of Madrid). To be defended on February 2011.

Soto, Alvaro. No title yet. University of Bologna, Italy. Date for defense to be defined.

Zibordi, Marco. Crop load control in apple via shading: exploring the potential of carbon unbalances to manage fruit growth and tree performance. Dipartimento Colture Arboree, (University of Bologna, Italy). Defended April 19, 2010.

Pillar 6

PhD Thesis – Pillar 6

1. Pagliarani G (2010) **Genomic and transcriptional analysis of allergen genes in apple**. This thesis was defended in May 2010 at the University of Bologna and is resulting of a close collaboration between the Univ of Bologna and DLO-PRI where Dr. Pagliarani stayed for one year.

2. Hättasch C. (2010) **Flower development in apple (*Malus domestica* Borkh.) and development of innovative strategies for production of genetically modified apple plants**. This PhD was defended at JKI Institute in Dresden.

3. Illa E. (2010) **Mapping candidate genes involved in peach fruit quality.** This thesis was defended in November 2010 at the University of Barcelona

4. Galvez-Lopez D. (2011) **Variability of cell wall determinants of texture and QTLs mapping in an apple progeny**. This PhD will be defended at the University of Angers in February 2011.

5. Granozio S. **Functional characterization of two ACS-1 promoters in apple**. This thesis is still running and will be defended at the University of Bologna.

Pillar 7

Schmidt-Andersen, M.B. Hypoallergenic fruits for fruit allergy sufferers. Review of existing research and design of a communication initiative. Supervisors: Lars Ove Dragsted, Sharon Hall, Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen. 2009.

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