

Bundesprogramm Ökologischer Landbau und andere Formen nachhaltiger Landwirtschaft

# Quality analysis of critical control points within the whole food chain and their impact on food quality, safety and health (QACCP)

FKZ: 07OE001

### Projektnehmer:

Universität Kassel (FB 11 Ökologische Agrarwissenschaften) Fachgebiet Ökologische Lebensmittelqualität und Ernährungskultur Nordbahnhofstraße 1a, 37213 Witzenhausen

Tel.: +49 5542 98-1715
Fax: +49 5542 98-1713
E-Mail: kahl@uni-kassel.de
Internet: htp://www.uni-kassel.de

### Autoren:

Kahl, Johannes; Kretzschmar, Ursula; Kristensen, Hanne L.; Lauridsen, Charlotte; Mengheri, Elena; Särkkä-Tirkkonen, Marjo; Seljåsen, Randi

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# **Final report**

for

Project no. 2006-1885

Quality analysis of critical control points within the whole food chain and their impact on food quality, safety and health (QACCP)

Period covered: 15.06.2007 – 30.06.2010

**CORE Organic** 

Contract no.	2006-1885	Contract Acronym:	QAC	СР	
Contract title:	Quality analysis of critical control points within the whole food chain and their impact on food quality, safety and health				
Coordinator information:					
Institution:	University of Kasse	el		Acronym	UniKa
Faculty/ Department/ Section/Unit	Organic food qualit	ry and food culture			
	Road name and number: Nordbahnhofstr. 1a P.O. Box:			P.O. Box:	
Address:	Town Witzenhausen	Post Code: 37213		Region:	Country: Germany
Coordinator:	Family name: Kahl	First name: Johanne		Title: PrivDoz. Dr.	
Address if different from above:			·		
	Phone:	Fax:		E-mail:	
	++49 5542 981715	++49 5542 981713		kahl@uni-kas	sel.de
Start of Project:	15.06.2007	End of proj	ect:	30.06.2010	

### **Project partners and contact persons:**

Partner no.	Country	Organisation name:	Functions*):	Involved in WP's:	Contact person:
1	Germany	University of Kassel	PC, WPM, WPCM, P	1,2,4,6,8	Johannes Kahl, Nicolaas Busscher
2	Switzerland	Research Institute of Organic Agriculture	WPM, WPCM, P	1,2,3,8,9	Ursula Kretzschmar
3	Italy	Universita Politecnica delle Marche	WPM	2	Raffaele Zanoli
4	Finland	University of Helsinki	WPM	3	Marjo Särkkä- Tirkkonen
5	Denmark	University of Aarhus	WPM, P	4,7,8	Hanne Kristensen, Charlotte Lauridsen
6	France	AgroParisTech	WPM	5	Ines Birluoez
7	Italy	Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione	WPCM, P	6, 7	Flavio Paoletti Elene Mengheri
8	Austria	Research Institute of Organic Agriculture	Р	6, 7	Alberta Velimirov
9	Germany	Federal Research Institute	Р	7	Bernhard Watzl
10	Norway	Norwegian Institute for Agriculture and Environmental Research	WPM	8	Randi Seljasen
11	Germany	Research Institute for Organic Agriculture	Р	3, 9	Alexander Beck
12	Switzerland	Hochdorf Nutritec	Р	3	Simone Eckstein

<sup>\*)</sup> PC: Project Coordinator, WPM: Workpackage Manager, WPCM: Workpackage Co-manager, P: Participant

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### Project Summary, including objectives and outputs

### Overall objectives of the research project

The overall objective of the project was to optimise organic production and processing in order to improve food quality and increase health promoting aspects in consumer products. The approach was a chain analysis approach which addressed the link between farm and fork and backwards from fork to farm. The objectives were to test food authenticity on farm level and food quality and health in processing. The carrot was chosen as the model vegetable since it is common for the involved partners from industry and is processed for baby food; hence the results are relevant for other vegetables and organic food in general as well.

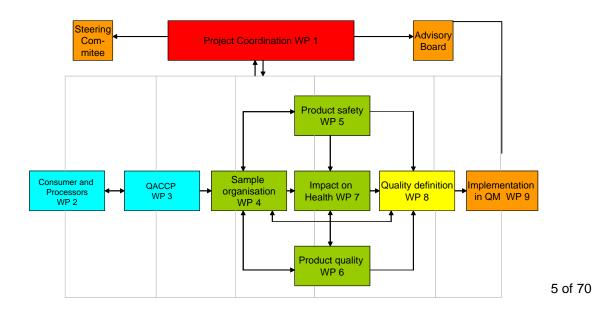
- Identify and define critical and essential product quality parameters useful to optimise organic food quality
- Compare products from different farming practices (conventional and within organic)
- Performance of QACCP (Quality Analysis Critical Control Point, similar to HACCP methodology)
- Test the impact of the food chain (focusing on processing techniques) on the product quality and safety
- Test the impact of organic food on health

### Project description and methodology

In order to achieve results of high scientific standard and high relevance for the practice, carrots were chosen as a vegetable product to be investigated, and carrot samples were taken from well defined field experiments that were designed with the aim to investigate the effect of different management practices on carrot quality. After the consumer and processor awareness had been identified by means of focus groups and two surveys, quality analysis was carried out at different European SMEs, and experimental field trials were sampled in Italy and Denmark. From the field trial in Italy, one representative of the organic cultivation system was processed in pilot plants testing the impact of different kinds of raw material (e.g. fresh, frozen) on the quality and safety of the final baby food product. Furthermore samples were taken at critical points at industrial scale.

The samples were sent around to different partner laboratories and analysed with a multi-method approach including safety and quality parameters. All samples were coded and analysed blind to get excellent scientific results. The methods applied include the detection of pesticides and nitrate, nutrients and secondary compounds, sensory analysis as well as microbiology and screening techniques like biocrystallization and fluorescence analysis. Furthermore the health impact was tested on different animal models.

The results were evaluated in relation to questions from practice and a quality definition is published on how carrots should be processed for optimum quality and safety. The results of the project were intensively discussed with possible stake-holders (through the establishment of an advisory board). In order to achieve an efficient and well-timed implementation of the project it had been divided into 9 work-packages. All WPs were sub-divided into different tasks. The 9 WPs were the following:



### Results

The overall results of the project are:

- A. A Quality Analysis Critical Control Points (QACCP) could be developed and applied in industry.
- B. Along the organic production chain of carrot baby food critical steps according to quality and safety could be identified.
- C. Although sterilisation of the product at the final production step, the selection of the raw material has a significant influence on the quality and safety of the baby food.
- D. Furthermore choice of raw material plays an important role for the willingness to pay of the consumer for the final product.
- E. The differentiation of carrots from organic and conventional farming systems is influenced by additional factors like year and variety. This was the same for the selected health markers. The measured single compounds seem not be sufficient for the differentiation but multivariate statistical analysis of the data increased the discrimination ability.

### Dissemination

Dissemination was carried out through the project website, scientific articles, scientific conferences and stakeholder workshops and presentation of the results by the involved SMEs. The research and development community is mainly addressed through publications of results in scientific journals.

The project results contribute to the improvement of the quality and safety of organic vegetables. In detail, the project results contribute to improvement of the food chain, focused on processing of organic vegetables, which will profit the SMEs and retailers in the global organic market. The analysis of the whole food chain regarding the aspects food safety, sensory quality and health gives important information for the product development in the future in accordance with the consumers expectations on organic food: environmentally friendly, healthy, tasty and safe. The project created an European network of research institutes from different disciplines and partners from practice. This is an excellent bases for efficient and interdisciplinary international research in the future in the organic sector.

### 1. Main results, conclusions and fulfilment of objectives

### 1.1 Summary of main results and conclusions

To maintain a high quality of organic products during processing is a crucial challenge. As a instrument for quality analysis and quality improvement the method of Quality Analysis Critical Control Points (QACCP) was developed and successfully tested at industry level. With this tool, the impact of selected processing steps (influencing factors) on important quality attributes of carrot baby food could be determined.

### Introduction

The demand for organic processed food is rapidly growing. With that the quality of organic product needs to be assured and optimized. The processing industry is looking for higher organic quality as well for instruments to optimize the processed quality. Therefore between June 2007 and June 2010, 14 research and industry partners from eight different European countries worked together to improve product-related quality management in organic farming and processing. Firstly, relevant gaps in quality attributes had been identified by a focus group survey and processors' survey, secondly a quality analysis was carried out at different European SMEs. Thirdly different field trials with different varieties, different ecological farming practices were done in Italy and Denmark to figure out the influence of the region, the variety as well the farming practice on the carrot quality. In order to achieve results of high scientific standard and high relevance for the practice, carrots were chosen as the vegetable product to be investigated, and carrot samples were taken from well defined field experiments, designed with the aim to investigate the effect of different management practices on the quality of the raw material.

### Results

### Field trials

The hypothesis for the authentication of fresh carrot samples was, that carrots from organic and conventional farming systems can be differentiated in a field trial or/and by comparing carrots from neighbouring organic and conventional farms. Moreover organic cropping should increase positive quality and health attributes and decrease negative effects on the safety of the carrots. Therefore we took samples from a defined field trial, where conventional farming practice was tested against three different organic regimes (WP4). This Danish VegQure field trial was sampled and registered according to the Standard Operation Procedure (SOP) in mid October 2007 and 2008, and the samples were coded and distributed to partners in WP5, 6 and 7 according to the sample plan. In addition carrots from two farm pairs, located in Italy were tested. Similarly the carrots from the Italian field trial were sampled, registered and distributed according to the SOP and sample plan in January and April (early and late varieties) 2008 and 2009. The sampling and distribution in Italy was performed by the AIAB (Italian Association of Organic Farming). Additional amounts were sampled by the Italian association (AIAB) and processed in the pilot plant in Helsinki.

The samples for the industrial trials were from a commercial grower, because the amount of carrots from the involved field trials was not enough according the SME's requirements (WP3). Samples were processed at the industrial partner (Hochdorf) in Switzerland. After sampling, all samples were coded, distributed to partners and analysed. Decoding of all samples has been performed according to a procedure of collection of results from all partners followed by official decoding by WP4.

In the VegQure field trial there were few differences in yield and harvest quality between the four cropping systems. Yields were generally higher in 2008 than 2007, and in 2008 highest in the conventional and one of the organic systems (O1).

The differentiation of carrots from organic and conventional farming systems was influenced by factors like year/climate and variety/harvest time which seem to have influences on the levels of

the selected quality criteria (e.g. terpenoids, carotene, sensory attributes etc.) as strong as the farming practice (WP6). The selected single compounds or criteria alone therefore seem not to be sufficient for the differentiation which was the same for the tested health markers (WP6,7). Multivariate analysis of the data seems to increase the possibility for the discrimination (WP8).

Quality characteristic of the fresh carrots were mainly affected by the seasonality that overshadowed the differences due to the farming practices. Moreover, an important influence of the variety on the parameters measured was detected when the carrots from the Italian farm comparison were considered. However, the carrots from the Danish field trial could be discriminated on the basis of the volatile compounds profile. In particular, the concentration of sesquiterpenes (beta-carophyllene was the main component of this group of volatile compounds) was significantly higher in the carrots from two organic systems (O2 and O3) than those from the conventional and the other organic (O1), thus allowing to hypothesize a relationship between the crop management system and this class of compounds (WP4, 6, 7).

According to the performed Principal Component Analysis (PCA) of samples from the Danish organic field trial (VegQure) conventional samples were separated from all organic fertilization regimes in 2007. The positioning of the conventional samples in the PCA score plot seems to be mostly determined by variables content of nitrate, carotene, fumaric acid, malic acid, pungent aroma, aftertaste, green flavor, soapy flavor, root diameter and weight as well as damages due to cracks and forks (WP6, 8).

For carrots from the Italian farm trial it was not possible to discriminate conventional from organic carrots by PCA. For those samples, variety had higher impact than cultivation system. When comparing results of Italian trial with Danish trial, location and variety were the main factors able to discriminate between samples and not cultivation method (WP4, 6, 8).

The several health biomarkers considered and the digestibility were similar in rats fed organic and conventional fresh carrots. Rodents as well as humans are born with taste predispositions including the preference of sweet and salty taste and the rejection of sour and bitter taste. Accordingly the paired comparisons of differently grown carrots as well as the consumed amounts of the test carrots indicated interrelations between sweet and bitter taste effects. Furthermore the food choices of the participating rats and mice coincided in most cases (WP7).

The intestinal immune system represents the first reaction to ingested food. Therefore the intestinal immune response is an important critical point to be considered. The results indicated no adverse effects on the intestinal and peripheral immune response after carrot consumption, both from carrots of the Danish and Italian trials; but a great variability was observed between the first and second year carrot harvests (WP7).

A very sensitive model was applied to evaluate the feed influence, i.e. the Reproductive Assessment by Continuous Breeding (RACB). The feeding studies with laboratory mice highlighted a slightly more successful breeding performance in the group fed with organic carrots (WP7).

### **QACCP**

The second hypothesis was that a Quality Analysis Critical Control Points (QACCP) can successfully be performed on carrot baby food and the effects of changes in selected critical control points can be determined. Therefore, the aim of the study was the development of a QACCP tool and to evaluate the quality influencing processing steps based on the production of carrot baby food. Once the processing conditions were identified the possibilities for alternative distribution ways and processing techniques were explored to improve the overall product and process quality. Next to the food safety (hazard) in the food processing the quality aspect is getting more and more important and an analyse system to optimise the process needs to be established (QualityAnalysisCriticalControlPoint, WP2, 3).

The QACCP approach was developed on several pillars:

- 1. Processors and consumer studies to evaluate the expectations regarding the quality of organic food.
- 2. Situation analysis of the 3 Industry partners: Hochdorf CH, Sunval DE, Rivier SAS FR
- 3. General spectral analysis of each production step of Hochdorf: markers were furosine, carboxymethyllysine and furan (marker for the Maillard reaction)
- 4. Evaluation of each production step regarding the aspects quality influencing factors, health and sensory quality
- 5. Rating of these factors
- 6. Comparison of the results with the consumer study, the processors study and the spectral analysis
- 7. Definition of the three most important quality points (qp), that were raw material, heat load and sterilisation. Decision to evaluate in detail the factor raw material
- 8. Pilot plant test with fresh, frozen and pasteurized raw material.
- 9. Industrial test with fresh, fresh stored and frozen raw material

Our research showed that based on the method Quality Analysis Critical Control Points (QACCP) quality factors for the improvement were determined and the product could be improved (WP2, 3, 5, 6). The methodology of QACCP could be implemented in the industry in the next step and an analyse matrix for the industry to evaluate sensory quality, food safety and health could be worked out (WP9).

The following figure illustrates the critical steps where quality could be changed during production of a baby food puree made from organic carrots. Within the project critical steps regarding processing were tested because of their degree to influence quality. The influences of three different agronomic systems on the quality as well as differences regarding varieties were analysed.



### Processing

Based on the successful QACCP carried out at industry level, we hypothesised, that along the organic production chain of carrot baby food critical steps according to quality, safety and health can be identified. For covering the different food quality aspects, various single compound measurements (e.g. antioxidants), sensory analysis as well as screening methods like biocrystallization and fluorescence analysis were applied, resulting in an amount of single criteria. Therefore data collection and data evaluation was done by standard protocols in order to guarantee the consistency of the results (WP3, 4, 8).

After performing a pilot plant test, an industrial test was done with fresh, stored and deep frozen raw material. Although sterilisation process followed, the pre-treatment of the raw material has significant influence on the quality. The baby foods produced by fresh carrots were clearly discriminated from the frozen raw material by several sensorial characteristics as well as by their content of health related compounds like of pro vitamin A (WP6).

In the processed carrots the heat treatments caused a clear reduction of the quality parameters measured with respect to the fresh carrots. The carotenoid content was reduced even more than 30% with lutein particularly affected. In addition, a significant effect of the pre-treatments and storage of the raw material was also observed. The results indicated that the freezing of the fresh carrots did not affect in itself the quality characteristics of the puree samples, but the frozen storage had more negative effects on the parameters measured (both chemical and sensory) than the other conditions tested (WP6).

Principal component analysis (PCA) was performed on data from the different experiments separately for a large amount of crystallisation studies, chemical analyses of flavor compounds and health parameters, sensory quality and external quality characters like size, form and yield. In some cases experiments form different years were also compared in the same PCA. The results for samples from different treatments of the raw material for processing (fresh, stored and frozen) indicate a clear grouping of the samples based on the measured end product quality variables. The groups correspond to the treatments of the raw material for processing, which is a clear indication that the treatments of the raw material for processing have different influence on the end product quality. The positioning in the PCA score plot of the puree samples from fresh raw material seems to be mostly determined by the quality variables specific aroma components, b-carotene, and colors (a, b, and c), while the positioning of the puree samples from refrigerated stored carrots seems to be mostly determined by the quality variables selected terpenes, orange brown color as well as sour and bitter taste. The positioning of the product made from frozen raw material seems to be mostly determined by the quality variables boiled carrot flavor and odor, color hue and watery texture (WP6, 8).

### Consumer's view

A consumer as well as a processor survey was carried out in order to identify awareness, expectations and demands on the quality of organic processed vegetable baby food. An exploratory qualitative analysis regarding the quality aspects as well the quality changment by the improvement of the product was performed by means of focus groups and in-Depht interviews (WP2).

The consumer response to the changes could be tested, in order to see if these changes are seen as improvements by consumer and if this affects their willingness-to-pay for the product The final consumer choice experiment showed that consumers are willing to pay more for baby food produced out of fresh raw material. Carrot baby food taste heterogeneity can be explained by socio demographic variables (WP2).

### Resumee/Conclusions

With this interdisciplinary research approach it was possible to go deeper in the question of organic food quality in special we got new knowledge regarding the questions:

- 1. Could carrots from organic and conventional farming systems be differentiated in a field trial or by comparing carrots from neighbouring organic and conventional farms?
- 2. Is organic cropping increasing positive quality and health attributes and decrease negative effects on the safety of the carrots?
- 3. How can we identify critical steps according to quality, safety and health systematically (QACCP)?
- 4. Are quality aspects relevant for the consumer and are they changing the willingness to pay for the organic product?

Those results are usable for the different players in the organic field like farmers, processors and as well retailers for their daily work (WP9).

Consumer demand for natural, sustainable and carefully processed food with a high quality is rising. Most of the consumed food is nowadays processed food. The impact of this intensive processing might threat the product quality unnecessary. Next to food safety and health aspects also the demand of an excellent sensory quality is rising in the organic food sector. The organic sector with the principles of limited use of additives as well minimal processing activated the discussion of quality. QACCP as a method for the systematic evaluation of the quality influencing production step was elaborated and basically tested with baby food. In a next step there is a big need to establish this concept in the industry broadly. A further development of the concept for the special need of the different product groups (milk, meat, fruit and vegetables, bakery products) is bases for a successful implementation. For performing a QACCP the whole food chain has to be evaluated in addition to processing also the farm level has to be included. On farm level the identification of critical points and/or critical control points and the quality improvement at these points are important. Variety and how the farming processes are managed within the organic EC-Regulations seem to be important.

In the food market more and more products have become complex multi-step processed products with high resource input. The impact of this intensive production might threat the quality of the product. Moreover natural food tends to preserve the nutritional value and health impact of the food. Therefore we suggest to compare natural production with multistep high processed food to show the potential of organic to be the natural way of production. Furthermore the production process will be compared based on sustainability (environmental impact). Moreover careful processing is a demand from the organic EC-Regulation 834/2007 and needs to be defined, processing techniques needs to be verified and evaluated as well. As the start for careful processing, the raw material quality is important and should further be tested on other products.

Consumers are supposed to pay for a 'plus' in organic product quality. It is a challenge to first define and second proof this 'plus'. In the EC regulation No 834/2007, organic production is defined as "a production method in line with the preference of certain consumers for products produced using natural substances and processes" (EC 834/2007, (1)). The specific principles which are applied to processing of organic food exclude substances and processing methods "that might be misleading regarding the true nature of the product" (Article 6, c). Furthermore processing should maintain the "vital qualities" and "organic integrity" of the product. These descriptional terms can be used as food claims which are essential for the further development of organic production, processing and consumption. Moreover these terms have no standardized and scientific definitions and analyses which are a requirement for consumer confidence. Therefore scientific definitions have to be investigated and additional strategies have to be developed how the claims can be tested and verified through criteria.

Stakeholders along the whole food chain (farmers, processors, traders) as well as representatives from associations should be involved in this process together with the experts from science. Once the terms and concepts of these relatively new aspects are defined, existing laboratory methods for quality testing need to be evaluated for their adequacy with respect to measuring them. Shortcomings in methodology need to be identified. An adequate testing methodology should be described. The methods applied in this project are a good start for covering the range of different measurement approaches from single compound detection, sensory analysis, biocrystallization to a wide range of potential health markers.

Organic products are defined by process aspects and in reality vary widely in quality. A stronger focus on organic product quality will stimulate producers to improve quality. The Quality Analysis Crictical Control Points, applied successfully in this project is a valuable tool for this.

### 1.2 Fulfillment of objectives

The project achieved nearly all its objectives. A Quality Analysis of Critical Control Points could be developed and applied. Critical Points and Critical Control Points within the organic food chain of carrot baby food could be identified and for selected points analysis of several quality and safety indicators was carried out. Only the improvement of the quality optimization at these points will be done at industry level after project life-time. All partners met several times during the project life-time and fulfilled their specific requirements/detailed objectives within the different work packages.

### 2. Milestones and Deliverables status

### Milestones:

Milestone no:	Description	Planned time	Final time
1.1	Kick-off meeting carried out	Month 1	Month 1
1.2	Consortium Agreement signed	Month 7	Month 18
2.1	Final 1° round processor questionnaire (End September 2007) and telephone survey done by partners (Mid September- End October 2007)	Month 2/3	Month 3/4
2.2	Focus Groups conducted in IT & DE (1-31 Oct. 2007)	Month 4	Month 5
2.3	Internal FG Reports completed and sent to partners (30 Nov. 2007)	Month 5	Month 6
2.4	Draft internal report on processor survey (31 Jan. 2008)	Month 7	Month 7
2.5	Choice experiment questionnaire completed & subcontract done (28 Feb. 2008 – postponed at late spring 2009 – May 2009)	Month 8	Month 23
2.6	Survey conducted (1-31 Mar. 2008) and econometric analysis finalised	Month 9/10	Month 24/25
2.7	Draft internal report on consumer survey (30 June 2008)	Month 12	Month 27
2.8	Final consolidated report incl. Processor analysis (31 Dec. 2008)	Month 18	Month 33
3.1	Selection of processes and distribution chain and identification of sample points	Month 4	Month 4
3.2	Definition of quality critical points + investigation of possible improvements ready	Month 24	Month 24
3.3	Description of feasible optimisations available.	Month 28	Month 28
3.4	Pilot tests with integration of results on individual SME-level conducted	Month 30	Month 30
4.1	Sampling and distribution scheme finalised	Month 2	Month 18

	and accepted by all partners		
4.2	Sampling of VegQure field trial finalised	Month 16	Month 16
4.3	Control of participants sampling and distribution of samples finalised	Month 18	Month 22
4.4		Month 27	Month 27
5.1	Selection of relevant indicators of pesticides and neoformed contamination	Month 3	Month 6
5.2	Contamination levels in organic carrots and proposals for improvements	Month 24	Month 24
5.3	Discussion on contamination of baby foods by neoformed contaminants and proposals for improvement	Month 24	Month 24
5.4	Discussion on contamination of organic baby foods by pesticides and nitrate and proposals for improvement	Month 24	Month 30
5.5	Evaluation of the potential of fluorescence for discrimination and prediction purposes	Month 27	Month 27
6.1	Product analysis plan for all product types	Month 3	Month 3
6.2	Data delivered according to the data delivery plan	Month 22	Month 32
6.3	Product quality analysis evaluation	Month 25	Month 34
7.1	Animal experiments of harvest year 1 performed	Month 14	Month 14
7.2	Animal experiments of harvest year 2 performed	Month 23	Month 23
8.1	Literature study on quality definition factors	Month 11	Month 15
8.2	Set up data format and organisation procedure of data evaluation.	Month 11	Month 11
8.3	Decide how to measure to fit our quality dimensions.	Month 14	Month 22
8.4	Analysis and discussions of results from WP 5-7	Month 28	Month 34
8.5	Implementation of structured data to WP9	Month 31	Month 35
8.6	Publication	Month 31	Month 36
9.1	Workshop with advisory board organised	Month 12	Month 12
9.2	Draft leaflet for food processors	Month 38 After project lifetime	
9.3	Presentation of the results at a final European seminar	Month 30	Wissenschaftstagung Ökologischer Landbau, D, 2011 International Organic Food Conference, CZ, 2011
9.4	Presentation of the results at a final European seminar	After project lifetime	
9.5	Presentation of the results at events of the label organisations and organic food industry	After project lifetime	Processors day, CH April and September, 2010
9.6	Publication of scientific articles in the most relevant journal	After project lifetime	See paper list

### **Deliverables:**

Deliverable no:	Description	Planned time	Actual time
1.1	Consortium Agreement	Month 1	Month 18
1.2	Short summary of the project	Month 3	Month 4
1.3	List of deliverables and milestones	Month 3	Month 4

1.4	Meeting plan	Month 1	Month 1
1.5	Internal web-page for communication and	Month 8	Month 18
	dissemination including uploading inputs for		
	organic Eprints database		
1.6	Mid-term Report	Month 19	Month 19
1.7	Final report	Month 31	Month 34
2.1	Internal report on the consumer analysis	Month 12	Month 27
2.2	Internal report on the expert survey on processors	Month 7	Month 7
2.3	DSS on alternatives & choice of attributes	Month 8	Month 27
2.4	Publishable consolidated report	Month 18	Month 33
3.1	Internal report on model product distribution	Month 5	Month 5
3.2	Report on the selection of specific product types and processes	Month 20	Month 20
3.3	Public report on the implementation of QACCP on SME Level		Month 30
4.1	Sampling and distribution scheme for field trials, SMEs and market samples		Month 18
4.2	Final WP report on sampling and distribution and relationships between carrot quality and agronomic conditions	Month 27	Month 34
5.1	Contamination level by pesticides and nitrates of conventional and organic carrots	Month 24	Month 24
5.2	discrimination models of the type of production (conventional or organic) with respect to pesticide and nitrate use	Month 27	Month 27
5.4	Key processing steps associated to production of neoformed contaminants	Month 24	Month 24
5.5	Cartogaphy of the exogenous and neoformed contamination in commercial samples	Month 27	Month 27
5.6	Calibration and prediction models for pesticides and neoformed contaminants using front face fluorescence.	Month 27	Month 27
6.1	Report on internal documentation and evaluation of methods applied for quality testing	Month 2	Month 2
6.2	Data sheet for further evaluation within WP8	Month 6	Month 7
6.3	Final WP report about the quality testing results	Month 27	Month 35
7.1	Scientific paper on rat experiment of Danish carrots	Month 27	Month 27
7.2	Scientific paper on gut immunology	Month 27	Month 27
7.3	Scientific paper on growth and preference test	Month 27	Month 27
7.4	Internal report and documentation	Month 31	Month 34
8.1	Presentation for the projects internet page	Month 11	Month 17
8.2	Data format defined and accepted by all laboratories	Month 11	Month 11
8.3	Organised and evaluated data	Month 14	Month 34
8.4	Scientific publication, presentation for internet page	Month 31	Month 37
9.1	Actualisation of the web site	continuous	continuous
9.2	Annual report to the user's board	Month 6 and month 18	
9.3	Leaflet for vegetable processors pdf, print version and CD	Month 30	Month 38

Most of the delays within year 2 were due to problems in finishing the Consortium Agreement. This was solved and had no consequences for the continuation of the project (budget, costs). The Internet homepage had been already set up but the revision and optimisation took longer then expected. This was also solved and had neither no consequences for the continuation of the

project (budget, costs). The delay of tasks within WP 2 was due to re-planning the consumer survey after discussions with partners. While the explorative analysis was correctly placed at the beginning of the research program, it was argued that the consumer choice test was better performed after the research preformed by other WPs could make clearer proposals on which product attributes and levels to test. The delay in year 3 was according one partner's problem in measuring the samples and delivering data in time. After discussion with the Core organic funding body network it was agreed to prolong the project until 30.06.2010, which was in line with most of the other running Core organic pilot projects.

### 3. Work package description and progress of the work:

### WP 1 | Coordination

### Responsible partner: P1, UniKa, Johannes Kahl

### **Description of work:**

The co-ordination included the monitoring of the overall project progress. This included management of communication and knowledge transfer between the project partners and board members. Co-ordination and administration was carried out according to the operational plan of the project. The work was divided into the following tasks:

### **Task 1.1 Making the Consortium Agreement**

The task dealt with making the consortium agreement between the partners and invitation of the advisory board members

Task 1.2 Planning and organisation of project consortium and advisory board meetings
The advisory board allowed partners from the praxis to discuss the different achievements of the
project and gave input to the different WPs. The project co-ordinator organised all project meetings
including careful and timely preparation of the agenda, meeting material and the minutes.

# Task 1.3 Project co-ordination, management, administration, supervision and quality control

The project co-ordination required communication inside the project. Each partner was required to submit a biannual status report on progress in their running tasks. The project meetings included a monitoring and evaluation process.

### Task 1.4 Internal communication

The regularly updated website (organised by FiBL) was of central importance for the internal communication of the project and its results. The web-page supported internal information, communication and data transfer and knowledge management with an intelligent database system accessible through the controlled-access section of the web-site. The project co-ordinator provided the means required to manage and support the web-site and database. The web page also comprised external functions which were considered in WP 9.

# Final report on work carried out, and progress of the work compared to the original plan: A- work carried out and results obtained:

The project started in time and the kick-off meeting was successfully carried out in Germany in June 2007. The yearly meeting 2008 was carried out in Italy in Rome, meeting 2009 in Denmark in Korsor and the final project meeting in Finland in Mikkeli 2010.

One additional project meeting was held in Austria in Vienna in fall 2009. On this meeting the main achievements of the project according measurement and statistical results and overall messages and publications were discussed among the partners. The answers to the hypothesis of the project were finally formulated together. It was decided to start writing scientific publications within project lifetime. The minutes, including decisions and key information were distributed among all partners. Time schedule of the meetings, work-tasks for the partners and responsibilities where discussed and agreed on. The homepage was set-up and completed.

A consortium agreement was sent to all partners and discussed during the kick-off meeting. The CORE funding body management board agreed on it and the CA was finally discussed on the project meeting in Italy 2008. The partners signed it within 2008, the Danish partner (P5) has objected to and has reservations to the CA as concerning "Acces rights to data and information" and to missing regulations concerning "Confidentiality", "settlement of disputes" and "Applicable Law". Here we found a solution by adding an amendment to the CA which is signed from the partners now. The list of deliverables and milestones was discussed with WP managers and sent to the CORE funding body. Difficulties with two national funding bodies could be clarified. The annual reports 2007, 2008 and 2009 and the mid-term report were sent to the CORE funding body in time. Because project partner P 7 could not carry out measurements on the samples in time (unforeseen prolongation of laboratory renovation), the consortium decided to prolong the project duration. On advice of the funding body network representative, the project duration was prolonged until 30.06.2010.

**B- comments on deviations from the original plan**The delays are explained above; there were no other deviations to the original plan.

# WP 2 Consumer and Processor Research on the quality of processed vegetable, in particular baby food

Responsible partner: "partner no, institution acronym and name of WP manager"

### Description of work:

- To investigate consumer awareness, expectations & attitudes on the quality of organic processed vegetables in special baby food
- To identify processor awareness, expectations and demands on the quality of organic processed vegetables in special baby food

### Task 2.1 Consumer analysis

Qualitative and quantitative market research methods were used in order to investigate:

- consumer awareness, expectations and attitudes with respect to sensory and nutritional quality characteristics of special baby food and wider societal issues (e.g. energy use, transport distance "food miles") regarding this food category
- consumer willingness-to-pay regarding each quality characteristics (attributes)

Exploratory qualitative analysis was performed by means of focus groups. Focus group discussions were conducted in IT & DE. Three Focus Groups were helt in each country on the basis of semi-structured guidelines prepared by UNIVPM. Recruitment was performed on the basis of a specific screening questionnaire. These discussions allowed to develop the survey items by: (a) capturing all the quality dimensions (attributes) that need to be measured in the survey: (b) by determining the levels of these dimensions/attributes.

On the basis of the FG results a Choice Based Conjoint questionnaire was developed in order to conduct a Choice Experiment survey. Alternative baby food was identified, as well as relevant attributes and the number of attributes levels. The choice sets were identified by means of an orthogonal design. The survey was administered on a total sample of 800 consumers in 4 CORE countries (DE, DK, FR, IT). The survey was administered by a subcontractor by means of RDD telephone interviews (CATI) over a representative sample of consumers. The results of the survey allowed to identify the willingness-to-pay of each attribute (quality characteristics). Moreover, a decision support system (DSS) was developed in order to predict the impact of the changes in the levels of the attributes on choice shares and absolute numbers of consumers choosing each alternative.

### Task 2.2 Processor expert survey

An expert questionnaire was prepared to be used in expert surveys. The survey focused on identifying challenges with respect to:

- maintaining food safety
- providing satisfactory shelf lives
- satisfying processors demands with respect to sensory and nutritional quality characteristics

The selection of the processors was done with all consortium members. The experts were technology specialists with experience in processing technologies used for vegetable in special baby food from the member countries from Core Organic (AT, DK, FI, FR, DE, IT, NL, NO, SE, CH, UK). These experts were from companies producing baby food (organic as well conventional) A first written survey with a semi-structured questionnaire involved approximately 30 baby food processing experts in the 11 participating countries. The key issues, points of controversy and needs for the further development of a quality definition for baby food was identified.

### Task 2.3 WP Consolidated report

The outcomes from Task 2.1 and 2.2 was compared and jointly analysed by UNIVPM & FIBL, who jointly produced a consolidated report, D2.3, on consumer & processor awareness, attitudes, expectations and demands with respect to the quality of organic processed vegetables in special baby food, which was the basis for the subsequent WPs.

### Final report on work carried out, and progress of the work compared to the original plan:

A standardised questionnaire and a simplified flow chart was prepared for the expert processor survey and translated into English, French, Italian and German language. The survey includes general open as well as specific closed questions and is focused on attributes of quality, raw material, sensory aspects, processing techniques and shelf life. Appraisements of customers as well as company characteristics were also covered.

Before starting the expert round, a pre-tested with the SME Hochdorf (P12) was performed by P2. The survey was carried out as a telephone interview and all project partners were asked for contacts to national experts. The expert selection was made up of representatives belonging to one of the following groups: organic baby food processing companies from the member countries, selling vegetable processed organic baby food under their own brand and technology specialists of the process or raw material pre-processing.

17 experts in 10 countries were asked to join the expert telephone interview, but only 10 expert interviews from six countries (I, D, F, NL, CH (3), FIN (3)) could be collected. The telephone survey was completed with the assistance of national Core organic partners in September and October 2007. The results of the interviews were analysed and supported the identification and definition of critical and essential control points as well as product quality parameters for WP3. An internal report on processor survey has been prepared at the end of January 2008 and presented at BIOFACH 2008 as results of a case study regarding expectations of quality of organic baby food processing experts.

A consumer FG guideline for the consumer survey has been prepared by P3. The guideline incorporates both changes proposed by P1 and some of the proposal of the guidelines circulated by P2 (this has been done in order to adapt similar directions of the both consumers and processor surveys). Therefore, the guidelines incorporate a question concerning new alternative technologies: P2 has prepared a "Product data sheet for product innovations for baby food" to be used during the FG. The discussion guide was translated and adapted to the countries (some small language changes were needed). Focus group discussions have been conducted in IT & DE. A recruitment scheme was also prepared by P3. The final selection criteria proposed were translated in a formal questionnaire by each partner.

In Germany 3 FG have been conducted.

In Italy, instead of three, only two focus groups did actually take place of the 3 foreseen, due to recruitment problems and many no-show of the prospect participants. In order to avoid further delay, one replication was considered sufficient, given the information collected was substantially similar in both FGs.

At the end of December 2007 the internal DE and IT FG reports have been finalized.

### Focus group data collection

- 5 group sessions
- in 2 countries (2 in ITALY and 3 in GERMANY)
- with 8-12 participants each group:
  - o Responsible for family food purchases (100% of consumers)
  - o Gender (100% female: mothers)
  - o Mothers (Children below 5 years and above 6 months of age)
  - o Employment (at least 1/3 and at most 2/3 full time or part-time worker and at least 1/3 and at most 2/3 housewives)
  - o Purchased vs home-made baby food (at least 1/3 and at most 2/3 consumers purchasing baby food and at least 1/3 and at most 2/3 self-preparing baby food)
- groups were made up of regular and occasional organic consumers (100% consumers buying organic 1 or more times/week or less than 1 time/week spending more than 5 euros/month)
- Participants working in market research, in agriculture sector, in food industry/food processing and food wholesale or retail have been excluded

### Results

Type of preferred vegetable baby food Most of the participants in IT and DE

- Uses home-made vegetables purée
- Or uses both home-made vegetables purée and purchased baby food
- Some mothers buy baby-food products only for convenience
- In some cases mothers reports that grandparents use purchased baby-food when the children are left with them (IT)
- A wide range of vegetable baby food are used
- Carrots has been mentioned as one of the preferred vegetable
- Some mothers stopped feeding their babies carrots in order to deal with their babies' allergies (DE)
- Mothers' preferences are influenced by what babies accept as well as mother's likes and dislikes

### Buying vs home-made

### Factors that make <u>home-made preparation preferred</u>:

- Safety: it is cooked freshly and not heated a second time
- Origin: you know how baby food products are made (IT)
- Convenience: (IT)
- Sensorial: better taste, texture and colour (DE)
- Low environmental impact: it helps to avoid a large amount of jars -waste (DE) and it considers the question of food miles (IT)
- Price: is assumed to be cheaper (DE)
- Control: it gives mothers the control over ingredients (combination of them and their quality)
- Educational: it is expected to make the transition of babies' separate diet to the family diet easier (DE)

### Factors that make purchased baby food preferred:

- Convenience:
  - o In travel or out of home situations (eating out of home: IT)
  - When they don't have time or for time saving purposes
- Variety: purchased baby food makes it possible to bring a wide variety of different dishes on the menu (DE)
- Safety: purchased baby food products are perceived as of higher food safety since they underlie very strict quality controls (DE)

### Buying criteria & product attributes

- Composition: salt, sugar, sweetener, sugar substitutes, spices and all sort preservatives, flavour enhances, thickening agents and dyestuffs should be avoided.
- Origin:
  - Local origin guarantees product freshness & immediate processing and preservation of environment (food miles)
  - o At same time, German baby-food brands have a very high brand image in IT
- Point of purchase:
  - o specialized organic shop preferred in IT
  - shops with a wide choice of different baby food purées preferred in DE: shop types other than discounters are more trustworthy.
- Packaging: only glass jars are accepted for packaging
  - o Transparent glass is preferred allowing the observation of food (DE).
  - o The packaging, in general, should be appealing and natural (DE).
- Serving size: most participants appreciate smaller size because the jar is open and used; in some other cases (family with many children: IT) also the big size can be useful
- Brands:
  - Brand awareness appear to be higher in DE than in IT, where only fewer brands exists and much less available in POS
  - German brands have good image in Italy for safety but less for taste ("German taste")
  - o In Germany brands are well recognized and have different positioning according to

the retailer: specialized, supermarket, discounter. Private label are appreciated too.

- Point of sale: Brands available in specialised organic shop are better trusted in IT
- Labelling:. Nutritional labeling is seen as important, though not all consumers actually read it.
  - In DE information on independent product tests results (Stiftung Warentest, Öko-Test) are perceived as positive
  - o Label should not be too colourful and carrot's images should show look healthy.
- Price: price perceptions and attitudes are vey different in DE and IT:
  - o In DE baby food puree prices are seen as indicator of quality ("food does have its price"): if price too low consumer do not trust the bay food
  - In IT price was mentioned only by one consumer: purchased baby food could be safer but they are also more expensive
- Aspect/colour/shape/smell/taste:
  - o IT: no relevant issues were mentioned on these attributes: most of participants think that baby food products in general do not have a good taste;
  - DE: purees should <u>appear</u> in a colour which is not brown or with any grey tones.
     The <u>texture</u> of baby purees should not be too watery or with any water separation.
     The <u>taste</u> should meet children's as well as parents' tastes.
- Best before date:
  - o IT: It should be longer because it is a guarantee of freshness (even if more important is the date of production, which is usually not shown)
  - DE: participants seem to like the idea of shorter shelf life as guarantee of freshness (no pasteurization)

### Product data sheet for product innovations for baby food

	Product 1: Cooled carrot purée	Product 2: Frreze-dried carrots	Product 3: Carrot powder to mix
Ingredient	fresh carrots, water	carrots	carrots
Raw material	special variety for processed carrots	Special variety for processed carrots	Special variety for processed carrots
Process	the product is pasteurized (heating time is shorter than with a sterilisation).	deep frozen carrots are dried under a small vacuum of about 6mbar. In this process the deep frozen water is sublimated directly to damp	Carrot pure eis dried on a heated waltz.
Shelf life	7-10 days	3 years	3 years
Storage	cooled	dry	dry
Product description	because of the pasteurisation instead of the sterilisation the product is careful processed and the loss of important ingredient, like vitamins could be minimized.	the freeze dried carrots is easy to prepare; it needs only to be added boiled water, then the carrots puree is ready to eat	the carrot powder is easy to prepare; it needs only to be added boiled water, then the carrots puree is ready to eat.
Sensory quality	because of the careful processing, the sensory quality should be better than the serialised product	the freeze drying keeps the structure and the colour of the raw material, the loss of important ingredient with a high nutritional value is minimized.	of important ingredient with a high
Packaging	glasses or PE cans with an aluminium cap	vapour deposited metal layer, reclosable	vapour deposited metal layer, reclosable
Product safety	to assure the product safety the cooling temperature has to be kept all the time		

### PRODUCT 1: COOLED CARROT PURE'E

- The most attractive one because is perceived as natural and fresher
- At same time, the necessity to store the product cooled is commented as impractical and unpleasant
- It has fewer production steps compared to the other alternatives (DE)
- Packaging in glass would be preferred while PE cans meet rejections (DE)
- In DE participants see this product as a good alternative to ordinary baby food jars while in IT it is perceived to be not very different from the actual baby food jars

### PRODUCT 2: FREEZE DRIED CARROTS

- Mixed feelings: some participants see this product as the less attractive one because is far away from the concept of fresh product; for some other is the most attractive one because it is very practical
- In general is perceived as unnatural and artificial
- Since it is light and easy to transport it could be used for travelling
- It is associated with known food products like instant mashed potato, instant soup, etc. (DE)
- Most participants did not know the difference between "freeze-dried" and "powdered" (IT)
- It can be used as a complement for baby food cereals which have to be mixed with hot water

### PRODUCT 3: CARROT POWDER TO MIX

- This alternative is described as being in no way concerned with carrots anymore and being out of question as a baby food alternative (DE); in IT is described to be disgusting
- It is described to be unnatural and artificial

### Conclusions:

- Organic is a source of quality guarantee, as some product and production attributes are granted, like: chemicals free, GMO free. It is also safer because of strictness controls
- Composition, Local Origin, Point of Purchase and Glass packaging appear to be important product attributes.
- Brand is also very important (DE) but just in supermarkets where there is no trust in the shop (IT).
- Vegetables Baby food are bought just in case of emergency (IT); in general, most of the participants in DE and IT use the home-made baby food
- Information on processing is low. Participants seem neither to be much informed, nor conscious about production methods used in conventional and industrial agriculture.

### B- comments on deviations from the original plan

At BIOFACH 2008 our final results on consumer and processor analysis have been presented and a "baby food workshop" performed.

At the end of May 2008 at the annual meeting in Rome, it was jointly decided to amend the original work programme, in order to allow a more efficient use of the choice experiment to gain useful information for the final steps of the project.

Therefore, the choice experiment was done to test how the improvements of the baby food products proposed by the other WPs was judged by the consumer.

# WP 3 QACCP Analysis Processing: Quality –driven distribution and processing chain analysis

Responsible partner: P4, UHEL, Marjo Särkkä-Tirkkonen

### **Description of work:**

The aim of the WP 3 was to select and focus on processes connected to specific carrot baby food (CH, D). Once the processing conditions were identified WP3 explored the possibilities for alternative distribution ways and processing techniques to improve the overall product and process quality.

### Task 3.1 Investigation and description of distribution and processing chains

The task started by getting into contact with SME's taking into account the specific products and processes involved in the production. The selected distribution and process chains were investigated and described in general by UHEL (University of Helsinki) in order to identify the criteria for quality evaluation. Based on this preliminary study UHEL in collaboration with P2 and P11 selected a limited number of specific products and processing steps for further investigation. Here the input of WP2, 5, 6 and 7 was required. Input was gathered from the involved SMEs regarding their current product range, market values, expected market potential as well as their current quality and health related policies. The final set of criteria was formulated at the kick-off meeting of this project. This assured a clear focus of the project.

### Task 3.2 QACCP Analysis of selected products and processes

The main part of this WP was the analysis of selected processes and distribution chains, identified in the two previous tasks with respect to their potential for improving performance. This task applied a distribution-chain (QACCP analysis). This analysis identified critical control points in the distribution and process chain where quality improvement was possible or accessed quality information may leads to improvements of the chain performance. The QACCP analyses was performed in close collaboration with the participating SME's. The QACCP analysis resulted in a selection of an adequate design of the process and distribution chain as well as of an overview about possible process chain alternatives. Hence some indications of improved vegetable processing to parties outside the project could be delivered from this task directly to WP 9. The report from this task was used for the first electronic communication with the advisory group conducted in WP 9 and gave the basic information for the sample plan design in WP 4. The standard HACCP analysis was done to fulfil the food safety regulations for baby food.

# Task 3.3 Optimization of the process regarding the QACCPs (microbiological, nutritional and sensorial quality ) in the pilot plant

The aim of the task was to identify the critical control points that have a substantial effect on quality attributes and knowledge about changes going on in the food in every chain element is needed. Technological knowledge was needed to control these points in the process. Therefore pilot plant trials were executed to optimize the process. Test material was carrot and model process was an autoclave sterilization process for baby food.

### Task 3.4 Implementation at SME Level

The process was optimized on SME level on the hand of the results of the QACCP analysis and the result of the pilot plant. The coordination of this step was done by University of Helsinki. These tests showed the results from improved vegetable processing and distribution for a specific case. This was a valuable demonstration material for WP 9. University of Helsinki was responsible for a public report about the implementation on SME level.

### Objectives of the WP3

The overall objective of WP 3 was to formulate and apply methodology, which can be used to perform distribution chain and process analyses leading more profitable and sustainable designs of production and distribution of food products. One important objective was to

confirm the plan concerning products and corresponding quality attributes with the participating industries. The specific objectives were

- O 3.1: Investigation and description of possible distribution and processing chains
- O 3.2: QACCP (quality analysis of critical control point, health and sensorial aspects) and additional standard HACCP analysis (actual situation)
- O 3.3:Optimization of the process regarding the QACCPs (microbiological, nutritional and sensorial quality aspects) in the pilot plant
- O3.4: Optimization of the process at SME level

# Final report on work carried out, and progress of the work compared to the original plan:

### A- work carried out and results obtained:

The first tasks in the project for WP 3 were to select and focus on processes connected to specific carrot baby food (CH, D, F). Once the processing conditions were identified (Tasks 3.1 and 3.2), the aim was to explore the possibilities for alternative distribution ways and processing techniques to improve the overall product and process quality. Therefore expected quality attributes of organic baby food, which were investigated by processor and consumer interviews (WP2), were defined. The processing and distribution chain was investigated by flow charts and personal interviews. After visiting the companies and production plants of Sunval (D) and Hochdorf (CH), Hochdorf was contracted and selected as the SME for the industrial trials. Samples were taken from each production step of Hochdorf and a general spectral analyses as well as analyses of furosine (marker for the maillard reaction) were done by LaSalle, France (WP5).-The analysis results of the Hochdorf samples were used as a basis for the planning of the pilot plant processing and definning the QACCP. During the WP3 meeting in Helsinki on November 2007 items concerning MS3.1. (Selection of processes and distribution chain and identification of sample point) were reflected and decisions made according the pilot plant processes conducted in Finland 2008. Based on the processors' survey (WP 2), the practical information of the involved SMEs, the general spectral analysis and the information of focus group interviews (DE + IT) three critical steps of quality were identified and the QACCP analyses was carried out. Specific processing steps were chosen for further investigation and samples were taken from each production step for a general spectral analyses done by LaSalle, France (WP5). Then the analysis results of the Hochdorf samples were used as a basis for the planning of the pilot plant processing. During the WP3 meeting in Helsinki on November 2007 items concerning MS3.1. (Selection of processes and distribution chain and identification of sample point) were reflected and decisions made according the pilot plant processes conducted in Finland 2008.

The pilot scale trials (Task 3.3) were performed in Finland. The trials were conducted using the organic carrot variety Maestro (the Italian field trial samples specially used for baby food production). The sterilized carrot puree was made from three differently handled batches: a) fresh cubes in order to make puree without any intermediate phases b) frozen cubes c) pasteurized puree. In table 1 a simplified description of the basic process for carrot puree in pilot plant is presented.

Table 1: The overall description of the process.

Process step	Description	Critical factors
1.Washing	Mashine wash in 3 kg batches	The water temperature
2. Peeling and cleaning	Mechanical peeling with karborundum.  Manual cleaning with knives.	Weight of the peeling waste, temperature
3. Dicing	5mm x 5 mm cubes	Cube size uniformity, temperature
4. Cooking	30 kg batch 30 min in 150 °C in steam convection oven (no pressure)	Temperature of the mass, process time

5. Milling	Speed 2880 u/min, Sieve 1, water added at this point to the mass	Temperature, structure and colour of the mass
6. Bottling	Manual bottling in 212 ml jars (net weight 190 g)	Weight and filling temperature
7. Sterilization	Sterilization in autoclave (full water) 118 °C, 45 min, rotation 6UpM, pressure 2,2 bar, F-value>6	Time and temperature of the process,(3 sensors), F-value, filling level,

The autoclaved samples from the pilot scale trials were sent to partners in WP 5, 6, 7 for analysis. Samples of extreme heat treatments (autoclaved samples made from frozen carrot cubes vs. made from fresh carrot cubes) were also sent for experiments conducted in FiBL/Austria. Some of the analysis have been conducted (CML, furosine, colour, dry matter and total soluble solids (Bx°). In the next step the analysis results were interpreted in order to optimize the process on SME level and to carry on the implementation of the process on SME-level.

The industrial scale tests (Task 3.4) were performed with Frigemo (pre-processing) and HOCHDORF (processing) Switzerland in January, March and April 2009 according the following test plan (figure 1). As a CCP "the quality of raw material" was checked and optimized at industrial level. Therefore Swiss Demeter carrots, which were grown in the same field (Basedingen) and were harvested at the same time, were used. The carrots were separated in three equal parts, stored (if necessary), pre-processed to fresh and frozen carrot cubes and processed to sterilized carrot baby puree in jars. After sterilisation samples were taken according to the sampling plan.

Fresh, frozen and stored raw material was pre-processed under controlled and documented circumstances. In January half of the processed carrot cubes were packed and immediately processed to puree of fresh carrots. The second half was blanched, frozen and stored until March when the frozen raw material was processed in HOCHDORF. The stored carrots were pre-processed and processed in April.

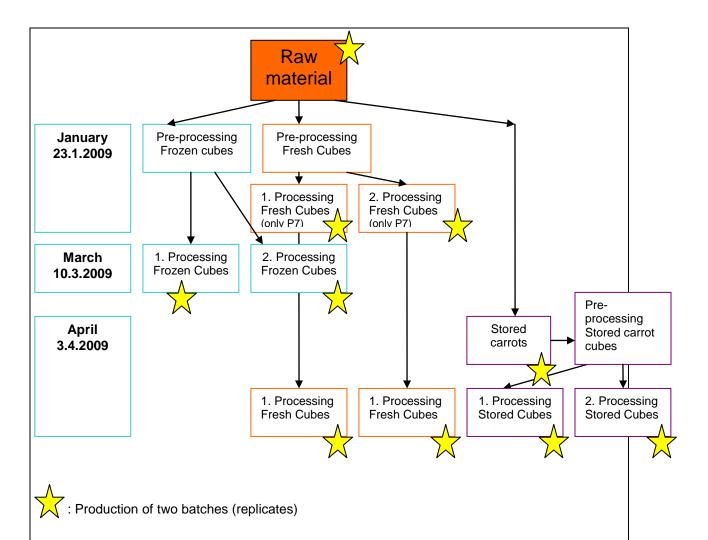


Figure 1: Test plan

Samples were taken according the sample plan and all samples were coded and sent as blind samples to the partners for analyses. The processed samples were shipped in April 2009 and analysed for CML, furosine, flurescence and polyphenols (partner 6), for sugar composition, organic acids, carotenoides, volatile compounds, dry mater, colour and sensory (partner 7), for biocristallisation, dry mater and sensory (partner 1) and for fungi growth (partner 8).

During processing back up were taken at the end of cooking, after milling and after bottling.

Raw material samples were taken as pooled samples before washing at AVAG in Kerzes. Sampling of processed baby food was conducted after sterilisation. Two batches (replicates) 1 000 kg of each raw material were produced. Batch 1 and 2 describes the replicates. One batch of cooked carrot puree produced 10 500 glasses which are one and a half fillings of an autoclaves. Samples of each autoclaving process were taken and coded A and B. i.e. 2 A indicates this sample is produced in the second batch/replicate and sterilized in the first autoclave. The aim was to take glasses which have the biggest heat impact during sterilisation process. Therefore the samples taken from the middle of the trolley (3 level) and from trolleys which were in the middle of the autoclave.

The pre-production of organic carrot cubes in fresh, stored and frozen quality was a standardised process, in which no differences were documented. The processing of, in each case 2\*800kg, carrot cubes to baby puree in jars at industrial level showed variable cooking procedures. Also replicates of processing showed differences. For industrial production small differences in processing are daily routine and accepted if the end

product specification is observed. Usually only frozen raw material is processed at HOCHDORF and all technical calibrations are adapted to these quality. Fresh and stored raw material has to be processed according other parameters and even with a different rate of water. If fresh and stored raw material should integrated in industrial processing new parameters have to be developed.

The end product analyse according HOCHDORF specification showed that only puree produced with frozen carrots comply the standardized requirements. Especially different sensory quality (intensive orange colour, sweeter taste) could be recognized in puree out of fresh and stored carrots. A analyses of the partners will shown, if also nutritional differences result out of these three different kinds of raw material. It may be stated that differences in the form of raw material (fresh, stored, frozen) also redraw through processing and sterilisation, and could be recognized in the quality of end product. So the QACCP is verified that we got differences with using different raw material. All samples were analyzed in detail by the partners and final conclusions was done based on those results.

Unfortunately HOCHDORF decided to quite the baby puree production so the improvement of the process at SME level in Switzerland couldn't be realized. Nevertheless the second SME Sunval in Germany was very interested in the results and also in changes of the process. At the moment the C.E.O. of Sunval is discussing the use of fresh raw material for processing organic carrot baby puree.

In addition to the pilot scale and industrial scale test procedure an overview report was done concerning different kind of sterilization methods (http://orgprints.org/17236).

### Final conclusion/summary of the work of WP3:

It may be stated that differences in the form of raw material (fresh, stored, frozen) also redraw through processing and sterilization, and could be recognized in the quality of end product. So the QACCP is verified that we got differences with using different raw material.

### B- comments on deviations from the original plan

It appeared that improvements of the distribution chain were within this project difficult to realize regarding the trade requirements. So the QACCP-analysis was carried on by reflecting the existing product flow and techniques of Hochdorf.

According the pilot scale trials the Italian carrot samples arrived ca. month later than originally planned (had to be ripe enough). This minor delay then was repeated in every stage hereinafter. Because of technical problems, P7 could not deliver the evaluated results of the pilot plant study in time to WP3. Therefore decisions about CCPs were taken based on results from P6 and measurements taken at P4.

Because of the limited resources in funding, partner 4 had to skip the second pilot test and the next step was decided to be conducted straight in industrial level in Switzerland by P2.

### WP 4 Sample organisation

### Responsible partner: P5, AU, Hanne L. Kristensen

### **Description of work:**

WP 4 organised sampling and distribution of carrot samples from field trials and SMEs. The field trials included the *VegQure* field trial in Denmark as well as a field trial in Italy. The Danish field trial was performed as part of the *VegQure* project aimed to investigate the effect of different crop rotations on vegetable quality (*VegQure* 2006-2010 Danish Research Center of Organic Farming, see reference). A detailed description of *VegQure* is found on this homepage.

This *VegQure* project included four different vegetable crop rotations, one conventional and three organic, with three full replications. The three organic crop rotations had increasing content of cover crops for nutrient management and intercrops to increase biodiversity and natural mechanisms for pest regulation. Carrot samples were sampled from all field replicates of the four crop rotations for analysis of fresh sample quality. The Italian field trial was runned by national partner and WP4 organised sampling through this partner (Italian Association of Organic Farming AIAB) of fresh samples for distribution and analysis in WP 5, 6 and 7. In addition fresh samples from the Italian field trial were processed at the pilot plant in Helsinki as well as by SMEs for distribution and analysis in WP 5, 6 and 7.

The sampling and analysis scheme followed in 2007 and repeated in 2008. In 2008 with adjustments of the Italian samples for processing at SMEs as well as samples after performance of storage test. The results were evaluated in relation to questions from practice. WP 4 contributed to the interpretation of results in WP 8 by relating differences in carrot quality to the agronomic field treatments and conditions.

The work was divided in the following tasks:

### T 4.1 Sampling of the VegQure field trial

Sample the *VegQure* field trial for fresh samples representing field replicates for analysis in WP 5, 6 and 7 in 2007 and 2008.

### T 4.2 Organising and distributing samples from field trials and SMEs

WP4 organised the sampling through national partners of fresh samples from the Italian field trial as well as samples from the pilot plant (Helsinki) and selected SMEs. These were distributed for analysis in WP 5, 6 and 7. The sample organisation was performed through the design of a sample and distribution plan (when, to whom, how much) assuring that samples from the same batch was measured by all participating laboratories. The sampling and distribution scheme was followed in 2007 and 2008 with adjustments in 2008 of the processed samples from SMEs and including a storage test.

### T 4.3 WP synthesis report

Interpretation of data was performed based on relationships to the agronomic field treatments and conditions in the field trials as part of WP 8.

### Linkages with other work-packages:

The sample plan for SMEs and to some extent field trials were built on the quality testing in WP 2 and 3. The sampling procedure was based on required sample conditions for high standard analysis in WP 5, 6 and 7. Information on agronomic treatments and conditions was input for WP 8.

# Final report on work carried out, and progress of the work compared to the original plan: A- work carried out and results obtained:

The sampling and analysis scheme has been followed in 2007 and repeated in 2008 including procedure of decoding by collection of results from partners. However, the milestone 4.1, 4.3 and deliverable 4.1 representing this finalising of this work, was delayed due to the following: The plan for processing of Italian samples at SMEs has been adjusted. WP 4 is contributing to the work in WP 8 of interpretation of results by relating differences in carrot quality to the agronomic field treatments and conditions as planned (milestone 4.4 and deliverable 4.2). Milestone 4.4 and deliverable 4.2 were delayed due to the postponing of the end date for the project.

The agronomic results showed that the yield of carrots harvested from the VegQure field trial in 2007 did not differ between cropping systems. In 2008 the yield was generally higher than in 2007, and in 2008 higher in the C and O1 systems compared to the O2 and O3 systems. The amount of total nitrogen and nitrate was higher in the C and the O2 systems in 2007, but not in 2008. No statistically significant differences were found in dry matter content, phosphorous and potassium between cropping systems. In the Italian field trial yields in the conventional system was approximately twice of those in the organic system, but this was due to double distance between rows in the organic system. The Italian yields were not tested statistically.

### References

Veg-Qure: Organic cropping Systems for vegetable production - product quality, natural regulation and environmental effects. Danish Agricultural Research Center for Organic Research III, 2006-2010. http://www.vegqure.elr.dk/uk/

### B- comments on deviations from the original plan

The sampling of SME samples made on carrots of unknown origin has been abandoned and instead the fresh samples from the Italian field trials was processed, distributed and analysed in WP 5, 6 and 7 in 2008. Also performance of a storage test on fresh samples prior to processing at SME has been included for spring 2009 followed by sampling and analysis at partners.

## WP 5 Assessment of food safety on fresh and processed carrots

### Responsible partner: "AgroParisTech - Inès BIRLOUEZ"

The overall objective of WP 5 was to assess some safety parameters and try distinguishing organic foods from conventional ones, for fresh as well as processed products. Although the first finality of organic agriculture is to avoid the use of polluting phytochemicals, and no obligation is required for the final product quality, it seems important to precisely control the level of phytochemicals with possible health impact in the final fresh or processed organic food products. On the other hand, food processing may be associated to the production of neoformed contaminants, depending on the severity of the heat treatment applied during sterilization. Monitoring of neoformed contaminants is of particular importance in processed baby foods, and key process steps or parameters influencing the final quality need to be identified.

The WP has the following specific objectives:

- O 5.1: Select the pertinent indicators for each aspect of the food safety in fresh and processed organic versus conventional products.
- O 5.2: Compare the levels of phytochemical contaminants between organic and conventional fresh products according to farming parameters.
- O 5.3: Assess that the microbiological safety is perfectly achieved by the process.
- O 5.4: Identify the key steps of the process and process parameters influencing the concentration of neoformed contaminants in carrots processed at the pilot plant.
- O 5.5: Assess the overall safety parameters in baby foods before and after processing at industrial level.
- O 5.6: Construct a cartography of the food safety parameters in commercial baby foods produced by organic and conventional lines.

### **Expected results:**

Characterization of the impact of organic practices regarding food safety Identification of the processing steps associated with neoformed contamination. Comparison of different processing regarding heat impact on food safety.

Implementation of a rapid and non destructive method allowing assessing the contamination of carrot products

# Final report on work carried out, and progress of the work compared to the original plan: A- work carried out and results obtained:

### 1.1 Pesticides

A deep screening of the pesticides expected to be present in the conventional carrots was done using GC-MS for the apolar and volatil ones and HPLC-UV for the polar and non-volatile ones. Possible known metabolites were also investigated according to the specifications given in the Guidelines for prediction of dietary intakes of pesticide residues (WHO, 1997).

Pesticides were divided according to their chemical structure into pure terpenes, terpene derivates, sesquiterpenes and triterpenoïdes.

No trace of pesticides were found neither in organic nor in conventional carrots of 2008 and 2009, except the presence of linuron and azoxystrobine in one italian organic sample in 2008. However it must be pointed out that carrots were peeled before analysis to account for the expected amount in carrot puree where peeling is one of the first process applied. Consequently, the fact that no pesticide was found in the peeled carrots does not mean necessarily that no pesticide was present on the peel. It has been reported that less than 20% pesticides are able to migrate from the peel to the flesh (Zohair et al., Chemosphere 63 (2006) 541–553), keeping more than 80% pesticides being present in the peel without being able to identify them in the flesh.

### 1.2 Nitrates

Strong differences in the nitrate concentration were observed between carrots of a same cultivar and farming. In 2007 VegQure organic carrots samples contained less nitrates than conventional ones whereas the contrary was observed for the Italian ones, which were globally more nitrate concentrated as the former ones. In 2008, nitrates in VegQure samples tended to have less nitrate than in 2007, while the concentrations also were slightly but not significantly lower in organic versus conventional carrots.

### 1.3 Neoformed contaminants in carrot puree

Neoformed or process contaminants (NFC) are the term generally used to name those compounds formed during heat processes from safe natural molecules in the product. Two main compounds are of interest in the carrot puree: the glycotoxins represented by fructosyllysine and carboxymethyllysine, and the carcinogenic compound furan.

Glycotoxins are formed during the Maillard reaction by condensation between reducing sugars and free amino acids, including peptide-linked lysine. Fructosyllysine is essentially metabolized in the gut by intestinal microflora and consequently is poorly absorbed. But the compound is of interest as indicator of the heat process. However, being an intermediary product in the Maillard reaction, a bell shape kinetic curve can be obtained for long heat treatments. Carboxymethyllysine is the most famous glycotoxin: it is known as being absorbed by the gut, and possibly interacting with receptors inducing oxidative stress and microinflammation. However CML is poorly formed in vegetables due to the low sugar and protein/amino acid content. Consequently, the toxicological significance of CML in processed carrots is low and CML can, as does fructosyllysine, be used as a marker of heat damage to the product with the advantage over fructosyllysine of being a stable product.

Furan is formed by heat degradation of carotenoïds, sugars, some amino acids and vitamin C. It is considered as a public health concern due to its high carcinogenicity and hepatotoxicity. The European commission has published a recommendation in 2007 to regularly survey the furan concentration in risky food products such as processed vegetables, especially in bottle sterilized baby food. The very volatile molecule has major risk to accumulate when the product is in bottle sterilized, as it cannot escape from the product. When opening the bottle before eating the product, furan seems not to escape from the bottle anymore.

Three assays were performed in 2007-2009: an industrial assay with unknown raw carrots in 2007, a pilot plan assay using Maestro 2008 carrots and again an industrial assay in 2009. The neoformed compounds (NFC) were analyzed during the process of puree manufacturing and were found to be formed during at two main steps: cooking of the carrot and in bottle sterilization of the puree. Mixing, crushing had no impact on the concentration of any neoformed compound. Filtration after mixing of the cooked carrots induced by contrast a significant decrease in the three NFC concentrations, suggesting that some were linked with insoluble fibers and furan could have disappeared by volatilization.

Among the contaminants studied, fructosyllysine, which was measured as furosine (product formed during acid hydrolysis of the sample) tended to be lower in the in bottle sterilized product after a previous accumulation during cooking. This is coherent with the expected degradation of fructosyllysine during severe and prolonged heat treatments. In contrast a cumulative effect was noted for carboxymethyllysine and furan (except for the filtration process), with furan accumulating especially in the last step of in bottle sterilization, whereas CML was formed earlier when cooking. This observation is in agreement with the knowledge on these two molecules; CML formation is associated with a low activation energy whereas furan needs high temperature to be produced, and needs a closed medium to accumulate.

Only few data are available on CML concentration in processed vegetables. The concentrations found in the cooked and pasteurized vegetables carrot puree prepared at Hochdorf or pilot plant were similar (between 4 and 8 mg/kg DM), a level close to that observed in common cooked vegetables (3-4 mg/kg DM). However, the CML concentration in the final in bottle sterilized product varied depending on the pretreatment and sterilization process applied: final concentrations were from 8 to 29 mg/kg DM, with puree processed from frozen carrots having either the lowest

concentration (pilot plant) or the highest (Hochdorf 2008). The reason for such inconsistency can be multiple: content in sugar (not known for Hochdorf assays), time-temperature of the blanching process before freezing, leading to variable vitamin C and sugar leakage. Hence, frozen carrots had very different CML levels in the pilot plant and in the Hochdorf industrial experiment (1 and 5 mg/kg DM respectively). Consequently carrot purees produced from fresh samples were either more concentrated or less concentrated depending on the assay. Carrots processed after pasteurization or after storage had intermediary levels. Furosine was strongly correlated with CML, as expected from two heat indicators. But this early Maillard compound was still more variable depending on the processing diagramme, as up to 8 times more furosine was found in Hochdorf 2008 sterilized carrots than in carrots processed at pilot plant. The difference must be related to the heat process applied and to the initial carrot sugar content (but this cannot be verified). No clear conclusion could therefore be deduced from these experiments regarding the impact of preprocessing on final CML content.

The project coordinator was notified by the partner from WP5, that the laboratory making the furan analyses had been a problem with their evaluation of the data and therefore the results they had provided could not be trusted. Partners from WP3 and 5 decided, that the laboratory has to reanalyze the samples to clearify the data evaluation (calibration) problem. The laboratory agreed to that and partners from WP3 sent stored baby jars from the Hochdorf trial to the laboratory. The results confirmed, that it was just an error in data evaluation and that the furan content of all jars is in line with the amounts reported in literature (far beyond the recommended limits). The samples made by frozen raw material showed lower amounts of furan content than the samples from fresh and stored raw material.

### 1.4 Fluorescence fingerprint as a global approach of quality monitoring

The UV-visible fluorescence signature of a food product is very sensitive to the physico-chemical composition and to the modifications occurring during processing. Moreover, front face fluorescence signature of the product is obtained non destructively using front face devices. Thanks to this approach a global view of the quality is possible. The fluorescence signature is analysed using multiway data analysis resulting in a description of the main information present in the signature (excitation-emission units referring to fluorophores). Each product is then characterized by the intensity of the fluorescence units.

Fluorescence fingerprints of the raw material was obtained and analyzed. 4 to 5 main fluorescence units were obtained. Final analysis of the impact of the couple variety-location, year of production and farming have been performed at the end of the project to evidence those factors mainly affecting the carrot physico-chemical state and global quality as evidenced by this optical approach.

During processing, a modification of the fluorescence was observed, especially during cooking and in bottle sterilization as previously observed for NFC. Decomposition of the image using PARAFAC analysis exhibited 3 main fluorescence units, one mainly due to proteins, phenols and vitamin E, the second one to the Maillard reaction products and the third one to riboflavin. The intensities of the decomposed fluorescence varied according to the processing steps, evidencing the strong impact of cooking and in bottle sterilization, as already evidenced from analysis of neoformed contaminants and nutritional markers such as carotenoïds and phenols.

As these general observations were repeated for the three processing experiments (Hochdorf industrial production in 2007, Pilot plant of Maestro carrots in 2008 and New industrial production by Hochdorf with a Bolero carrot variety in 2009), the information contained in the carrot fingerprints and changes along the processing chain seems robust.

To confirm this, a global PCA was done to analyze globally all the samples produced at different steps of the process following different conditions, from differently processed raw material and different carrot varieties. This PCA clearly discriminates, in the first principal component (explaining 70% of the variability), the three independent experiments. The difference comes from the raw material (different carrot varieties and year/place of production) and from the different processes applied. In the second principal component (explaining 24% of the variability), the impact of process step or heat damage becomes obvious: fresh raw material at the bottom, frozen and

defrosted material higher, then cooked or pasteurized carrots with mixing and smashing operations together, and in the top, the ready to eat sterilized carrot purees. Inside the latter group, the order of heat damage is the following: PPfrozen, HD07, PPPast and HD08stored fresh, PP and HD08 fresh and HD08 frozen (with PP pilot plant, HD Hochdorf, and indication of the state of the raw material, fresh or stored as fresh or frozen or pasteurized).

The information brought by the fluorescence fingerprint corroborates those obtained from the quality indicators, such as CML, furan and nutritional markers, with some small differences however: the differences between the different raw material preprocessing are smaller than those found with the contaminant furan, and closer to those obtained for the Maillard product CML. Briefly, the carrots processed as fresh material suffer more than the carrots submitted to a preprocessing. But freezing has a different impact in the PP trial (where it is the best), and in HD08 where it is the worst, similarly to what was found for CML.

Analysis of correlations between the conventional analysis of quality indicators regarding the sensorial, nutritional and safety aspects reveals strong correlations with the fluorescence intensities obtained after PARAFAC decomposition. The highest correlation levels were observed with phenols and carotenoïds for nutritional parameters, sugar, Brix, volatile compounds, texture, for sensorial parameters, and with various organic acids. Consequently, we can conclude that the fluorescence images evolve similarly to those different parameters upon processing. Based on these correlations, calibration models could be built to fit the quality parameter of interest optimally. This was done for example for furan and phenols, where highly satisfactory calibration models were obtained. These results evidence the potential of replacing conventional analysis by the "in real time" measures using a fluorescence sensor, such as the Fluoralys sensor developed by the SME Spectralys Innovation, a start up from AgroParisTech. Once the calibration models built and validated for any parameters correlated with the fluorescence, the sensor is implemented with a software allowing translating in real time the fluorescence image of the sample (25 sec) in concentration or level of indicator. Such a sensor could make it possible to monitor any heat damage on the product sensorial profile, nutritional value and safety.

Regarding pesticides, it should be outlined that conventional farming was carried out in a very controlled way explaining the absence of pesticides in the harvested carrot. Furthermore, some pesticides could have been lost with the peel as the carrots were peeled before analysis. These two remarks let suppose that safety benefit of organic farming regarding exposure to pesticides could be in reality higher than these results are suggesting.

As far as neoformed contaminants are concerned, it was confirmed that heat treatment steps are the most critical ones, and filtering allows decreasing the concentration probably by adsorption on the fibres. In addition, it was shown that processing frozen carrots tend to decrease the final concentration in neoformed contaminants, very possibly because of the leakage of the hydrophilic substrates, glucose and vitamin C in water during the blanching process.

### B- comments on deviations from the original plan

There were no deviations from the original plan

### WP 6 | Product quality

### Responsible partner: P1, UniKa, Johannes Kahl

### **Description of work:**

Through communication with the project partners the WP translated the quality key issues and problems into analysis. Together with WP 4 a sample plan was performed for analysis.

Different methods measured in different laboratories in the EU resulted in analytical and systemic as well as consumer oriented variables.

The work was divided in the following tasks:

### T 6.1 Definition of quality testing methods

The choice of methods was based on the inputs from WP 2 and WP 3 on the kick-off meeting. These methods were chosen from 3 different aspects, a: human health related parameters (e. g. biophenols, secondary metabolites, vitamins), b: structure related and systemic parameters (e.g. fluorescence spectroscopy, biocrystallization, dry matter) and c: sensory testing. The list of parameters (variables) tested within WP 6 was visible via intranet.

### T 6.2 Product analysis of different product groups

The information of all partner laboratories was the basic information for designing a sample plan together with WP 4. Always samples from the same batch were measured from all participating laboratories. The samples from both field trials were measured in field replicates and the raw material for the processing will be measured in bulk-samples. The measurements were carried out in 2007, 2008 and 2009.

### T 6.3 WP synthesis report

Evaluation of data was performed. Some result were used in WP 7 selecting the samples for the health studies and all results were finally transfer to WP 8 in a suitable format decided upon in agreement with WP 8.

# Final report on work carried out, and progress of the work compared to the original plan: A- work carried out and results obtained:

### Field trials comparing organic and conventional farming

Generally there were high yields obtained in all four systems in both years. The yields were higher in 2008 than 2007. In 2008 the yield and the total root biomass were higher in the C and O1 systems compared to the O2 and O3. The same differences were found when the yields were tested for total root biomass (discarded included).

There were only few differences in harvest quality and chemical contents between systems and the differences were not the same in 2007 and 2008. There were more discarded in the C system in 2007, whereas more were discarded in the O2 and O3 systems in 2008. There were more large carrots in system O2 and O3 in 2008, and more carrots were discarded in all systems in 2007 due to more pests, flies and worms compared to 2008 (*not statistically tested yet*). More roots were discarded from the C system than from the other three systems in 2007 due to more cracked and forked roots.

The content of total nitrogen and nitrate was highest in the C and O2 systems in 2007 compared to the O3 system. The same trend was seen for nitrate in 2008 (O3 being lowest). In 2008, P was close to be significantly higher in O2 and O3 compared to C and O1 (p<0.06).

No differences between the samples of the I year, while in the second year the organic carrots showed slightly higher moisture values than the conventional ones (O1 > O2 = O3 > C). The moisture content of the carrots of the second harvest year was slightly higher than those of the I year carrots.

In the literature it has been reported that the organic plant food tend to show a low moisture content than the conventional. However, this trend has not been shown by our results.

No differences between organic and conventional carrots resulted for the soluble solids content. The values of the titratable acidity of the second year were higher than those of the first year. The sample O1 showed lower levels of titratable acidity than the other samples, while no differences resulted between C and O2 and O3 in both the harvest years.

From the ratio between soluble solids content and titratable acidity resulted that the maturity degree of the carrots was homogeneous and comparable among C, O1, O2 and O3 for each year, but the carrots of the second year resulted slightly less mature than those of the first one.

Significant was the effect of the field replicates for each parameter as also the interactions among the variables.

Malic, citric, fumaric and ascorbic acid were the organic acids detected in the carrot samples, the malic acid being the most representative.

Malic and ascorbic acid contents were not affected by the harvest year, while no differences resulted among the field replicates for the fumaric and citric acid.

Differences between the samples related to the growing method were statistically significant but negligible for citric and malic acid. An inconsistent trend was observed for the fumaric acid. Both the harvest years the sample O1 was characterized by the highest levels of ascorbic acid. However, the content of this acid in the samples was so low to make the observed differences of no relevance from a nutritional point of view.

Significant interactions between the variables resulted for all the organic acid determined.

Fructose, glucose and sucrose were the sugars individuated and quantified. Sucrose was the most representative (about the 50% of the total sugars), followed by glucose (about 28%) and fructose. The concentration of fructose and sucrose was higher in the carrots of the first year than in those of the second year, while the pattern was not equally clear for the glucose content. These data, along with those of the organic acid composition, confirm that the carrots of the first year were slightly more mature than those of the second year.

Even though significant from a statistical point of view, The significant interactions between the variables made impossible to individuate a clear effect of the growing method on the sugar composition that can distinguish between the organic and conventional carrots.

Climate conditions can affect the sugar content in carrots. Carrots grown at low temperature are considered to have higher sugar content than those grown in more temperate conditions. Precipitation, light intensity, day length are other climate factors that may affect carrot quality. Precipitation leads to a reduced solar radiation and then in a lower air temperature. Water stress might increase the sugar (and dry matter) content of carrots. Light is an other important factor. At northern latitudes low temperature is compensated by long periods with photosyntetic activity. In our study it seems not to exist a relationship between air temperature and sugar content, because the temperature profiles observed in the two growing seasons were the same, while differences resulted in the sugar composition in our samples between the two harvest years. Differences instead have been observed in the monthly precipitation during the field experiment, with the first year being more rainy than the second one. However, in our experiment all the fields were irrigated, thus eliminating much of the effect of the fluctuation in the precipitation.

A clear difference between the first and second year samples resulted for the color parameters, with the exception of h° that showed the same value for all the samples and the harvest years. In particular, the carrot samples of the first year showed the same color characteristics, while in the second harvest year samples some difference was individuated. The L\* values of the carrot samples of the first year were higher than those of the samples of the second year. On the contrary, the first year carrot samples tended to show lower values of the a\*, b\* and C\* parameters. In the second year, the C and O2 carrots resulted characterized by higher values of the parameters a\*, b\* and C\* than the carrot samples O1 and O3.

Texture characteristics determined on the carrot samples resulted not affected by any of the variables considered.

Lutein,  $\alpha$ -carotene and  $\beta$ -carotene were the carotenoids detected and quantified.  $\beta$ -carotene was the main compound representing about the  $\frac{3}{4}$  of the total carotenoids. Clear differences resulted for the  $\alpha$ -carotene and  $\beta$ -carotene contents between the two years, with the samples of the second year showing higher values than those of the first year. These two main carotenoids resulted unaffected by the growing method, with the samples within each year showing the same contents. Only small differences resulted instead for the lutein content, but it was impossible to attribute them

clearly to the effect of the single variables tested. Again, the field replicates resulted to affect significantly the results obtained and the significance of the interactions made impossible to individuate a consistent pattern among the results.

Carotenoids are a group of natural pigments responsible for the yellow, orange or red color of many foods. In particular, the color of carrots is mainly caused by  $\alpha$ -carotene and  $\beta$ -carotene. The best documented and established function of some of the carotenoids is their provitamin A activity, especially of  $\beta$ -carotene.  $\alpha$ -carotene and b-cryptoxanthin also possess provitamin A activity, but to a lesser extent than  $\beta$ -carotene. Carotenoids have been shown to be effective antioxidants in vitro. The in vivo antioxidant behavior depends on the concentration and localization in the actual target cells, tissues or cellular compartments, as well as on many other factors, such as the nature of the reactive oxygen species (van den Berg et al., 2000). Carotenoids have also been reported to have immunomodulatory effects, such as the reduction in UV-induced immunosuppression. They have also been associated with lowered risk of developing degenerative diseases such as cancer, cardiovascular diseases, cataract and macular degeneration.

In plants carotenoids play indispensable roles in photosynthesis. In all photosynthetic organisms, carotenoids serve two major functions: as accessory pigments for light harvesting and in the prevention of photo-oxidative damage.

Carotenoid content is genetically determined; then different carrot varieties are characterized by different carotenoid composition. However, many other factors can affect quail- and quantitative carotenoid composition.

Fertilisation practices also affect the accumulation of carotenes in vegetable crops. Normally, the  $N_2$  availability in the organic fertilization is lower than that in the conventional one. As a consequence, the organic carrots should be characterized by a lower content of carotenoids than the conventional carrots. However, the results from studies comparing different fertilization methods (organic and conventional, are not conclusive. In fact, differences in  $N_2$  content of the four crops rotations between the two years were negligible. In both the growing seasons the O3 system resulted characterized by lower  $N_2$  content, however, this did not affect in a different way from the other systems the carotenoid content of the carrots obtained.

In our study we identified and quantified 15 monoterpenes, 7 sesquiterpenes, 2 phenylpropanoids, 2 aliphatic aldehydes (1-octanal, tr-2-nonenal), 1 oxygenated terpenoid (bornyl acetate), one carotenoid degradation product ( $\beta$ -ionone), 2 monoterpene ketones ((E)-6,10- dimethyl undeca-5,9-dien-2-one and 4'-methylacetophenone), 1 oxygenated terpenoid (thymol methylether). Volatile profile obtained in the present study was similar to those reported in previous papers, with terpinolene being by far the main component of the monoterpene fraction and  $\beta$ -caryophyllene and  $\gamma$ -bisabolene the most abundant components of sesquiterpenes.

The results obtained were analysed in order to investigate the effect of the four cropping systems on volatile compounds level. The same volatile profile was observed in all the samples of the two harvest years but, in general, a significant effect of the year on the amounts of volatile compounds was observed, with the carrots of 2008 showing significantly higher values than those of the 2007. A significant and consistent effect over the two years crop production was observed on total sesquiterpene and  $\beta$ -caryophyllene content. In both years total sesquiterpene level was significantly higher in the organic O3 sample than in the conventional one: about +20% and +52% in 2007 and 2008, respectively. These differences were associated to those observed on  $\beta$ caryophyllene, which was by far the main component of the sesquiterpene group (64% and 66% of the total content in 2007 and 2008, respectively). Total sesquiterpenes were also higher in the organic O2 than in the conventional carrots in both years (about + 31% and +28% in 2007 and 2008, respectively), but the differences were significant only in 2007 samples. Again, these differences were due to correspondent differences in the  $\beta$ -caryophyllene levels. These differences could also be correlated with significant differences observed on 2008 carrot samples in sensory bitter aftertaste, which was significantly higher in O3 sample with respect to C sample. On the other hand, total sesquiterpene and  $\beta$ -caryophyllene levels in carrots obtained by the organic cropping system O1 were not significantly different from those observed in conventional samples.

A similar effect of the cropping system was not observed on  $\gamma$ -bisabolene, another important constituent of the sesquiterpene group.

Regarding monoterpenes, the other main class of carrot volatiles, we did not observe consistent

and significant effects associated to the cropping system. Really in samples from 2007, variability in total monoterpene level among field replicates from the same cropping system was higher than variability associated to the cropping system. The same was true for terpinolene, which is by far the main component of the monoterpene group.

From the analysis of the biocrystallization pattern the carrot samples did not show clear differences. According to variable *sum variance* of the texture analysis, in both years the conventional sample C was different from the organic sample O1 and O3, whereas not from O2 in 2007. The variation within treatments of organic and field reps was greater than that within treatments organic vs conventional. In 2008 field replicate nr 3 of the C sample resulted different from the other field replicates of this treatment.

According to variable *diagonal moment*, when plotted against the Region of Interest of the crystallization pictures (ROI), the slope of the sample C was lower compared to the organic treatments in both years.

In conclusion, the O1 system (minimal organic) carrots showed most difference compared to C, maybe because of N-fertilization, whereas the organic treatments varying between years.

From sensory analysis results the following differences were perceived:

- the sample "O3" had a significant higher burning mouthfeel (P < 0,05), in tendence (P > 0,05) it showed a more green and bitter flavour than the other samples, also a more green aftertaste and higher burning afterfeeling;
- the sample "O1" grew significant (P < 0,05) less dark after cutting into slices;
- other differences were only shown in tendencies (P > 0,05). Sample "C1" had least intensity in a soapy aroma and most intensity in a fruity and nut flavour (reference: fresh hazel nut, parsnip) and sweet aftertaste. Sample O2 showed most dark-stained slices, had least soapy but highest sweet flavour.

According to the slight differences detected in the sensory profiles, a differentiation of management systems could not be done.

The results showed only few significant differences of the 4 carrot samples:

- O1 was significant sweeter than C1. In tendency, it had a higher pungent mouthfeel than all other samples:
- C1 was at least pungent in aroma and mouthfeel; it was at least green in flavour and had the highest intensity in a nut flavour referring to significant differences. In tendency, it was also at least bitter and most sweet in aftertaste;
- O3 was significant less firm than the other samples. In tendency, it was slight more bitter in flavour than the other carrot samples;
- O2 showed no significant differences to the other samples. It was close to O3 in profile except firmness and bitter flavour.

Comparing the product profiles, due to the slight differences in basic attributes it is difficult to differentiate the management systems. In general, all samples were less intensive in aroma and flavour attributes.

The assessment of dry matter loss in the decomposition test indicates storage quality. But this test also demonstrates potential differences in microbial decay due to the physiological state of the test product. Furthermore fungal growth tests on processed samples serve as indicators of nutritional properties. The carrots originating from the VegQure field trial had a higher storage quality then the varieties grown in the Italian farm comparisons due to genetic disposition. But within the same variety growing methods can still have an inpact on after harvest behaviour and storabilty.

Over the two test years the VegQure carrot variants showed only minor differences concerning dry matter loss and ergosterol contents due to a high variation between the field replicates. Concerning the Italian farm comparisons the organic variants displayed higher dry matter loss results compared to their conventional comparators with the exception of Excelso in the 2<sup>nd</sup> year, where the results were to the contary.

Dry matter loss overview:

Danish carrots

2007: C > O3 > O2 > O1 2008: C > O1 > O3 > O2

Italian carrots

2008: Morg > Mcon; Eorg > Econ 2009: Morg > Mcon; Econ > Eorg

In the VegQure samples the ergosterol contents, reflecting fungal growth, did not coincide with dry matter loss data. But the ergosterol contents of the Italian samples corresponded with the dry matter loss results.

Ergosterol contents overview:

Danish carrots

2007: O1 > C > O3 > O2 2008: O3 > C > O2 > O1

Fungi: high species abundance, low growth rate

Italian carrots

2008: Morg > Mcon; Eorg > Econ 2009: Morg > Mcon; Econ > Eorg

Fungi: one species dominant, high growth rate

## **Processed samples**

With respect to the Maestro organic fresh carrots the processed samples showed a higher moisture content (91-92% vs. 89.3%) and a lower soluble solids content (7.7-8.0 °Brix vs. 8.8 °Brix), while the titratable acidity values were about the same, apart for the batch replicate 601 (sample A) that showed a significant higher values than the fresh carrots and other processed samples. Even though some statistically significant difference resulted among them (lower values of the moisture content in sample C than in A and B), generally the processed samples showed the same characteristics.

In the following table, the carotenoid composition of the fresh carrots and the processed samples is shown. Lutein, alpha-carotene and beta-carotene were the carotenoids individuated and quantified. With respect to the fresh carrot, the processed samples showed a lower carotenoid content, with reduction that ranged from -54% to -62% for the lutein and from -25% to -29% for the beta-carotene, while amounted to -34% for the alpha-carotene. Lutein resulted to be more sensitive to the heat processing than the other two carotenoids.

A comparison among the processed samples indicated that the sample A (puree obtained from fresh carrots and then autoclaved) showed the higher lutein content, while no particular differences were detected between the sample B and C. The alpha- and beta-carotene content was similar in all the processed samples. However, for the sample C significant differences resulted between the two batch replicates for all the three carotenoids determined.

Compared with the fresh carrots, all the processed samples resulted characterized by lower values of the color parameters, with the exception of the hue angle.

Among the processed samples, L\* values were higher for the sample A (fresh carrot + puree + autoclaving), but the sample from frozen carrot cubes (C, 294-847) showed higher values of a\*, b\* and chroma parameters.

As expected it was observed a quite lower level of both monoterpenes and sesquiterpenes with respect to fresh carrot samples, probably due to losses mainly produced by the heat treatment (see the following table). The reduction of monoterpenes content was about 90%, while that of sesquiterpenes was 70-80%. Differences in volatile composition between processed carrots and raw carrot were found to be mainly quantitative rather than qualitative, as observed also in a previous study on canned carrots Nevertheless some compounds, which were not present in fresh

samples, seemed to be formed as a result of processing: styrene, a dimethyl substituted styrene compound,  $\beta$ -cyclocitral (tentative identification to be confirmed). Also  $\beta$ -damascenone, found at trace level in pilot plant trial samples was not detected in fresh samples, whereas  $\beta$ -ionone was present in a higher level in processed samples than in raw carrot: these two compounds are known to be formed by carotenoids degradation and their appearance or increase in processed samples might be associated to the heat treatment effect on the carotenoid fraction. Moreover the presence of these two compounds might be relevant to the aroma properties of the puree due to the their markedly low odour threshold.

A higher level of both monoterpenes and sesquiterpenes resulted in the sample A "fresh + puree + autoclaving" (601-173) (somewhat reduced in the process repetition 173); the type "fresh + puree + pasteurized + autoclaving" (307-587) (sample B) showed intermediate values of both monoterpenes and sesquiterpenes, whereas for the sample from "frozen cubes + puree + autoclaving" significant differences were found between the process replicates (847 being characterised by markedly higher amount of all determined volatile compounds in comparison with 294). In these samples a higher level of some compounds formed as a result of processing ( $\beta$ -ionone,  $\beta$ -cyclocitral, the dimethyl substituted styrene compound) was found. Anyway quantitative differences were not quite marked so they are not expected to produced marked differences in the sensory properties.

The processed samples of January 2009 resulted characterized by a higher moisture content (about 91.5 vs. 89.0%) than the raw carrots.

The ascorbic acid content was particularly low in the fresh carrots, confirming that carrots are not a good source of this vitamin. After the processing, the ascorbic acid was not detected because of the heat treatment. The other organic acids were not affected (malic acid) or showed an increased content as for the fumaric acid.

With respect to the raw carrots, the processed samples showed a decrease of the values of the color parameters. Only the values of the hue angle parameter in the processed were higher than that of the fresh carrots. Some significant differences resulted among the replicates but they were due to the narrow range of variation of the data and can be considered of no relevance in practice.

A general reduction of the carotenoids was observed in the processed samples in comparison to the raw carrots. In particular, the reduction of lutein content was about 30%, that of alpha-carotene about 17% and for beta-carotene content about 25%. For the beta-carotene content some significant differences resulted between the batch replicates.

The processed samples from April 2009 showed a higher moisture content with respect to the fresh carrots, thus confirming the trend showed by the samples of January. However, the moisture content of the processed carrot F resulted higher than that of the puree samples of January.

As expected, the ascorbic acid was not present in the processed samples D, E and F. The malic acid content of the samples D and E was similar to that of the fresh carrots, while the fumaric acid content was higher, as it occurred for the puree samples of January. In comparison with the samples of January no difference resulted for malic and fumaric acid content in the samples D and E; however, the sample F was characterized by a significant lower level of malic and fumaric acid with respect to both fresh carrots and puree samples of January.

All the color parameter values resulted lower than those of the fresh carrots but comparable with those of the puree samples of January.

Slight, even though significant differences in the parameter values resulted between the samples D, E and F, with the sample E showing lower values of L\* and the sample F the highest values of L\* and the lowest of a\*. However, for the color characteristics many significant, although very slight, differences were detected between the batch replicates of the three puree samples.

A significant reduction of carotenoids content with respect to the raw carrots was shown by the processed samples of April. In particular, lutein content resulted about 45% lower than that of fresh carrots, alpha-carotene about 30% and beta-carotene about 35%.

Compared to the processed sample of January 2009, the reduction of the carotenoids content ranged from 3% to 25%, with the beta-carotene less affected than lutein and alpha-carotene.

The content of lutein was lower in the sample E in comparison with the other ones. The samples F

was characterized by a lower content of alpha-carotene, while the samples D showed a higher level of beta-carotene than the other puree samples of April

Concerning the volatile compounds, the same considerations made for the "pilot plant" samples are good also for the Hochdorf samples: a dramatic reduction of the monoterpenes and sesquiterpenes content in comparison with the fresh carrots; the formation of new volatile compounds, not present in the raw carrots, due to the heat processing.

With respect to the samples of January, the puree samples D (processed fresh and stored) did not show any particular difference for the main monoterpenes and sesquiterpenes compounds content. However the samples E and F, obtained from stored raw carrots and stored frozen carrots, respectively, shown an evident reduction of the content of both the main classes of volatile compounds. In particular, the reduction of the main monoterpenes content was significantly high in the sample E, while the sample F showed a more clear decrease in the content of the main sesquiterpenes compounds.

A complex mixture of phenols were observed in the chromatogramme from which only a part was identified. Some were derivates from a major phenol and were therefore quantified together, such as caffeoyl quinic acids and esters of hydrocynnamic acid. Three main phenols were identified and quantified: caffeic acid, chlorogenic acid and coumaroylquinic acid. The main stages in processing associated with changes in the phenols profile and concentration are step 3 corresponding to carrot cooking and stage 7 referring to autoclaving. In addition to the first three phenols identified in the raw carrots, two new compounds derived from caffeic acid appeared during carrot processing, probably due to caffeic acid degradation. Caffeic acid itself accumulated during the process as a probable result of hydrolysis of the glycosylated conjugate. In contrast, chlorogenic acid and coumaroyl quinic acid decreased by a 40% during cooking and 50% during autoclaving.

The evolution of the phenol profile during the process is complex. Major changes concern the production of high amounts of chlorogenic and caffeic acids, as well as caffeolyquinic acids and a modification of the type of esters of hydroxycynnamic acid. The raw carrot did not contain caffeic acid which was exclusively produced from the heat treatment.

Poor information is available concerning the degradation reaction of such compounds. But very probably free phenols such as chlorogenic, caffeic and caffeoylquinic acids could be released from more complex structures. One cannot however exclude the possibility that the rupture of the cellular wall could favour phenol extraction.

The nutritional and health consequence of such changes are therefore very difficult to interpret. More work is needed to understand the chemical reaction mechanisms responsible for phenol thermoxidation.

On the other hand, the heat treatment could have inhibited some phenol oxidase activity inducing a better preservation of the phenol content. It must be kept in mind that the raw material was frozen for a long period before analysis and freezing can be associated with oxidation processes.

For sensory analysis, statistically significant differences were found for 11 attributes, except harsh, sour and astringent which were scored very low values and did not change among the puree samples.

Deep frozen processed samples (D samples) differentiated for lighter global odour and flavour, canned odour and flavour, sweetness, saltiness and texture parameters. All the attributes were strongly positively correlated to the first principal component (PC1) of PCA, which alone describes 94% of the total variance. The model shows clear separation of the frozen+stored+puree+autoclaved samples and good reproducibility across replicate and highlights a reduced impact of flavour and texture properties.

In the 1<sup>st</sup> year the carrot purée samples from the Pilotplant were tested as to their potential ability to sustain fungal growth. The purée variants differed concerning the raw material used: fresh, pasteurized and frozen carrots. The test fungi *Penicillium sp.* and *Fusarium sp.* were isolated from decomposition tests with the VegQure carrots. The detected differences concerned colony diameter, sporulation and colony appearance after one week of incubation.

In the 2<sup>nd</sup> year the pocessed sample from Hochdorf only included fresh and frozen carrots as raw material. In this case both fungi showed a significantly better growth on the samples made from fresh carrots. This result migth reflect differences in nutritional quality. Therefore this test could be an useful vitality test for future investigations.

With the biocrystallization method the puree samples from different treatments from pilot plant studies and Hochdorf trials could be significantly discriminated. Whereas at pilot plant level pasteurization had the biggest influence on the texture parameters, at Hochdorf the puree from frozen raw material was different from the fresh and stored. Moreover the biocrystallization was the only method, which could detect the influence of temperature loadings within the processing chain on the final puree quality also after sterilisation. The method is not more expensive than other sensitive tools like new mass spectrometry methods. The biocrystallization shows something which is not reflected by the other methods, so we have a new indicator for organic food quality. Specially in processing it is a very valuable tool, because with biocrystallization the treatments of the raw material could be clearly differentiated on the final food product. Moreover the biocrystallization was the only method, which was able to detect a problem during processing (temperature levels), so very sensitive to technological impacts on the food. Therefore it should be used in every investigation on organic food processing from now on.

## B- comments on deviations from the original plan

There were deviations compared to the original plan, because one partner could not deliver results from measurement in time. Therefore, the project was prolonged from December 2009 to June 2010..

## **WP 7**

## Responsible partner: P5, AU, Charlotte Lauridsen, Elena Mengheri

## **Description of work:**

The following research activities were listed in the proposal:

- 1) To characterise the different qualities of the test products obtained from controlled field trials by studying several biomarkers of health (growth, clinical evaluation, immune defence, antioxidant status, food preference and bioavailability) using a sensitive rat model (partly funded from the Danish DARCOF III "OrgTrace")
- 2) To study the effects of organic and conventional grown fresh products on intestinal Immune function of laboratory mice
- 3) To perform a preference test with mice on fresh and processed test products, and specifically to test the quality of selected test products by carrying out a growth study with baby laboratory mice

Final report on work carried out, and progress of the work compared to the original plan:

#### A- work carried out and results obtained:

With reference to the above mentioned research activities:

1)

### **Methods**

Danish VegQure carrots, organic (O1, O2 and O3) and conventional (CV) from 2007 and 2008 harvests were used. Carrots were freeze-dried and chemical analysedwere performed. The freeze dried carrots were included at 40% in an Altromin (chow) diet, which was given to weaned female GKMol rats, in groups of five rats per diet. At arrival the rats were put in a balance trail, where they were given their assigned diet (CV, O1, O2, O3) and CO (control/Altromin)) for a week. Faeces and urine were collected individually and digestibility of dry matter and protein was determined. Afterwards they were re-grouped again and given their assigned diet, for approx. 2.5 months (Except for O3). Throughout the experimental period the rats were monitored and weighed each week (Figure 1 and 2). At the end all blood was drawn by heart puncture for measuring some biomarkers, including glucose, cholesterol, triglycerides, NEFA in plasma, and activity of alkaline phosphatase. The plasma was also analysed for vitamin E and A, and immunoglobulins. All the organs (heart, lungs, kidney, spleen, ovary, pancreas, stomach and adipose tissue) were removed and weighed. The liver and fat tissues was frozen and further analyzed for vitamin contents. The spleen and Pevers Patches from the small intestine were sent to Bernhard Watzl's lab for cytokine production analysis and flow cytometry analysis of CD4/CD8 lymphocytes and NK cell activity. Finally, a preference test was performed on 15 rats, during two separate weeks with one washout week in between (Table 1).

## **Results & Conclusion**

As expected a higher content of protein (N) and nitrate in dry matter was found in the conventional system (CV). From the digestibility and balance experiment it can be seen that the carrot dry matter was highly digestible whereas protein was less available. There was not found any difference between the growth curves of rats fed the carrot+chow diets, and only the growth curve of the chow fed rats was somewhat higher. Analysing statistically the data from 2007 and 2008 harvest, the main conclusion is that harvest year rather than diet has an effect, and if any effect of diet, this is ascribed to the Altromin differing from the experimental diets. This means that there is no difference between experimental diets, i.e. no impact of the cultivation systems on the health biomarkers of rats after eating the carrot diets. The preference test showed that rats are able to differentiate between the diets and that there is a field influence on the preference for certain diets.

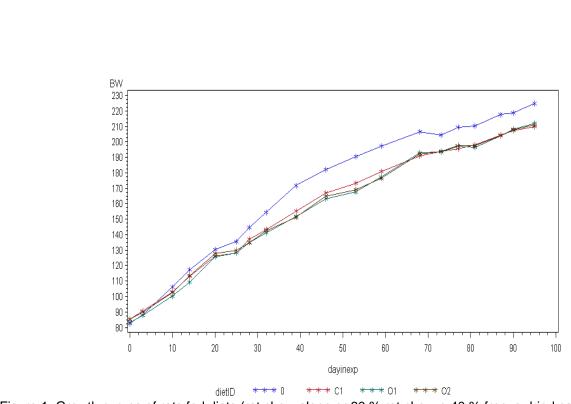


Figure 1. Growth curves of rats fed diets (rat chow alone or 60 % rat chow + 40 % freeze dried carrots) from harvest 2007.

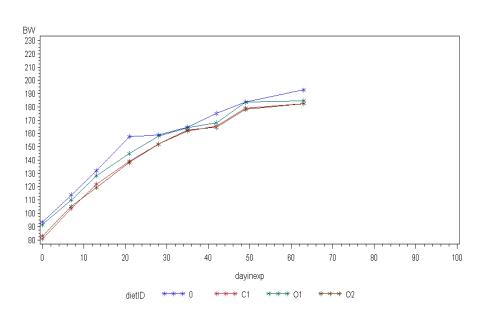


Figure 2. Growth curves of rats fed diets (rat chow alone or 60 % rat chow + 40 % freeze dried carrots) from harvest 2008. In harvest 2008 the experiment had to terminate after 63 days because of lack of carrots.

Table 1. Preference test on the three diets (60 % rat chow + 40 % freeze dried carrots) from the three field replicates and two harvest year

			field				
		N107	N109	N111			
		intake	intake	intake			
		Mean	Mean	Mean			
year	system						
2007	C1	2.0	6.1	3.0			
	01	3.4	1.9	5.1			
	O2	6.6	4.5	5.5			
2008	C1	3.6	5.6	5.3			
	01	5.4	5.8	5.3			
	O2	5.6	4.2	4.8			
syste	m						
C1		2.8	5.9	4.1			
01		4.4	3.8	5.2			
O2		6.1	4.4	5.1			

Rats could choose between the three diets (C1, O1 and O2) from one field replicate. Intake of diets is average of four days.

## 2) Methods.

Organic and conventional Danish and Italian carrots from field trials of 2007 and 2008 harvests were used. The Danish carrots, Bolero variety, consisted of three organic O1, O2 and O3 and 1 conventional (CV) variants; the Italian carrots were the organic (org) and conventional (CV) Maestro and Excelso varieties. Freeze-dried carrots were prepared to be included in a standard diet (70 g/kg diet) for mice. Balb/c mice 21 days old were fed for 1 month with the carrot diets. A standard reference 20% casein diet was also used as control. At the end of the experimental period, blood, spleen and small intestine were removed from the animals. Blood lymphocytes, splenocytes and *lamina propria* and intraepithelial lymphocytes (LPLs and IELs, respectively) were isolated and their immune phenotypes were freshly analyzed by flow cytometry (FACS): CD4 $^+$ , CD8 $^+$ , CD4CD8 $^+$ , CD19 $^+$ , CD25 $^+$ Foxp3 $^+$  (Treg cells). Cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)- $^-$ , interleukin (IL)-1 $^-$ , IL-2, IL-4, IL-5, IL-6, IL-10, IL-17 and tumor necrosis factor (TNF)- $^-$  were analyzed from sera by a cytometric assay: Th1/Th2 10plex Flow Cytomix Kit.

The significance of the differences was evaluated by 1-way analysis of variance (ANOVA) followed by Fisher's test. Differences with P values < 0.05 were considered significant.

### Results.

**Body weight and food consumption.** After consumption of Danish carrots, the body weight of mice fed the organic carrots did not differ from that of mice fed the conventional ones. The year production did not influence body weight. Similarly, there were no differences on the body weight after 1 month feeding with the Italian organic or conventional carrots, independently of the year production. The food intake was similar among all mice.

*Immune phenotypes.* In general, some differences on lymphocyte subsets were observed between organic and conventional carrots, that however were not similar between the first and the second year of cultivation.

Relating to the **Danish carrots** (Table 1).

### Gut

Intraepithelial compartment (IELs).

First year: the consumption of O2 and O3 carrots resulted in a lower CD4+ and higher CD8+ percentage, as well as higher CD19+ after O2, as compared to the subset distribution after the CV carrots consumption. Interestingly, the CD4+ and CD8+ populations after O2 and O3 diets were similar to those after the control diet. After the O1 diet, the main changes were an increase of CD4+ and CD19+ cells as compared to CV diet.

Second year: the lymphocyte populations of IELs did not differ from those of the first year, with the exception of CD8+ cells that were similar between organic and conventional groups.

Lamina propria compartment.

First year: higher percentages of CD8+, CD4+CD8+ and CD4+CD25+Foxp3+ cells were observed after O2 and O3, as compared to CV consumption. After the O1 diet, an increase of CD8+ and CD4+CD25+Foxp3+ cells was seen as compared to CV diet.

Second year: CD8+ remained higher, and O2 diet induced a higher percentage of CD19+ and a small but not significant increase of CD4+CD25+Foxp3+ cells. After the O1 diet, the CD4+CD25+Foxp3+ cells remained higher.

## Spleen

First year: a higher percentage of CD4+ and CD4+CD25+Foxp3+ cells, while a lower percentage of CD8+ were seen after O2 and O3 as compared to the CV consumption. After the O1 diet, an increase of CD4+CD25+Foxp3+ cells was seen as compared to CV diet.

Second year: these changes were not maintained. The values of CD8+ and CD4+CD25+Foxp3+ cells after O2 and O3 diets were similar to those obtained after the control diet.

## <u>Blood</u>

First year: the only change found was an increase of CD19+ after the O2 and O3 as compared to the CV and control diets.

Second year: these changes were not maintained.

In conclusion, the consumption of O2 and O3 both on the first and second year of carrot production had similar effects on the distribution of lymphocyte populations in intestine, spleen and blood, and differed from the effect of O1 consumption.

## Concerning Italian carrots:

**Maestro** carrots (Table 2).

#### <u>Gut</u>

First year: org carrots consumption induced a decrease in CD4+, an increase of CD8+ subpopulation in the intraepithelial and *lamina propria* compartments, and an increase of CD19+ subpopulation in the *lamina propria* as compared o the CV carrots.

**Table 1.** Lymphocyte populations of blood, spleen, IELs and LPLs of mice fed the Danish organic (O1, O2, O3) and conventional (CV) carrots, *Bolero* variety.

				BOLERO				
		1 <sup>st</sup>	year			2 <sup>nd</sup>	year	
	01	02	О3	CV	01	02	О3	CV
Blood								
CD4 <sup>+</sup>	$77.3 \pm 2.5$	$79.0 \pm 1.4$	78.7 ±1.5	76.1 ± 1.9	$76.6 \pm 1.4$	$76.5 \pm 3.4$	$74.6^{\#} \pm 3.7$	$75.5 \pm 2.9$
CD8 <sup>+</sup>	$19.7 \pm 2.3$	19.8 ± 1.8	$20.2 \pm 1.5$	$20.4 \pm 2.2$	21.0 ± 1.5	$20.0 \pm 2.4$	$21.5 \pm 2.8$	$21.8 \pm 3.0$
CD4 <sup>+</sup> CD8 <sup>+</sup>	1.1 ± 0.5	$0.8 \pm 0.3$	$0.9 \pm 0.3$	$0.9 \pm 0.4$	1.1 ± 0.3	$0.8 \pm 0.2$	$0.8 \pm 0.4$	1.1 ± 0.6 29.7 ±
CD19 <sup>+</sup>	17.1 ± 4.2	$26.2^* \pm 8.2$	$31.9* \pm 5.6$	$16.3 \pm 5.8$	$22.2 \pm 7.8$	$26.0 \pm 10.2$	$25.8 \pm 13.0$	19.0
Spleen								
CD4 <sup>+</sup>	57.1 ± 6.2	$73.3^* \pm 2.9$	$72.8* \pm 4.0$	$58.7 \pm 3.8$	$59.7 \pm 2.8$	$57.8^{\#} \pm 2.6$	$55.3^{\#} \pm 5.2$	$50.4 \pm 14$
CD8 <sup>+</sup>	$34.0 \pm 7.4$	$23.0^* \pm 3.6$	$24.0^* \pm 4.0$	$33.8 \pm 2.2$	$26.8^{\#} \pm 3.7$	$33.5^{*\#} \pm 2.8$	$36.2^{*\#} \pm 4.2$	$26.6 \pm 8.9$
CD4 <sup>+</sup> CD8 <sup>+</sup>	1.2** ± 0.5	$2.0 \pm 0.4$	$2.4 \pm 0.6$	$1.9 \pm 0.8$	$0.6^{**}\pm0.2$	$1.0^{\#} \pm 0.2$	$1.1^{\#} \pm 0.3$	$1.9 \pm 1.0$
CD19 <sup>+</sup>	48.6 ± 16.2	$47.7 \pm 8.2$	$56.5 \pm 4.4$	$56.6 \pm 5.5$	$54.1 \pm 9.9$	61.5 <sup>#</sup> ± 4.1	$60.9 \pm 3.4$	$54.3 \pm 5.5$
CD25 <sup>+</sup> Foxp3 <sup>+</sup>	11.4 ± 1.1	14.1* ± 1.0	15.1* ± 0.9	$9.0 \pm 1.3$	$11.2 \pm 2.0$	12.6 ± 1.3	$13.0 \pm 1.3$	11.0 ± 1.5
IELs								
CD4 <sup>+</sup>	19.3** ± 4.2	10.5*± 1.6	8.2* ± 2.1	$13.4 \pm 2.7$	$6.0^{\#} \pm 4.0$	$3.6^{*\#} \pm 0.6$	$3.6^{*\#} \pm 0.8$	$5.1^{\#} \pm 0.9$
CD8 <sup>+</sup>	$60.7 \pm 9.9$	81.2* ± 2.2	$85.8^* \pm 2.7$	63.4 ± 10	81.1 ± 3.0	$83.4 \pm 3.6$	$82.8 \pm 3.8$	$81.4 \pm 2.4$
CD4 <sup>+</sup> CD8 <sup>+</sup>	$7.4 \pm 4.0$	$5.2 \pm 1.9$	$4.2 \pm 1.4$	$6.2 \pm 4.0$	$3.3^{\#} \pm 1.0$	$3.0 \pm 0.8$	$2.8 \pm 0.6$	$3.8 \pm 1.2$
CD19 <sup>+</sup>	26.8** ± 5.4	13.1 <sup>§</sup> ± 5.6	$9.6 \pm 4.6$	$7.9 \pm 0.4$	4.5 <sup>#</sup> ± 1.8	8.0\$\$ ± 4.2	$4.9 \pm 0.9$	$5.8 \pm 3.3$
CD25 <sup>+</sup> Foxp3 <sup>+</sup>	$3.9 \pm 1.0$	$4.3 \pm 1.5$	$3.4 \pm 0.8$	$3.3 \pm 0.9$	$3.8 \pm 1.9$	$3.7 \pm 1.3$	$4.3 \pm 1.5$	$3.4 \pm 1.6$
LPLs								
CD4 <sup>+</sup>	62.6** ± 3.2	$42.0 \pm 4.3$	$48.8 \pm 4.6$	$42.3 \pm 7.9$	62.6 ± 8.2	$52.0^{\#} \pm 2.4$	49.0 ± 17.2	59.3 <sup>#</sup> ± 11
CD8 <sup>+</sup>	$25.0 \pm 5.5$	52.3* ± 3.2	$42.5^* \pm 3.4$	$22.6 \pm 2.5$	26.6 ± 6.1	37.6* <sup>#</sup> ± 1.9	$31.4^{*\#} \pm 3.9$	$23.9 \pm 2.9$
CD4 <sup>+</sup> CD8 <sup>+</sup>	$3.3 \pm 1.2$	$7.3^* \pm 0.2$	$6.6^* \pm 2.4$	$2.9 \pm 1.0$	6.2** <sup>#</sup> ± 1.7	$3.0^{\#} \pm 1.0$	4.1 ± 1.2	$4.4 \pm 1.6$
CD19 <sup>+</sup>	$47.4 \pm 0.8$	42.0 ± 1.9	45.2 ± 11.5	$54.5 \pm 9.8$	26.9 <sup>§§#</sup> ± 3.2	27.3 <sup>§§#</sup> ± 4.9	13.1 <sup>#</sup> ± 4.2	17.9 <sup>#</sup> ± 5
CD25 <sup>+</sup> Foxp3 <sup>+</sup>	12.8\$\$ ± 0.5	7.7§\$ ± 2.8	7.4 <sup>§§</sup> ± 2.2	$4.9 \pm 0.9$	10.1**± 4.7	7.0 ± 1.2	5.5 ± 1.1	5.6 ± 1.1

Data represent means  $\pm$  SD of at least 8 mice per group. **Blood.** \*P < 0.05 O2 and O3 vs O1 and CV. **Spleen, 1**<sup>\$\sigma\_t\$</sup> **year.** CD4<sup>+</sup>and CD8<sup>+</sup>: \*P < 0.01 O2 and O3 vs O1 and CV; CD4<sup>+</sup>CD8<sup>+</sup>: \*\*P < 0.05 O1 vs all; CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>: \*P < 0.05 O2 and O3 vs O1 and CV. **2**<sup>nd</sup> **year.** CD8<sup>+</sup>: \*P < 0.05 O2 and O3 vs O1 and CV; CD4<sup>+</sup>CD8<sup>+</sup>: \*\*P < 0.01 O1 vs CV. **IELs, 1**<sup>\$\sigma\_t\$</sup> **year.** CD4<sup>+</sup> and CD19<sup>+</sup>: \*\*P < 0.01 O1 vs all; CD4<sup>+</sup> and CD8<sup>+</sup>: \*P < 0.05 O2 and O3 vs O1 and CV; CD19<sup>+</sup>: \( \frac{\sigma\_t}{\sigma\_t} \) **year.** CD4<sup>+</sup>: \*P < 0.05 O2 and O3 vs O1 and CV; CD19<sup>+</sup>: \( \frac{\sigma\_t}{\sigma\_t} \) **year.** CD4<sup>+</sup>: \*P < 0.05 O2 and O3 vs O1 and CV; CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>: \( \frac{\sigma\_t}{\sigma\_t} \) **year.** CD4<sup>+</sup>: \*\*P < 0.05 O1 vs O2 and O3; CD8<sup>+</sup>: \*P < 0.05 O2 and O3 vs O1 and CV; CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>: \( \frac{\sigma\_t}{\sigma\_t} \) **year.** CD4<sup>+</sup>: \*\*P < 0.05 O1 vs O2 and O3; CD8<sup>+</sup>: \*P < 0.05 O2 and O3 vs O1 and CV; CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>: \*\*P < 0.05 O1 vs all. **AII**: \( \frac{\sigma\_t}{\sigma\_t} \) **year.** same treatment.

Second year: any difference was seen between lymphocyte subsets of org and CV fed mice. Spleen

First year: the CD4+, CD8+ and CD19+ subpopulations increased whereas CD4+CD8+ decreased in the org carrots fed mice as compared to the CV mice groups.

Second year: any difference was seen between lymphocyte subsets of org and CV fed mice.

Blood

First year: the CD19+ cells increased after the org diet as compared to the CV die.t

Second year: an opposite trend was found in the blood CD19+.

## Excelso carrots (Table 3).

Gut

First year: the org carrots induced a decrease of CD8+ in the intraepithelial and *lamina propria* compartment as compared to the CV carrots.

Second year: only a decrease of CD4+ subpopulation in the intraepithelial compartment was observed after organic carrots consumption.

## Spleen.

First year: a decrease of CD19+ and CD8 was seen after the org as compared to the CV carrots.

**Table 2.** Lymphocyte populations of blood, spleen, IELs and LPLs of mice fed the Italian organic (Org) and conventional (CV) carrots, *Maestro* variety.

		MAEST	RO	
	1 <sup>st</sup> )	/ear	2 <sup>nd</sup>	year
	ORG	CV	ORG	CV
Blood				
CD4 <sup>+</sup>	$78.4 \pm 1.7$	$76.1 \pm 3.3$	77.5 ± 2.1	$78.8 \pm 2.8$
CD8 <sup>+</sup>	$20.0 \pm 1.3$	$21.7 \pm 2.6$	$19.7 \pm 2.6$	$18.9 \pm 2.0$
CD4 <sup>+</sup> CD8 <sup>+</sup>	$1.3 \pm 0.5$	$0.9 \pm 0.2$	$1.1 \pm 0.6$	$1.0 \pm 0.3$
CD19 <sup>+</sup>	14.1* ± 2.4	$20.8 \pm 6.4$	21.2* <sup>#</sup> ± 6.6	$14.3^{\#} \pm 3.9$
Spleen				
CD4 <sup>+</sup>	61.2* ± 2.7	$52.0 \pm 4.3$	$60.1 \pm 4.6$	$60.6^{\#} \pm 6.8$
CD8 <sup>+</sup>	$34.6^* \pm 2.7$	$30.6 \pm 1.4$	$34.1 \pm 2.4$	$34.0 \pm 5.9$
CD4 <sup>+</sup> CD8 <sup>+</sup>	$1.3^* \pm 0.4$	$2.2 \pm 0.8$	$1.7 \pm 0.6$	$1.8 \pm 0.7$
CD19 <sup>+</sup>	$65.3^* \pm 3.4$	57.9 ± 1.8	$59.2 \pm 5.2$	$63.3 \pm 5.8$
CD4 <sup>+</sup> CD25 <sup>+</sup> Foxp3 <sup>+</sup>	$9.4 \pm 2.0$	10.1 ± 1.7	10.4 ± 1.6	$10.3 \pm 1.5$
IELs				
CD4 <sup>+</sup>	$10.6 \pm 2.6$	$12.9 \pm 2.5$	$6.0^* \pm 3.3$	10.4 ± 1.8
CD8 <sup>+</sup>	87.6* ± 1.3	$78.8 \pm 6.8$	$85.6^* \pm 3.9$	$69.8 \pm 6.7$
CD4 <sup>+</sup> CD8 <sup>+</sup>	5.4 ± 1.5	$4.6 \pm 2.5$	$2.7 \pm 1.3$	$4.6 \pm 1.9$
CD19 <sup>+</sup>	5.1 ± 2.1	$6.4 \pm 3.2$	$3.0 \pm 2.9$	$5.6 \pm 2.4$
CD4 <sup>+</sup> CD25 <sup>+</sup> Foxp3 <sup>+</sup>	$3.3 \pm 0.9$	$3.6 \pm 1.1$	$3.6 \pm 1.6$	$2.7 \pm 0.8$
LPLs				
CD4 <sup>+</sup>	$66.4 \pm 4.1$	$64.4 \pm 6.4$	$45.3^{*}\pm 9.5$	$24.3^{\#} \pm 10.3$
CD8 <sup>+</sup>	25.5* ± 4.1	$17.9 \pm 4.8$	$42.9^{\#} \pm 5.2$	57.4 <sup>#</sup> ± 10.5
CD4 <sup>+</sup> CD8 <sup>+</sup>	$3.5 \pm 1.3$	$2.8 \pm 1.6$	$6.8 \pm 2.4$	$5.7 \pm 1.2$
CD19 <sup>+</sup>	$47.4^* \pm 4.8$	$36.8 \pm 1.9$	$24.5^{\#} \pm 8.1$	$24.1 \pm 8.8$
CD4 <sup>+</sup> CD25 <sup>+</sup> Foxp3 <sup>+</sup>	9.2 ± 1.3	$8.2 \pm 2.3$	$6.2 \pm 0.8$	$5.9 \pm 2.8$

Data represent means  $\pm$  SD of at least 8 mice per group. \*P < 0.05 organic (ORG) vs conventional (CV), same year; \*P < 0.05 2<sup>nd</sup> year vs 1<sup>st</sup> year, same treatment.

Second year: no differences were seen between lymphocyte subsets of org and CV fed mice <u>Blood</u>

First year: the CD19+ increased after the org consumption as compared to the CV consumption. Second year: no differences were seen between lymphocyte subsets of org and CV fed mice

In conclusion, concerning the Italian carrots, more differences between org and CV carrots were observed in the first year, as compared to the second year.

**Serum cytokines.** The analysis of pro- and anti-inflammatory cytokines did not reveal any significant differences among organic and conventional Danish and Italian carrots, indicating the absence of any inflammatory status.

**General conclusion**. Although a great variability was observed between the first and second year carrot harvests, no adverse effects were found after the organic carrots consumption, both from Danish and Italian trials.

## 3)

## **Methods**

All animals are kept in an air-conditioned animal husbandry (22-23°C) with a 12 hours light program at the Veterinary University in Vienna. Carrots used were from the Danish VegQure field trials (O1,O2 O3, C) and from the Italian farmer comparisons organic and conventional Maestro and Excelso cultivar.

**Table 3**. Lymphocyte populations of blood, spleen, IELs and LPLs of mice fed the Italian organic (Org) and conventional (CV) carrots, *Excelso* variety.

	EXCELSO				
	1 <sup>st</sup>	year	2 <sup>nd</sup> year		
	ORG	CV	ORG CV		
Blood					
CD4 <sup>+</sup>	$74.5 \pm 3.4$	76.2 ± 1.9	$74.0 \pm 2.6$ $77.4 \pm 3.2$		
CD8 <sup>+</sup>	21.1 ± 1.9	19.7 ± 1.2	$21.8 \pm 1.8$ $19.4 \pm 3.2$		
CD4 <sup>+</sup> CD8 <sup>+</sup>	$0.9 \pm 0.3$	$1.0 \pm 0.3$	$1.5 \pm 0.8$ $1.4 \pm 0.7$		
CD19 <sup>+</sup>	$22.4* \pm 7.8$	14.1 ± 6.2	$27.5 \pm 8.5$ $21.8 \pm 7.4$		
Spleen					
CD4 <sup>+</sup>	$65.7 \pm 2.3$	$64.4 \pm 2.5$	$57.9^{\#} \pm 5.7$ $60.5 \pm 4.3$		
CD8 <sup>+</sup>	23.1* ± 2.0	$26.7 \pm 3.3$	$31.9^{\#} \pm 2.8$ $33.5^{\#} \pm 3.0$		
CD4 <sup>+</sup> CD8 <sup>+</sup>	$0.8 \pm 0.3$	$0.9 \pm 0.4$	$3.1^{\#} \pm 1.3$ $2.6^{\#} \pm 1.1$		
CD19 <sup>+</sup>	$59.0^* \pm 4.7$	$63.5 \pm 2.0$	$57.6 \pm 6.9$ $54.1^{\#} \pm 5.5$		
CD4 <sup>+</sup> CD25 <sup>+</sup> Foxp3 <sup>+</sup>	13.0 ± 1.1	$12.3 \pm 1.0$	$12.6 \pm 0.5$ $10.9 \pm 1.8$		
IELs					
CD4 <sup>+</sup>	$4.2 \pm 0.9$	$3.9 \pm 1.0$	$5.5^* \pm 1.1$ $11.3^* \pm 4.4$		
CD8 <sup>+</sup>	$74.2* \pm 7.6$	$82.7 \pm 3.7$	$73.8 \pm 11.8$ $69.2^{\#} \pm 7.9$		
CD4 <sup>+</sup> CD8 <sup>+</sup>	$6.3 \pm 5.2$	$4.4 \pm 1.2$	$4.5 \pm 2.2$ $2.4 \pm 0.7$		
CD19 <sup>+</sup>	$4.7 \pm 2.4$	$5.2 \pm 1.6$	$6.9 \pm 2.6$ $10.8^{\#} \pm 6.8$		
CD4 <sup>+</sup> CD25 <sup>+</sup> Foxp3 <sup>+</sup>	$3.0 \pm 1.6$	$2.7 \pm 1.2$	$4.7 \pm 0.7$ $5.5^{\#} \pm 1.4$		
LPLs					
CD4 <sup>+</sup>	$30.5 \pm 4.4$	$35.9 \pm 6.6$	$25.8 \pm 5.0$ $22.6^{\#} \pm 5.3$		
CD8 <sup>+</sup>	$32.1* \pm 6.3$	$37.7 \pm 2.4$	$58.9^{\#} \pm 17.2$ $45.0 \pm 10.4$		
CD4 <sup>+</sup> CD8 <sup>+</sup>	$6.1 \pm 2.5$	4.1 ± 1.2	$4.9^* \pm 1.6$ $2.9 \pm 1.0$		
CD19 <sup>+</sup>	$31.7 \pm 10.2$	31.4 ± 13.2	$20.2^{\#} \pm 10.6$ $19.2 \pm 6.0$		
CD4 <sup>+</sup> CD25 <sup>+</sup> Foxp3 <sup>+</sup>	$6.8 \pm 2.0$	8.5 ± 4.1	$8.1 \pm 2.4$ $5.5 \pm 1.4$		

Data represent the means  $\pm$  SD of at least 8 mice per group. \*P < 0.05 organic (ORG) vs conventional (CV), same year;  $^{\#}P < 0.05 \ 2^{nd}$  year vs 1<sup>st</sup> year, same treatment.

**Food Preference Tests.** The food preference tests were conducted with 20 female laboratory mice (OF1) and 20 male laboratory rats (Strain Longevans) *kept single in Makrolon cages* for 5 days. A partition, containing the water bottle, divided the feeding rack into a right and left section, into which defined amounts of the two test products were apportioned simultaneously. The remainders of the feed were weighed 24 hours later and new feed was supplied. The sides were changed with every meal in order to prevent the effect of "position preference". The commercial rodent feed was also apportioned in the feeding rack to avoid any deficiency syndrome. The fresh carrots were washed and cut into cubes (about 1cm³). For processed carrots, the carrot purée was mixed with water and added to the commercial pellet feed (500g pellets + 250ml water).

For the preference tests the statistical method used was a nonparametric test, the Wilcoxon-test for pair differences (SPSSwin).

**Feeding Trials (mRACB).** Two mice groups were used: group A supplied with CV carrots and group B with organic ones. In the 1<sup>st</sup> year, organic and CV Maestro were used, while in the 2<sup>nd</sup> year the Danish CV and O3 were used. During the test period of 18 weeks three litters were produced. The Reproductive Assessment by Continuous Breeding (RACB) is a very sensitive model to

investigate feed influences and consists of 1 week exposure of the animals to test feed and then housing of the animals as breeding pairs for 14 weeks; during this time litters are produced and newborn pubs are removed and killed. For this project a modification was introduced concerning the prolongation of each reproduction cycle by including the rearing period of the offspring until weaning after 3 weeks. This modified RACB test allows for a number of parameters such as feed intake, mating success and delivery, litter size, litter weight and pub loss, growth development of pubs during the lactation period, rearing success, number of pubs weaned and intermediate period between litters.

## Results

### Food preference tests

Carrots from Denmark, harvest 2007. For the food preference tests, 3 replications of the variants C, O1, O2 and O3 were used as bulk samples. Preference tests of the 12 foods were conducted: 6 on 10 male rats and 6 on 10 female mice. The rat-preference tests showed no significant differences between the variants, the only trend occurred between the two organic variants O1 and O2, with a slight preference for O1. The mice preference indicated that CV was preferred to O1, but O3 was preferred to CV. In addition O2 was favoured to O3. The consumed amounts showed the following ranging: Rats: C>O1=O2>O3; mice: O3>C>O1=O2.

Carrots from Italy, harvest 2008. Four food preference tests were made with rats and mice.

In the test with Maestro only the rats distinguished org from CV carrots with a significant preference of the org variant. In the tests with Excelso both animal species preferred the org variant (Table 4).

Table 4: Overview food preference tests with the Italian carrot samples, var. Maestro and Excelso

	sample pair	s compared	% amounts		
test animals	1 <sup>st</sup> sample	2 <sup>nd</sup> sample	1 <sup>st</sup> sample	2 <sup>nd</sup> sample	p-value
rats	Org Maestro	Conv. Maestro	70	30	0,000
mice	Org Maestro	Conv. Maestro	50	50	0,742
rats	Org. Excelso	Con. Excelso	61	39	0,000
mice	Org. Excelso	Con. Excelso	63	37	0,000

*Processed Carrots.* The food preference tests with processed carrots were conducted with 20 laboratory rats. One carrot purée was made from fresh raw material and the comparative sample from frozen carrot cubes. The tests revealed no significant preference for one of the two production processes.

Carrots from Denmark, harvest 2008. The food preference tests were performed as in the 1<sup>st</sup> year. The mice did not distinguish between the Danish variants, but there was a trend of CV preference over O2 carrots (P = 0,082). The rats showed a significant preference of the CV as compared to O1 and O3 carrots. The consumed amounts showed the following ranging: rats: C>O1=O2>O3; mice: C>O2=O3>O1.

Carrots from Italy, harvest 2009. The tests were performed as in the 1<sup>st</sup> year In the test with Maestro the rats distinguished the org and CV carrots with a trend of preference versus the CV variant. In the tests with Excelso both animal species preferred the CV variant

To a certain degree food preferences can be attributed to taste properties and rodents have comparable inborn unconditioned stimuli for sweet and against bitter. "Sweet" is connected to nutritious, whereas "bitter" could indicate a toxic substance. Comparing the sugar and sesqi-and monoterpenes contents of the test carrots a good correlation between these components and food consumption could be found in most cases (Fig. x 1+2). The tests with the Italian Excelso showed contrary results in the 2<sup>nd</sup> year.

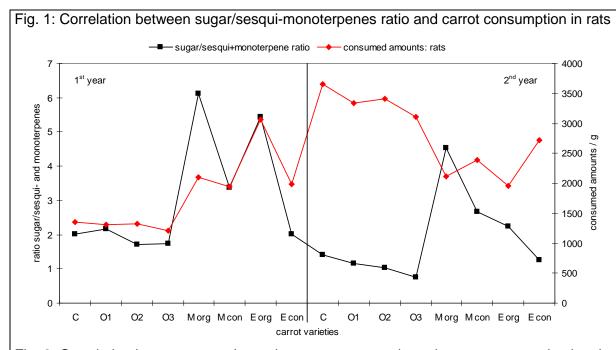
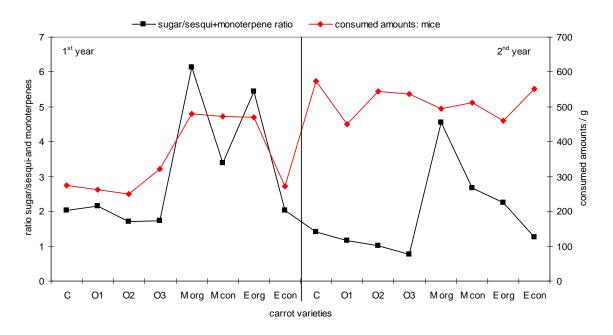


Fig. 2: Correlation between sugar/sesqui-monoterpenes ratio and carrot consumption in mice



Apart from genetic determination (unconditioned stimuli) postingestive consequences and subsequent learning play a major role in the food selection of animals (conditioned stimuli). But in this study all test animals were fed with a balanced commercial diet in addition to the test carrots. Thus taste influences could play a more important role than physiological needs.

The significant preference of the conventional variety Excelso by rats and mice in the 2<sup>nd</sup> year is difficult to understand in this context. Comparing the two Italian variants Maestro and Excelso from different cultivation systems the trends were comparable. The contents of titratable acids as well as the brix values were higher in the organic variants, whereas the contents of carotenoids were lower. The contents of sesqi- and monoterpenes were significantly higher in the conventional variant Excelso, which according to the other preference tests should have been a consumption reducing factor.

Summarizing the results, some influences of taste effects could be attributed to the food choices, but no clear differentiation according to growing methods could be achieved. The choice tests with the carrots originating from the VegQure trial did not show very decisive distinctions, but indicated a preference for the more conventional variants, whereas the tests with the carrots originating from

the Italian farm evidenced a tendency towards the conventional growing methods only in the 2<sup>nd</sup> vear.

## **Feeding experiments**

## **Reproductive Assessment by Continuous Breeding**

Results 1<sup>st</sup> year

The feeding test took place from April to August 2008. During this time 3 litters (F1) were produced. 22 breeding pairs of the inbred mouse strain Balb/c were used per feeding group. All animals were supplied with commercial rodent feed, and org or CV Maestro carrots were added daily *ad libitum* to the base feed. Group A obtained conventional carrots, group B organic ones.

Feed consumption. During the  $1^{st}$  litters, group B consumed significantly less commercial feed (P = 0,000) than group A. During the  $2^{nd}$  litters, group B still consumed less, but not on a significant level.

Reproductive performance. Only 20 of the 22 mice had deliveries in group B. Since this was the start of the experiment, this difference is due to coincidence and not a feed effect. 21 mice of group A and 19 of group B had 2<sup>nd</sup> litters. The time span between the 2<sup>nd</sup> and 3<sup>rd</sup> litters was generally longer and only 5 mice of group A and 7 of group B had pups.

The body weight increased with time in both groups and there was no significant difference between the groups.

No statistically significant differences concerning the number of deliveries, number of pups, litter size and weight were found. In the  $3^{rd}$  litters, group A had more pups per litter (p=0,090) and heavier litters (p=0,142). On the other hand there were 2 more females with pups in group B.

The 1<sup>st</sup> delivery occurred after 35 days in both groups, but the 2<sup>nd</sup> delivery of mice of group A took longer. The time between 2<sup>nd</sup> and 3<sup>rd</sup> deliveries was generally longer than the first one and after the average period of 40 days only 5 mice of group A had litters as compared to the 7 mice of group B. However, no significant differences were found.

## Results 2<sup>nd</sup> year

In this year the O3 and CV Dutch VegQure carrots were tested. Group A received the CV variant, and group B was fed with O3. The feeding test took place from the middle of November 2008 to the end of March 2009. During this time 3 litters (F1) were produced. 20 breeding pairs of the inbred mouse strain Balb/c were used per feeding group.

Feed consumption. There were no significant differences in feed consumption between the groups.

Reproductive performance. All the 20 female mice of each group had 1<sup>st</sup> litters. 19 mice of group A and 20 mice of group B had 2<sup>nd</sup> litters. The 1<sup>st</sup> and 2<sup>nd</sup> deliveries of group A and B lasted 32 and 35 days respectively. This period was comparable with the feeding test conducted in spring and summer 2008 with the Italian carrots (var. Maestro). But the time span between the 2<sup>nd</sup> and 3<sup>rd</sup> litters differed greatly in this RACB as compared to the last year test. To deliver the 3<sup>rd</sup> litters 28 and 31 days occurred for mice of group A and B respectively. Last year the difference was 40 days. Similarly more 3<sup>rd</sup> litters were delivered in 2009 than in 2008, although the test period was 18 weeks each year. In 2009 12 litters were delivered from mice of group A and 9 from those of group B, whereas only 5 litters were delivered in 2008. These differences could possibly be attributed to seasonal influences.

The weight of the parents did not show any statistically significant differences between the groups nor between the years.

The litter size and weight of 1<sup>st</sup> litters were comparable as well as the total litter weight gain during lactation. Only a very slight tendency to a more successful breeding performance of group B was observed. The  $2^{nd}$  litters of group B showed a statistically significantly higher litter weight at weaning (p = 0,035) and total weight gain during lactation (p=0,029) as well as a tendency of more

pups per litter (Table 5).

Table 5: Overview of reproductive parameters

parameters	deliveries %	n dead pups	n pups weaned	average litter size (weaning)	average litter weight (weaning)	avg litter weight gain	
	/0		4 81 114		(wearing)		
			1 <sup>st</sup> litter	S			
Group A	100	6	104	5,20	45,20	37,05	
Group B	100	1	111	5,55	47,95	38,80	
			2 <sup>nd</sup> litte	'S			
Group A	95	2	102	5,10	46,36	37,48	
Group B	100	3	131	6,55	59,82*	48,75*	
	3 <sup>rd</sup> litters						
Group A	60	10	63	6,30	54,93	43,75	
Group B	50	1 + 11 <sup>1</sup>	59	6,56	56,81	46,80	

<sup>\*</sup> P-value < 0.05

Only 12 mice of group A and 10 mice of group B had 3<sup>rd</sup> litters after the test period of 18 weeks. One mouse of B cannibalised all her pups. Neither weight gain nor litter size were significantly different in the last litters, only a slight tendency comparable to the 1<sup>st</sup> litters.

For the statistical evaluation one-way Anova was used.

Summarizing the results it could be shown that this test design is sensible enough to evidence even slight diet differences. The amount of carrots added to the basic diet was only about 20% of the total daily diet. This modified reproductive assessment of continuous breeding has never been used in comparative quality research focused on production methods. But it is a promising test design which could be useful in future feeding studies with differently produced whole diets.

## Growth studies with newly weaned pups

Three-weeks growth study with Danish carrots (Variants C and O3). The CV and O3 Danish carrots were used. The weight development of 23 (group C) and 21 (group O3) pups randomly chosen from 2<sup>nd</sup> litters of the same size was monitored during 3 weeks after weaning. The weight increase was not significantly different between the groups. When the pups were 5 weeks old their sex was determined and they were then weighed separately and revealed 13 males and 10 females in group C, whereas 10 males and 11 females were present in O3 group. At the end of the experimental period, the males were slightly heavier in group O3, whereas the females were slightly heavier in group C.

One- week growth study with differently processed carrots. This test was conducted with 29 (group 1) and 27 (group 2) pups randomly chosen from litters of the same size. Feed 1 was from fresh carrots (codes 294 and 847); feed 2 from frozen carrot cubes (codes 173 and 601). The feed was offered in open feeding troughs, since there was not enough feed to fill and close them. This fact caused the pups to spoil their feed and spill a great amount into the cage. Therefore only 1 test week was possible. During this week the pups of group 1 (fresh carrots) had a slightly higher but not significant weight increase as compared to the pups from group 2 (frozen carrots).

## B- comments on deviations from the original plan

There were no deviations from the original plan.

<sup>&</sup>lt;sup>1</sup> Female 13 B cannibalised all her 11 pups

## WP 8 Quality definition

## Responsible partner: P8, Bioforsk, Randi Seljåsen

## Description of work:

The work of WP 8 concentrated on quality definition by investigations on quality dimensions, their criteria and measurement methods. Literature studies and treatment of results from WP 5, 6 and 7 were used to find the most important variables that could be used to define and control the requested quality.

## Task 8.1 Quality definition - selection of variables to define quality

A literature study on the quality concept of fresh and processed carrots was performed to investigate appropriate quality dimensions, criteria and measurement methods for the project. This was presented and discussed by all participants by arrangement of a two day meeting in the beginning of the project period.

## Task 8.2 Evaluation of results

Set up data format and organisation procedure of data evaluation.

## Task 8.3 Procedure of data organisation and evaluation

Meetings to define procedure of data organisation (P1, P6, P10). Discussions on how to measure and fit the dimensions. Discussion and decisions which evaluations that should be performed to meet our quality dimensions. Selection of data evaluation methods (P6, P10).

## Task 8.4 Analysis of results for implementation in quality management system (WP 9).

Discussion meeting (WP 8 and 9) on what WP 9 was expecting from WP8: selection of the presentation form that were used for all results. Meetings were arranged with WP 3, 4, 5, 6, 7, 8 to discuss results during the result evaluation period. Presentation of a short result overview and discuss results from the Danish trial. The relationship (correlation) between the different methods used wwas analysed (P1) and presented on internet and discusses on the meetings.

## Task 8.5 Analysis of results from WP 5-7 and study correlation between different quality parameters.

By the end of project period (2009) the results were structured and delivered to WP9. Principal component analysis (PCA) was performed to map out factors (farming practice or processing factors) that are most correlated to high or low product quality. A two days meeting was performed with all participants to discuss the results. The results were presented in a scientific publication and to the project web page.

# Final report on work carried out, and progress of the work compared to the original plan: A- work carried out and results obtained: Task 8.1.

Literature study is performed and a review paper was written. Title: Fresh and pureed organic carrots (*Daucus carota L.*) – quality aspects and their influencing factors from an European perspective . Journal: Food Reviews International.

## Tasks 8.2, 8.3. and 8.4

Meetings has been conducted in connection with the project meetings and in two separate WP8 meetings.

### WP 8.2.

## Task 2.1 Contribution to the summary of the main results.

The organizing of the measurement data and adaptation of the existing software and the gathering process needs was performed. Based on the first draft of data format and the software specifications, that was worked out in 2007, a second draft and first coding was done between March and May 2008. The software was tested with the 2006 data from the german BÖL 020E170F project. During the

meeting in Rome the participants got a second information round on the data format and the handling. In July 2008 the first data were sent and received and checked and in response the needed changes were made. The data were re-sent until there were no detectable errors. After the decoding the data were merged and the first correlations were made visible through the intranet. As a result of this first data sending round the data checking process was adapted to the response of the data-sender The errors with the highest frequencies were reduced. In special we decided that for the future the so called "data headers" will be generated and send by the checking system (P1). When the coded samples will be send to the Laboratories the codes (not the decoding) should be send in parallel to the University of Kassel as the data receiver to prepare the checking. In 2009 the adapted procedure was repeated for the results from the second year. In parallel the merge procedure was developed further.

So far the data from samples measured as field repetitions (e.g. VegQure 4 treatments, 3 field replicats= 12 samples) or as bulk samples (VegQure: the 3 field replicats were bulked which results in 4 samples [only the treatments]), were merged in two files each. Some of the health study measurements were due to cost reasons done only on bulk samples. To be able to compare with measurements done on field replicats the data were merged into one file. In the case of missing data, e. g. the sample was not delivered or the measurement failed, usually the method was excluded from the merge. The merge function was extended to a reduced sample set, which allows to merge for a reduced sample set with all methods. Another problem was to merge different types of methods. In case of the panelist type (e. g. sensory analysis, which have data from panelists, which are not real repetitions) the mean and the sd value was build, to merge the data. In case of the control group type (e. g. Lymphocyte subpopulations in the blood) the control group was excluded for the merge. In the case of pairwise type (e. g. Mouse food preference test) no simple merge with the "normal" methods was possible. This has to be reserved for further development. The multivariate PCA statistical analysis needed the data as mean values over the laboratory repetitions. The file was added to the zip file including the data file with the data description files and the data format documentation. As the last the merge functionality was expanded to be able merge different trials (e. g. data from VegQure and data from the AIABTrial) and different years, in our case 2007 and 2009 data. In total 12 different merge files were used for the multivariate PCA evaluation.

## 8.2.2. Contribution to the WP description and results of the work

## A) Adaptation of the existing software

The adaptation of the <u>Quality Data Integration Facility</u> (QDIF) to the needs of the core-oganic 1885 project was accomplished. The handling for the users who send the data was improved, according to the most repeating errors in the handling. The handling for the user who receives and merge the data was improved from a development level to a administrator level, still not reaching a user interface level. Especially the merge facilities were enhanced to deal with the daily requirements.

## B) Receiving and merging the project data

The data from the project partners from WP 5, WP 6 WP 7 were received, checked and improved . After decoding the data were merged for both years. The basic anova statistics for a method variable and the correlations between the method variables were calculated. The anova and the correlation results, as well as the merged data were accessible through web pages for all project partners.

## C) List of merged data files

In the following table the merged data sets for the PCA evaluation are listed.

Table Data.1) List of merged data sets used in the PCA analysis. Column Year is counting the saw year, not harvest year. Column Source is the source of the samples e.g. a field trial. Subset is describing the way the samples are handled (e. g. FieldReps or Bulk). When different ways of handling are combined the names are connected with the text AND. Mapping is describing the set of the samples from a trial or from a subset of a trial. Column Date shows the date, when the data set is generated.

Year	Source	Subset	Mapping	Date
2007	VegQure	FieldReps	all	2010.03.11
2007	AIABtrial	FieldReps	all	2010.03.11

2007	AIABTrial	PilotPlantBasicANDFieldReps	AutoclavF	2010.03.11
2007	AIABTrial	PilotPlantBasicANDFieldRepsANDBulk	AutoclavF	2010.03.11
2008	VegQure	FieldReps	all	2010.03.11
2008	AIABTrial	FieldReps	all	2010.03.11
2008	HochDorf	Processed	all	2010.03.11
2008	HochDorf	ProcessedANDFresh	all,onlyFresh	2010.03.29
2007	VegQure+AIABTrial	FieldReps	all	2010.03.11
2008	VegQure +AIABTrial	FieldReps	all	2010.03.11
2007+2008	VegQure+AIABTrial	FieldReps	all	2010.03.11
2007+2008	VegQure	FieldReps	all	2010.04.21
2007+2008	AIABTrial	FieldReps	all	2010.04.21
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## Task 8.4 Analysis of results

Data were organized to get them into a form that is possible to use in the multivariate statistical analyses. The datasets were used to study mainly the hypothesis number 1: "Carrots from organic and conventional farming systems can be differentiated in a field trial or by comparing carrots from neighboring organic and conventional farms". The statistical method we use is a principal component analysis. This work was done separately for the available AIAB-data and VegQure-data from both 2007 and 2008. Results were presented and discussed by all project participants on the project meeting 26-27 Nov. this year. The results indicate that it may be possible to differentiate carrots from organic and conventional farms by using combinations (that is principal components) of the measured variables. Besides it seems possible to say something about which of the measured variables that contribute most to this separation. When all data will be available next year we plan to do some more detailed multivariate analyses based on the same methods, but perhaps on some sort of a reduced set of variables.

## Tasks 8.5 and 8.6. Analysis of data by multivariate statistics (principal component analyses).

First we have organized the available data to get them into a form that is possible to use in ordinary multivariate statistical analyses. That means to have the datasets organized with observations as rows (one such observation will typically be a group, and typically one such group is a cultivation method / some sort of treatment combined with a variety and/or a field/processing replicate, in some situations the groupings also include the year). In some other situations we have different raw materials as a grouping variable. The means of all the measured/observed values of the variables from the actual group (row) are given in separate columns in that row. The datasets on this form were used to study mainly the hypothesis number 1: "Carrots from organic and conventional farming systems can be differentiated in a field trial or by comparing carrots from neighbouring organic and conventional farms". In all situations we want to investigate if it may be possible to separate, distinguish, recognize or identify interesting groups by using simultaneously values observed for a lot of different variables.

We have used principal component analysis, which is a multivariate statistical method. These analyses are based on the ordinary Pearson correlations between all pairs of the actual variables. The use of correlations, instead of covariances, is equivalent to use variables standardized to have mean equal to zero and standard deviation equal to one. This standardization is done because we have variables with very different locations, scales, variations etc.

The results from the principal component analyses are presented by means of the three ordinary plots: the scree plot, the score plot (which is the main plot for our main objective looking for groups of observations), and the loading plot. All score plots and the loading plots are solely based on the first and second principal component.

In all analyses we have decided to leave out the variables with coefficient of variation less than 1 % (in absolute value), mainly because of computational difficulties. In addition, variables with small variation

will probably nor contribute significant to separate or discriminate the observations using any statistical method.

The analyses described above were done separately for several different datasets. For some of these datasets there seems to be possible to separate, distinguish, recognize or identify interesting groups based on the actual variables used in the principal component analyses. For other datasets this is difficult or even impossible. By using both the score plot and the loading plot in combination we are for some of the datasets able to say something interesting about which variables that contribute to the positioning of the observations (groups) in the score plot.

For those datasets where the principal component analysis based on all actual variables seems to indicate some interesting groups of the observations we have in addition done some corresponding principal component analyses based on some particular groups of the variables. The results from these analyses indicate, among other things, that we may manage with only a relatively small number of variables to separate, distinguish, recognize or identify groups of observations.

#### Results

According to the performed Principal Component Analysis (PCA) of samples from the Danish organic field trial (VegQure) conventional samples was separated from all organic fertilization regimes in 2007, and in 2008 the highest intensity of organic was separated from conventional carrots. The positioning of the conventional samples in the PCA score plot seems to be mostly determined by variablescontent of nitrate, carotene, fumaric acid, malic acid, pungent aroma, aftertaste, green flavor, soapy flavor, root diameter and weight as well as damages due to cracks and forks.

For carrots from the Italian farm trial it was not possible to group conventional from organic carrots by PCA. For those samples variety had higher impact than cultivation system. When comparing results from Italian trial with Danish trial, location and variety was possible to group, but not cultivation method.

PCA of samples from different treatments of the raw material for processing (fresh, refrigerated stored and frozen) indicate a clear grouping of the samples based on the measured end product quality variables. The groups correspond to the treatments of the raw material for processing, which is a clear indication that the treatments of the raw material for processing have different influence on the end product quality. The positioning in the PCA score plot of the puree samples from fresh raw material seems to be mostly determined by the quality variables specific aroma components, b-carotene, and colors (a, b, and c), while the positioning of the puree samples from refrigerated stored carrots seems to be mostly determined by the quality variables selected terpenes, orange brown color as well as sour and bitter tast. The positioning of the product made from frozen raw material seems to be mostly determined by the quality variables boiled carrot flavor and odor, color hue and watery texture.

### B- comments on deviations from the original plan

There were deviations from the original plan, because partners from WP 6 could not deliver data in time. Therefore part of the work was done during prolongation of the project in 2010.

## WP9

## Responsible partner: P2, FiBL, Ursula Kretzschmar

## **Description of work:**

The work of the WP concentrated on the external relations of the project and the implementation of results in relevant industrial situations. This concerned conveyance of information to key stakeholders being the participating SME's, the vegetable industry at large, public institutions (in the Core Organic member countries) as well as the research community in general. A special target group was the project Board Committee which comprised representatives from the stakeholder. The results were set to work in practice through continuous web-based information, a concluding conference, and presentations at events of the label organisations as well the organic food industry.

## Task 9.1 External communication of project results

P2 assisted from P1 was responsible for the development of a webpage for external communication. Results from the project were published on the web page as regulated by the consortium agreement. All relevant project results were published in scientific journals and food industry press when appropriate.

## Task 9.2 Consultation with the Advisory Board

P1 assured a periodical electronic consultation of the board committee in order to validate intermediate results and obtain recommendations from the committee to the relevant project partners.

## Task 9.3 Organising a final European seminar with recommendations.

In order to finalize the proposed strategies and recommendations from the different WP's of the project a European conference with all partners, the scientific advisory board, invited experts and interested vegetable processors will be organised within Wissenschaftstagung 2011.

## Task 9.4Presentation of the results at events of the label organisations and organic food industry

P1 and P2 presented the results on events of the label organisations (e. g. Day of organic processors organized from BIO SUISSE) and events of the organic food industry (e.g. BIOFACH). The presentation contained the specific needs of SME.

## Final report on work carried out, and progress of the work compared to the original plan:

## A- work carried out and results obtained:

The following actions have been made according this task during the reporting period (06/07-05/10).

A general description of the project as well the main objectives of the work packages (WP) are uploaded on the project web site http://www.qaccp.organic-research.org/qaccp0.html.

During the project the following dissemination activities were done:

- 1. A first work shop took place at Biofach 2008 in Nürnberg: "Organic baby food: demands for quality and product range"
- 2. Participation at the Wissenschaftstagung ökologischer Landbau 11-13. February 09 at the ETH in Switzerland with the following workshop: Qualitätsbeeinflussende Prozessschritte in der Verarbeitung
- 3. Review paper Organic quality of carrots from field to fork is finalized.
- 4. Workshop at Biofach February 18<sup>th</sup> 2010: Quality of organic baby food; Consumer demand and quality optimisation based on the principle of QACCP
- 5. A detailed paper outline connected to the hypothesis is worked out and approved by all partners. The papers should be finalized until end of June 2010. Paper outline:

## Hypothesis 1

- 1. Sensory results on fresh carrots
- 2. VegQure (single plus multivariant) Journal: JAFC
- 3. AIAB (single plus multivariant) Journal: IB JAFC

## Hypothesis 2

- 1. VegQure and AIAB
- 2. VegQure
- 3. Preferences

## Hypothesis 3

- 1. Hochdorf Journal: EFRT
- 2. Pilot Plant Journal: RAFS

## Hypothesis 4

- 1. QACCP (model)
- 2. QACCP (applied)

## Hypothesis 5

Focus group and processing survey Journal:FQP

Consumer study Journal: FQP or RAFS

Measurement results

- 6. FiBL presented the QACCP concept on the processors day of SQS in Morschach Switzerland in April 27<sup>th</sup> . 150 Processors joined the meeting.
- 7. Every partner has worked out a detailed publication plan to publish the final results
- 8. FiBL will present the QACCP concept on the organic processors day September 21th in Basel.
- 9. At the final project meeting it was decided to join the following conferences:

**Wissenschaftstagung Ökologischer Landbau** 2011 in Gießen Germany 15. bis 18. März 2011 call of paper 1. September 2010

First International Conference on Organic Food Quality and Health Research 18 - 20 May, 2011, in Prague Oral presentation deadline abstract submission March 3, 2011 Poster: deadline poster submission March 30, 2011

After project life time of 30. June 2010 the following activities will still be done:

- 1. Actualisation of the Core Organic homepage continuous
- 2. Publish the papers until End of 2010
- 3. Preparing a PWT presentation of the most interesting results of the project, which could be presented by each partner in his own country until end of August 2010?
- 4. Preparation of the papers for the calls of the **Wissenschaftstagung Ökologischer** Landbau 2011 in Gießen as well the First International Conference on Organic Food Quality and Health Research, Prague 2011

## B- comments on deviations from the original plan Task 9.2 Consultation with the Advisory Board

Instead of the previous working plan the members of advisory board were invited to join the project meetings in Kassel, Rom, Arhus and Mikkeli. There were no separate workshops, because the group of partner was quite small.

## Task 9.3 Organising a final European seminar with recommendations.

Instead of the organisation of a final European seminar it is decided to join **Wissenschaftstagung** Ökologischer Landbau 2011 in Gießen as well the **First International Conference on Organic Food Quality and Health Research** and to have there a workshop as well oral presentations and poster of the different evaluated results. The interdisciplinary workshop with processors, retailers and researchers is already declared at the Wissenschaftstagung.

## 4. Publications and dissemination activities

## Project website(s)

Address		Authors:	When was it last updated	Language	Comments
		(name + institution acronym)			
http://www.qaccp.organic-research.org/qaccp0.html		Helga Willer, FiBL	22/09/2010	English	

## Reviewed papers

Planned / actual date	Title:	Authors:	Name of Magazine	Comments
June/July 2010	Health biomarkers in a rat model after intake of carrots grown under different cultivation systems.	Maja Jacobsen		Draft
	Running title			
	Quality of organic food- health and safety.			
July 2010	Fresh and pureed organic carrots (Daucus carota L.) – quality aspects and their influencing factors from an Europan perspective	Randi Seljasen	Food Reviews International	Under review
	Quality data integration and evaluation	Nicolaas Busscher	Computers and Electronics in Agriculture	Draft
July 2010	Multi-method comparison of carrot quality from conventional and three organic cropping systems with increasing levels of nutrient recycling and biodiversity	Flavio Paoletti	Journal of Food Science and Agriculture.	Under review
	Preference of organic grown carrots in 1 a rat/mice model	Henry Jorgensen	Journal of the Science of Food and Agriculture	Submitted
	Evaluation of the impact of organic and conventional carrots consumption on intestinal and peripheral immunity.	Marianna Roselli	Journal of the Science of Food and Agriculture	Submitted
	Comprehensive approach of influencing factors on phenol distribution in carrots: interest of organic farming	Stephane George	JAFC or Biochim Biophys Acta.	
	Quality aspects of processed organic baby food - Industrial test with fresh, frozen and stored raw material	Kathrin Seidel	European Food Research and Technology	Draft

Planned / actual date	Title:	Authors:	Name of Magazine	Comments
	QACCP: tool for a systematic quality optimization in the processing line	Ursula Kretzschmar	European Food Research and Technology	Planed until the end of 2011
	Consumer and producer perception of organic baby- food quality: A qualitative study",	Daniela Vairo	Organic Agriculture.	submitted , under review
	Sensory assessment of conventional and organic carrots: consistent or confounding results?	Fiorella Sinesio	Food Quality and Preference	
	Impact of processing on carrot baby food nutritional quality and safety Potential of fluorescence as in real time monitoring tool	Ines Birlouez	Biochim Biophys Acta	Submitted, under review
	Study of the quality of carrots grown on-farm: comparison of organic and conventional management systems".	Flavio Paoletti	Organic agriculture	In preparation

## Conference papers and book chapters

Planned / actual date	Type and Title of contribution:	Book title / Conference:	Partners involved:	Type of audience	Size of audience	Countries addressed
	Conference Paper: Futtwerwahlversuche mit Nagern zur Überprüfung der Qualität von Produkten aus biologischem und konventionellem Anbau <a href="http://orgprints.org/14437/">http://orgprints.org/14437/</a>	Conference: 10. Wissenschaftstagung Ökologischer Landbau, Conference in Zurich	P8	Researchers		German speaking countries
01.2010		Conference in Rapid Methods, Netherlands	P6			
11 -13 February 2009	Conference Paper: "QACCP Analyse in der Verarbeitung:Qualitätsbezogene Beurteilung der Verarbeitungskette " <a href="http://orgprints.org/14586/">http://orgprints.org/14586/</a>	Conference: 10. Wissenschaftstagung Ökologischer Landbau, Conference in Zurich	P2 and P4	Researchers, advisors	600	European countries
11-13 February 2009	Conference Paper: "Qualität von verarbeiteter biologischer Babynahrung aus Konsumenten- und Herstellersicht" <a href="http://orgprints.org/19409/">http://orgprints.org/19409/</a>	Conference: 10. Wissenschaftstagung Ökologischer Landbau Conference	P2 and P3	Farm sector		Switzerland, German- speaking countries

Planned / actual date	Type and Title of contribution:	Book title / Conference:	Partners involved:	Type of audience	Size of audience	Countries addressed
		in Zurich				
11-13 February 2009	Conference Paper:"Authentizität und Sicherheit von Möhrenproben im Rahmen des CORE-Organic QACCP-Projektes" <a href="http://orgprints.org/14187/">http://orgprints.org/14187/</a>	Conference: 10. Wissenschaftstagung Ökologischer Landbau Conference in Zurich	P1 and P6	Farm sector		Switzerland, German- speaking countries
11-13 February 2009	Conference Paper:" "Organisation und Zusammenfassung quantitativer und qualitativer Messdaten im Rahmen des CORE-Organic QACCP Projektes".	Conference: 10. Wissenschaftstagung Ökologischer Landbau Conference in Zurich	P1	Researchers		Switzerland, German- speaking countries
06.2008		F&V International Summit Paris	P6			

## Deliverable reports, proceedings, internal reports, newsletters, web communication etc.

Planned / actual date	(No.) and title	Туре:	Partners involved:	Type of users addressed	Countries addressed
December 2007	Consumer and Processor Research on the quality of processed vegetable, in special baby food WP2 – Reports on Focus Group – Italy	Internal report	P3 (Daniela Vairo and Raffaele Zanoli)	Researchers	Not applicable
January 2008	CORE Organic Project (QACCP) Consumer and Processor Research on the Quality of Processed Vegetables, in Special Baby Food WP2 - Report on Focus Group Discussions – Germany	Internal report	P1 (Angelika Riefer and Ulrich Hamm)	Researchers	Not applicable
November 2009	Quality aspects of processed organic baby food - Report of Industrial test with fresh, frozen and stored raw material	Internal report	P 2 (Kathrin Seidel Dipl. oec. troph with contributions from Ursula Kretzschmar, Marjo Särkkä- Tirkkonen,	Researcher	This is an internal report which includes confidential data and information of our SME; therefore the report is not public on

Planned / actual date	(No.) and title	Type:	Partners involved:	Type of users addressed	Countries addressed
			Hanna-Maija Väisänen and Alex Beck)		organic Eprints
Mai 2010	Overview on different sterilization techniques for baby food <a href="http://orgprints.org/17236/">http://orgprints.org/17236/</a>	Public report	P4 (Marjo Särkkä- Tirkkonen and Hanna-Maija Väisänen) with contributions from Ursula Kretzschmar and Kathrin Seidel and Alex Beck	Researchers and SME's	

Popular articles and other dissemination activities (presentations at workshops or meetings, leaflets, posters, press releases, interviews etc.)

Planned / actual date	Title of contribution:  Type of contribution		Partners involved:	Type of audience	Language	Countries addressed
9. Aug. 2007	Ny forskning skal give bedre øko-mad. (New research will give improved organic food) <a href="http://orgprints.org/15783/">http://orgprints.org/15783/</a>	Dagbladet Børsen (news paper article based on interview)	AU (Hanne L. Kristensen)	General public	Danish	Denmark
9. Aug. 2007	Er øko-gulerødderne sundere? (Are organic carrots healthier?) http://orgprints.org/15784/	Dagbladet Børsen (news paper article based on interview)	AU (Hanne L. Kristensen)	General public	Danish	Denmark
21-24 February 2008	BIOFACH presentation on baby food consumer & processor research <a href="http://orgprints.org/19411/">http://orgprints.org/19411/</a>	Presentation	P1,P2, P3	General public	English	
Planned for spring 2009	Le caratteristiche qualitative negli alimenti dei bambini: un'indagine esplorativa sui piccoli consumatori Not in Organic Eprints, please upload if finalised	Daniela Vairo and Raffaele Zanoli UNIVPM	AzBio	In process	Italian	
June 2008	Frisch ins Glas – Verbraucheranforderungen an die	Angelika Riefer	Biowelt, no. 6	Results of the German Focus Group	German	

Planned / actual date	Title of contribution:  Type of contribution  Partners involved:		Partners involved:	Type of audience	Language	Countries addressed
	Verarbeitung von Babykost aus Öko- Gemüse. In: Biowelt, Jg. 2008, Nr. 6, S. 14.			published in a German non- scientific journal for the organic market		
30. October 2009	Er økologiske gulerødder sundere end konventionelt dyrkede?.	Charlotte Lauridsen	Økologisk Jordbrug, nr. 442, page 20	Type of audience: general public. Language: Danish.		
July 2010	Das Ausgangsmaterial entscheidet: Studie zur Qualität ökologischer Lebensmittel	Johannes Kahl	Several Newspapers	General public	German	Germany
July 2010	Økologisk kvalitet i hele produktionskæden (Organic quality in the whole food chain) http://orgprints.org/19556/	Økologi og Erhverv (magazine article)	AU (Hanne L. Kristensen)	Farm sector	Danish	Denmark
Aug. 2010	Økologisk kvalitet - fra jord til bord (Organic quality - from farm to fork) http://orgprints.org/19559/	Landbrugsavisen (news paper article)	AU (Hanne L. Kristensen)	Farm sector	Danish	Denmark

## 4.2 Further possible actions for dissemination

- List publications/deliverables arising from your project that Funding Bodies should consider disseminating (e.g. to reach a broader audience)

Fresh and pureed organic carrots (Daucus carota L.) – quality aspects and their influencing factors from an Europan perspective. This publication gives a very good overview of all different kind of quality aspects for the production of organic food from field to fork; Randi Seljasen et al

QACCP: tool for a systematic quality optimization in the processing line, paper available Spring 2011

This tool is a systematic approach to optimize organic food quality Ursula Kretzschmar et al.

- Indicate publications/deliverables that could usefully be translated (if this has not been done, and indicate target language)

The concept of QACCP for the application in the industries.

## 4.3 Specific questions regarding dissemination and publications

- Is the project website up-to-date?

The home page was hacked in Augusts 2010 and all data's were deleted. We reactivate the extranet http://www.qaccp.organic-research.org/ but not the intranet. All documents of the intranet are stored by University of Kassel and are available at University of Kassel PD Dr. Johannes Kahl.

- List the categories of end-users/main users of the research results and how they have been addressed/will be addressed by dissemination activities
- A) Project reports for funders
- B) Articles in professional magazines for farmers and food processors
- C) Articles in peer-reviewed journals for researchers in the agricultural as well food processing sector
- D) Articles in professional journals for researchers in the agricultural as well food processing sector
- E) Proceedings in connection to international conferences like Wissenschaftstagung Zürich 2009, Göttingen 2010, First International Conference on Organic Food Quality and Health Research May 2011 in Prag for researcher
- F) Proceedings in connection to national conferences for researcher
- G) Project leaflets yes on work QACCP for the processing industry
- H) Workshops with stakeholders and end-users of results: Biofach 2008, 2009, 2010, Processing day SQS April 2010, Organic processing day BIO SUISSE, FiBL, Demeter, bio.inspecta September 2010
- I) Internet pages for all target groups like students, researcher, processors, farmers and founders
- J) Power Point presentations on Internet: final power point is planed for all project members for dissemination for the use of all target groups
- K) Posters (at congresses, fairs,) mostly for researchers
- Impact of the project in relation to main beneficiaries of the project results

Note: for the different categories of end-users/main users of the research results, explain how well the project has been able to reach these target groups, and any known impact

This project had the following different research approaches which have a different importance for the different target groups:

- Identify and define critical and essential product quality parameters useful to optimise organic food quality
- Compare products from different farming practices (conventional and within organic)
- Performance of QACCP (Quality Analysis Critical Control Point, similar to HACCP methodology)
- Test the impact of the food chain (focusing on processing techniques) on the product quality and safety
- Test the impact of organic food on health

## The overall results of the project are:

- A Quality Analysis Critical Control Points (QACCP) could be developed and applied in industry. With the QACCP we meat the needs of the industry as well the consumer, to optimize final product quality systematically on existing processing lines.
- B. Along the organic production chain of carrot baby food critical steps according to quality and safety could be identified. Those results are important for the farmers, food processors as well consultants.
- C. Although sterilisation of the product at the final production step, the selection of the raw material has a significant influence on the quality and safety of the baby food. This information is important for the farmers as well for the industry. Because with that the selection for the raw material got a new priority.
- D. Furthermore choice of raw material plays an important role for the willingness to pay of the consumer for the final product. The consumer research supports the market decisions of organic trade companies and with that the consumer.
- E. The differentiation of carrots from organic and conventional farming systems is influenced by additional factors like year and variety. This was the same for the selected health markers. The measured single compounds seem not be sufficient for the differentiation but multivariate statistical analysis of the data increased the discrimination ability. New input for the farmers is given how the different cultivation systems influence the product quality and what kinds of limitations are given.

## ANNEX 1: CHANGES IN WORKPLAN AND PROBLEMS ENCOUNTERED

## Changes in the work plan or consortium

On the kick-off meeting we decided not to measure baby food products from the market (benchmarking) but to focus on defined samples from field-trials, pilot plant studies and from industrial partners of the project. Furthermore we concentrated to work with 2 instead of 4 SMEs for the QACCP because of the already working partnership between research partners (P2) and SMEs (P12).

Because of the amounts needed and the technical possibilities at P4 for the pilot plant studies, we select one organic variety from the carrots produced in Italy instead of samples from the field-trial in Denmark.

Because of problems at P4, we could not repeat the pilot plant study there. Partner P2 took this task over and will do a pilot plant study or experiments directly at industry level at P12. This was in line with the project goals, because industrial partners were directly involved in the QACCP application, including sampling and measurements.

## Problems encountered, delays and corrective actions planned or taken, if any:

Because of the different administrative expectations from the different partners, the sign of the consortium agreement had a delay. The problem was solved.

The revision of the homepage took much more time than expected. Therefore the revised version was uploaded before the end of the first period.

Because of technical and financial problems at P4, the pilot plant study could not be performed as planned. For the repetition P2 took over this task with an industrial test.

Because of technical problems, P7 could not deliver the evaluated results of the pilot plant study in time to WP3. Therefore decisions about CCPs were taken based on results from P6 and measurements taken at P4 which was no problem as the measured results indicated later.

WP2 performed the last task after the results of WP3 had been available in 2009.

WP8 needed additional meetings in order to structure the work within the different tasks. The first publication planned as a review on carrot quality was sent to the journal before the end of the project.

For the final report the WP7 management went from P5 to P7.

One of the most important problems is, in comparison to EU-funded projects, that the budget is discussed only with the national funding body. The coordinator of the project has only little or no possibility to check money transfer with the finished work tasks. This has to be optimized in future projects.

### ANNEX 2: COST OVERVIEW AND DEVIATIONS FROM BUDGET

## Project Budget and Costs in €

Partner no.	1	2	3	4	5	6	7	8	10
TOTAL						101'			
BUDGET	422'006.51	152'552.00	177'216.00	42'000	84'372	970	234'300	138'974	131'479
	452'624.8					64'			137'030
Spent		131'507.80	176'207.00	42'000	85'012	666	234'300	142'879.16	
						+37'			
<b>DEVIATION:</b>	-30'618,29	+21'044.20	+1009.00	0	-640	304	0	- 3'905.16	-5'551

Person months (PM) spent on the project:

Partner no.	1	2	3	4	5	6	7	8	10
TOTAL PM									
budgeted	50	14.4	36	5	10.8	9.5	78	22.9	12
Spent	50	13.25	24.68	5	10.8	16	78	23	12,2
<b>DEVIATION:</b>	0	+1.25	+11.32	0	0	-6.5	0	-0.1	-0.2

## Reasons for major deviations in spending compared to original budget:

#### Р1

Prolongation of the project duration from 31.12.2009 to 30.06.2010 including project and data management. The difference between what was applied from a theoretical table and the praxis within the personal costs.

## P 2

The final research results were available at the end of April 2010. So some of the the dissemination activities have to be done after the project lifetime. For that the work of the work package 9 dissemination is prolonged until 30 of March 2010 (contract with swiss government of agriculture) that includes the following work: paper QACCP, Hochdorf, leaflets, dissemination of the results by the participations at the conferences like Wissenschaftstagung Ökologischer Landbau 2011 in Gießen Germany 15. bis 18. März 2011 call of paper 1. September 2010

First International Conference on Organic Food Quality and Health Research 18 - 20 May, 2011, in Prague Oral presentation deadline abstract submission March 3, 2011 Poster: deadline poster submission March 30, 2011 and other dissemination (see WP 9 description)

## P 3 Deviations depend from the delaying and re-planning of the consumer choice-test.

Savings were obtained in subcontracting for survey services. At the same time, higher costs were incurred in consumable since new software was acquired for data analysis.

Less PM were spent due to hiring of more qualified staff for part of econometric analysis of data.

### P 4

Pilot scale trials were supposed to simulate the industrial scale trials as well as possible and to be sure to achieve this goal more resources had to be transferred to the first period than was originally planned.

## P 5

With regard to the contributing partners with main responsibility in WP7: it should be mentioned that the original budget included one-third of the salary for a PhD-student, however, instead the

scientific work has been undertaken by senior scientists. Furthermore, 2 technician PM's were transferred to operational expenses due to the change of parameters to be tested in the animal experiment. Overall, this explains the deviation in total PM spent in comparison to the total PM budgeted.

## P 6

Note on the lower MM used as previously planed: Due to the moving of the research team of Inès Birlouez-Aragon in the new SME Spectralys on October 2008, the work was carried out for AgroParisTech in Spectralys Innovation.

Firstly researchers were engaged by AgroParisTech. But later, those persons became personal of Spectralys Innovation. Consequently, the 6,5 MM expended for the project were financiated by Spectralys. To compensate these expenses, it was agreed that expenses in chemicals and environment would be charged to AgroParisTech.

Today AgroParisTech is engaged in refunding the corresponding costs up to 37 300 euros. This refund was not given at the moment because ACTA and INRA did not pay the remaining due amount of 37 300 euros.

## FINAL REPORT Addendum:

## A. Added value of the transnational cooperation in relation to the subject

The project contributes to the improvement of the quality and safety of organic vegetable products of the European market. The project contributes also to improvements of the food chain, focused on processing of organic vegetables, which will profit the SMEs and retailers in the global organic market. Within the European project team an exchange of national expectations and differences of quality is possible as well as a platform for discussion and at last implementation of the QACCP concept is implemented. The project creates a European network of research institutes from different disciplines and partners from practice which will be a contact point for future project in the organic sector and international questions.

## B. Impact of the project in relation to main beneficiaries of the project results

## The main users of the research results are

- o Firstly the <u>involved SME's</u> in the project and with them the consumers of the products of these companies. → optimization of the process according the QACCP analyses at 2 SMEs
- o <u>in general the babies, as a consumer,</u> because the research work supports the production of a more suitable and high-value product for the babies.
- o <u>the vegetable processors</u> because they get scientific information about the kind of raw material and its different influence on product quality → electronic info-letter
- the food industry in general; they are informed about the method and the principles of QACCP and could be sensitized that the optimization of the product quality should be also implemented in the process and not only on the basis of the recipe.
- the <u>national competent authorities and the European Commission</u>, because first instruments to evaluate careful processing techniques are worked out
- o and last but not least <u>organic research institutes</u>: for the further development of criteria and instruments to determine careful processing techniques.

The target groups are addressed individually by the information of the project: the processors of processing events, a part of the consumers by the presentation at Biofach, the researchers by the presentation at the scientific congress like Wissenschaftstagung and the publication of the elaborated results in a scientific journal, and the EU Commission by the final report and all interested by the project home page.

## C. Recommendations to the CORE Organic Funding Body Network in relation to launching and monitoring of future transnationally funded research projects

Practical experiences showed some needs for improved project management practices i.e. controlling instruments, payment linked to fulfilled performance mandate etc. The main problem occurs, when one partner is not able to carry out/finish the planned research tasks (for various reasons). Then, the consortium/the coordinator should get the possibility to transfer the work tasks to another partner, which not necessarily have been part of the consortium before. In that case the consortium/the coordinator should have the right to transfer the planned money for these tasks to the new partner. Because the payment is linked to national requirements and through a direct dialogue between the national partners, the consortium/the coordinator should have the possibility to check money transfer with the finished work steps, as usual in EU-funded projects.

The EC regulations on organic production are focussing on practical agronomy but are very limited on processing. In the growing organic market more and more products have become complex multi-step processed products, so called convenience products like deep-frozen pizza or breakfast cereals. It is supposed within the organic movement that the impact of this intensive processing

might be a threat for the product quality. The gap between consumer expectations and how regulations may guarantee the different food claims underline the importance of this topic.

If consumers are supposed to pay for a 'plus' in organic product quality, it is a challenge to first define and second proof this 'plus'. In the EC regulation No 834/2007, organic production is defined as "a production method in line with the preference of certain consumers for products produced using natural substances and processes" (EC 834/2007, (1)). The specific principles which are applied to processing of organic food exclude substances and processing methods "that might be misleading regarding the true nature of the product" (Article 6, c) and the processing should be done with care (Article 6, d). In addition to the exclusion of two processing methods (GMO, ionising radiation) and several food additives (Annex of the regulation), it seems to us, that all other processing technologies available and applied in the market are not regulated and also possibly allowed to be applied within EC 834/2007.

Therefore we suggest the following arguments for transnationally funded research projects:

The focus of future research activities in organic food quality should lay on the whole process from field (defined raw material) to fork with a focus on the processing of the food. Here technologies have a very high impact on the different quality aspects. Furthermore processing is less defined in EC 834/2007. Furthermore the process should be holistically evaluated in that product related aspects (authenticity, naturalness, health etc.) as well as the impact of the production process on the environment and society should be evaluated.

Possible research tasks are reviews on the existing technologies towards their potential for fulfilling consumer expectations/guaranteeing an organic food claim. New technologies should be evaluated in this way. Research methods should be quality analysis of critical control points on industry level followed by pilot plant studies on the critical steps. Studies should be carried out comparing different production methods for 2-4 important organic food products and define and evaluate criteria for testing careful and natural organic production.