

Preserving Raw materials into Excellent and Sustainable End products while Remaining Fresh

Final report

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¹ Usually the contact person of the coordinator as specified in Art. 8.1. of the grant agreement

² The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index en.htm; logo of the 7th FP: http://ec.europa.eu/research/fp7/index en.cfm?pg=logos). The area of activity of the project should also be

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1 Executive summary

Three novel food processing technologies have been studied to increase sustainability within the food chain without compromising food quality/ freshness. These are (i) freeze protection technique, (ii) CO2 drying, and (iii) pasteurization with pressurized CO_2 (CPT).

Freeze protection resulted in improved cell viability when freezing spinach, strawberry or parsnip down to temperatures of -11°C. Further research is needed to completely understand the relation with food structure and the process parameters, to allow extension to a wider selection and to conventional -18°C storage temperatures. The final process contained first a vacuum infusion step, with cryoprotectants, followed by a PEF impregnation step. After a certain resting period the materials could be frozen. Two main new insights on the process are: (i) vacuum impregnation of spinach with cryoprotectants increased its metabolic activity, and (ii) the metabolisation of trehalose seems to be linked with the plants stress response system.

CO₂ drying showed to produce dry products that meet freeze-dried quality parameters for a series of vegetables and fruits. Overall quality evaluations on basil, raspberry and tomato are good; mushrooms quality still requires improvement. In-situ pasteurisation during drying also contributes to food safety. By using liquid instead of solid sorption materials to recycle CO₂, a continuous processing mode was designed to allow improved ease of operation and substantial reduction of both energy costs (operation costs) and investment costs.

CPT has proven to be successful as a technique to pasteurize at close to ambient temperatures, but good structure preservation is restricted to materials with a firm product consistency, such as coconut, carrot and ham. Besides as a means to eliminate spoilage bacteria, extending shelf life, pathogens could be inactivated, contributing to food safety. CPT processing of the products spiked with Escherichia coli, Salmonella, and Listeria monocytogenes, resulted in their inactivation to undetectable levels. All products showed satisfactory and promising results in terms of overall quality and sensorial attributes. During 4 weeks of storage no regrowth of bacteria or other signs of spoilage were observed.

HACCP analyses were performed on each of the three technologies and Critical Control Points were determined. The analysis for CPT confirmed that in a food chain it would be possible to extend shelf life for about 1 week.

A 3-stage LCA approach was developed to allow the users to integrate the sustainability aspects early in the process development and to select the right level of detail required for the case of interest. Freeze protection allows a reduction of the impact of transportation during off-season. CO₂ drying results in a substantial decrease of the carbon footprint, up to 70%, for the case of drying of basil. CPT could result in less consumer waste and less used chemicals.

A pilot equipment for CO_2 drying and CO_2 pasteurisation was constructed to investigate scale-up issues and providing larger samples for application trials. The equipment has been functionally tested at an equipment builder (Separex) and demonstration tests are being done on the site of a producer of vegetables and herbs (VNK). The capacity of the unit has been designed for 40-70 kg batches, for demonstration trials at interested parties. The unit comprises a number of elements to reduce processing costs at a larger scale.

During the project contact has been made with a wide range of stakeholders, among which industrial partners interested in applying the technology. This is valuable for industrial and societal feedback with the aim of shortening the time-to-market of these 3 novel technologies. On the basis of the developments two spin-off companies have been established for further exploitation of the freeze-protection technique and the CO₂ drying technology. The spin-offs have already attracted the interest of investors and potential users, ensuring that the work performed within PRESERF will continue.

2 Project context and objectives

2.1 Project concept and objectives

European consumers are becoming increasingly aware of the importance to consume healthy products in order to maintain a healthy body weight and prevent diseases like cancer, diabetes and cardiovascular diseases. Healthy products are mostly associated with fresh products (vegetables, fruit, herbs and meat), and as a result, an increasing amount of food products is consumed fresh. However, providing a constant and diverse supply of fresh food products in the supermarket is often not sustainable. Products are transported over large distances and need to be cooled or frozen during transport. As globalisation progresses, food products are more and more imported from countries outside Europe, which amplifies unsustainability in the food chain. A second aspect herein is that the shelf-life of fresh products is limited, resulting in much waste material.

Preservation of food products by freezing, pasteurization/ sterilization or drying improves the shelf-life of the products, and can result in a reduction in transport costs. For drying, this cost reduction is due to a decrease in product weight during transport, while freezing and pasteurization give the possibility to store seasonal, locally produced products for a longer time.

However, fresh food products are difficult to preserve without a significant loss of product quality, which reflects the need for mild preservation techniques that preserve the quality of 'fresh' products. Other methods aim at preserving as much of the initial structure and nutritional aspects present in the food. Particularly important, while maintaining or creating nutritional value, are texture and flavour in preserving its value as fresh food.

Apart from loss in quality associated with the current processing methods, the production chain is inefficient and the processes used are expensive. For instance, the wet product, including the associated water, is usually transported over several hundreds of kilometres before drying. Processing that strives at maintaining the product quality is generally expensive both in terms of processing cost and in terms of energy consumption.

Based on the problems associated with quality loss, sustainability and consumer needs, the following objective was defined for this project:

The main objective of this project is to develop novel process technologies for the preservation of fresh food products without affecting the product quality (nutritional value, texture and flavour) and product safety. These technologies are to be implemented into a sustainable food chain, where implementation will focus on small-scale production (SMEs).

The PRESER has developed three novel technologies to preserve solid food stuff, mainly groups of vegetables, fruit, herbs and meat that are currently difficult to preserve either for reasons of food quality, sustainability or costs. A complete assessment for the production chains applicable (frozen foods, dried foods and ready-to-eat packed foods) has been performed in order to establish sustainability, product quality and safety throughout these food production chain.

A number of specific objectives has been defined to function as milestones in the project (Table 1). This report will elucidate how and to which extent the PRESERF project has met these objectives.

Table 1 Objectives of PRESERF project

Specific objective(s)

Develop freeze protection technique to allow freeze preservation of relevant vegetable materials

Develop and extend applicability of a novel drying technique (sustainable alternative for freeze drying) to soft tissues, such as fruits herbs and meat

Develop novel pasteurization technique to solid food stuffs (ready-to-eat applications)

Obtain valid data on nutritional value, food structure and food safety using the above techniques, to support acceptance of food industry and consumers

Design and construct a mobile pilot equipment unit for comparative studies and demonstrations

Evaluate products from comparative studies and develop new product concepts using the novel technologies

Establish a scientifically based integrated modelling technique for sustainability evaluation of the food chains of interest

Evaluate the environmental impact of developed process lines in a life cycle perspective

Evaluate the novel techniques on technical, economic and sustainability potential

Asses exploitation opportunities through results dissemination

2.2 Approach and execution

Fulfilling the above mentioned objectives was performed by a team of six project partners. The project partners and the ways how their work was interconnected to reach the projected results are presented below.

2.2.1 Project partners

- FeyeCon Development & Implementation bv, Weesp, The Netherlands (SME/coordinator)
- University of Trento, Trento, Italy (University)
- University of Zagreb, Zagreb, Croatia (University)
- SIK Institute for Food and Biotechnology, Gotenburg, Sweden (Institute)
- University of Lund, Lund, Sweden (University)
- VNK, Biddinghuizen, the Netherlands (SME)

In addition, during the project Separex (Champigneulles, France) was added as a third party associated to FeyeCon, for manufacture of the pilot plant.

2.2.2 Project organization

In the project, three separate workpackages were devoted to the development of the three novel technologies, where the University of Lund focused on developing the prefreezing technique, FeyeCon the CO₂ drying technique and the University of Trento the CO₂ pasteurisation technique (CPT). Additional work packages were devoted to assessment of the food product quality, led by the University of Zagreb, the process sustainability, by SIK, the pilot upscaling of the (CO₂) techniques and demonstration of this technique, where FeyeCon collaborated with VNK. Additional work packages focussed on the management of the project and to dissemination and exploitation of the generated results. For this purpose an Interest Group was established of interested companies to be informed and consulted during the project.

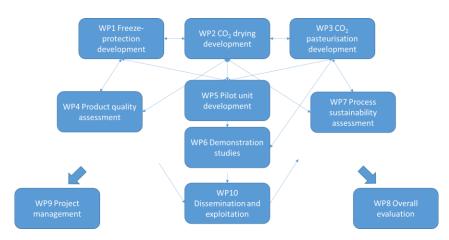


Figure 1. Simplified project structure

For all three processes a similar development structure was employed. First a screening was performed on products that could benefit from the technology. From these studies candidate products were selected that were used to optimize product quality and determine suitable process execution and process settings. Considering that in all processes food structure conservation at cellular levels was key to preservation of nutritional value and taste also combinations between the processes and extensions of these were investigated by clusters of partners. Assessing good process conditions for good quality preservation of the product candidates was considered as major milestones in the project.

To decide on the product quality, including product safety, in a quantitative controllable manner, first - similar to the process development work packages – a screening was performed for each of the candidate products, followed by assessing a limited set of quality indicators. For these protocols were established for further use in the project.

To decide on the process sustainability, sustainability indicators were listed and reduced to a limited number that enabled process comparisons and scenario development for situations were companies would need to decide on the use of processes, location and the use of fresh versus conserved foods.

Processes with considerable progress were taken up as functionality in the design and construction of a pilot equipment. This pilot equipment was to serve scale-up and demonstration studies on relevant product categories.

Final work packages served to draw conclusions on combining results on processing, product quality and safety, scalability, process sustainability and processing costs and to disseminate these results.

3 Main project results

3.1 Development of three novel food preservation techniques

3.1.1 Freeze protection

3.1.1.1 Introduction

Frozen storage of food products results in inhibition of microbial growth and slows down other degradation processes (chemical and enzymatic reactions and physical changes). The main disadvantage of freezing is that it can lead to damage of the tissue of the product due to the formation of ice crystals. To overcome this problem, plant cells can be infused by osmotically active substances with freeze protection action, such as sucrose and trehalose.

The University of Lund developed a novel process that comprises impregnation of freeze protection agents in cells of food products by means of pulsed electric field (PEF) and vacuum processing. As a result, products with a soft tissue can survive freezing and thawing and recover their natural turgor. The PEF treatment makes the cell membranes permeable, while the vacuum treatment infuses the cells with freeze protection agents. Initial successful tests on spinach leaves have been extended in this project, to assess whether the application range is broader than spinach alone.

3.1.1.2 Product quality

Product categories that were investigated included soft red fruit, leafy vegetable and root vegetables. After potato and rocket appeared not to be successful, good results were found for strawberry, spinach and parsnip.

Principle product quality indicator was the cell viability of the structure after freezing and 2thawing, mainly investigated by microscopy analyses (Figure 2), but also expressed in an indicator like drip loss and strongly influencing the texture and nutrient loss.

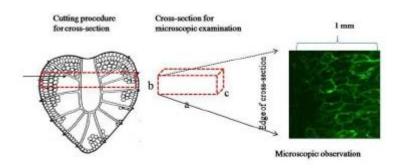


Figure 2 Preparation and result of cross-section for microscopic observations.

After optimization, cell viability increased from 0% to almost 100% for strawberry and most parsnip important tissues, as well as for spinach, with the remark that freezing temperatures could not yet be reduced to the currently common value of -18 C. A minimum of -11C was nevertheless suitable. However, the cryoprotection effect was influenced by the heterogeneity of the tissues. Tissues close to the surface of the products often did not survive thawing. Cortical tissue, vascular tissue and pith survived the freezing/thawing cycle. Further research is therefore needed to fully understand the influences of all process variables in conjunction with all tissue types that are present as well as the effects of cultivar and plant growing conditions.

Other important product quality indicators were colour and a range of sensory indicators, resulting from a panel survey. For strawberry no colour change was observed, but spinach and to a lesser extent also for parsnip some colour change was observed between untreated and pretreated materials. The spinach colour darkens upon each processing step (fresh, VI, PEF, thawed product), which is expected when air is replaced by a liquid (solution of cryoprotectants) in the structure. For spinach, the fresh leaves further lost reflectance in the green and yellow colour regions as an effect of vacuum impregnation and freezing/thawing.

For parsnip, the lightness of the product changed after each of the treatment steps. Other colour coordinates results were quite close to each other.

In the sensory testing the colour differences were not remarked as negative. For spinach further no significant differences in flavour and texture properties were detected between fresh leaves and treated leaves. For parsnip a difference was perceived on hardness when the product was frozen at -18°C. This result correlates with the cell viability not being 100% survival of the cells. Nonetheless, the panel did not find the difference in hardness to affect the perception of freshness.

3.1.1.3 Process development

The order of process steps involved in the chain of processing proved to be essential for a good result. Successful trials were performed by first employing a vacuum infusion steps, where pores of the tissue are being filled with an aqueous solution of non-sweet sugars and optionally a wheat grass extract. These components were shown to serve as cryoprotectants after having them subsequently being impregnated into the cells by employing pulses of electric fields (PEF), intended to reversely permeabilise the cell membranes. After a resting period, the food materials can then be frozen.

Figure 3 Series of processing steps involved in the novel freeze protection process

The number of pulses within a trail, the number of trails, and the width and amplitude of the pulses are just some of the variables that were optimized for the candidate products.

During the investigations novel insights have been obtained:

- It was shown that vacuum impregnation with cryoprotectants increases the metabolic activity of spinach leaves. This increase is related with the metabolization of part of the impregnated trehalose and involves oxygen consuming pathways.
- Metabolization of trehalose by the cells provokes the accumulation of trehalose-6-phosphate
 which is a well-known signal molecule for stress responses. Activated genes due to stress responses
 might contribute to increase the cryoprotection of the treated tissue.
- Another consequence of impregnation and the application of PEF might be the influence of the cryoprotectant on supercooling, ice propagation and freeze-related injury.

3.1.2 CO₂ drying

Introduction

Drying enables foodstuffs to be stored at room temperature. Drying processes that are often used are (hot) air drying, vacuum drying and freeze drying. Air drying leads to tissue and heat damage and oxidation, and is normally only applied for low-value applications. Better results are obtained with vacuum drying, but the collapse of product structure is still considerable. The quality standard for dried products is freeze drying, which preserves the overall appearance of the original product. However, tissue damage due to the formation of ice crystals is a regular occurring problem, and upon rehydration, the product becomes soggy and has no good bite. Furthermore, freeze drying is much more expensive in term of processing costs (particularly energy costs).

FeyeCon had performed lab-scale and bench-scale drying experiments by using CO_2 at elevated pressure and water adsorbents as drying media. CO_2 drying can be performed at mild temperatures of 30-40C whereas regeneration of the water adsorbents can be done efficiently at high temperatures in an oxygen-free environment. As a result, there is no heat damage to the product. Low interfacial tension in such CO_2 environment further prevents excessive collapse of the product structure. For many vegetables, herbs and other foodstuffs, the process was thought to hence be an affordable alternative for e.g. freeze drying to produce a premium dried quality product.

Within the project, the feasibility of the process has been investigated for a wider set of products will be investigated (vegetables, mushroom, fruit and herbs), with the focus on keeping the structure of the material intact and maintaining the nutritional value, while obtaining a microbial safe product. Data obtained on the drying kinetics will give input to process scale-up. Additionally also the in-process recycling of CO₂ was investigated, in order to further exploit the energy cost reduction and improve on scalability of the process

3.1.2.1 Product quality

In the project, FeyeCon extended the results for a range of categories. Initial results were obtained for relatively hard vegetables such as broccoli, bell pepper and leak. In the PRESERF project, also soft fruits and vegetables such as raspberry and tomato, as well as mushroom and herbs such as basil were investigated.

The evaluation of texture has shown that CO₂ drying is able to maintain the structure of the evaluated products relatively well, resulting in products with an open and porous structure. When rehydrated, the appearance resembled in many cases freeze-drying, except for mushroom which showed more shrinkage, also after minimization employing pretreatment steps. The colour preservation appeared to be different per product, where tomato and raspberry showed no colour loss and little changes were observed for the other two products. After optimizing operating conditions, the nutrient preservation during CO₂ drying was comparable or better to freeze drying. With respect to microbial activity CO₂ drying has shown the advantage over freeze-drying in having a substantial inactivation capacity for most of the products tested. From sensorial evaluations basil, raspberry and tomato showed good sensorial properties, whereas the mushrooms still remains a challenge.

3.1.2.2 Process development

Process development focussed for a large part on the CO_2 recycling. The drying process comprises basically of two pressure vessels where in the first the food materials are inserted to be dried by CO_2 that is passing through towards the second vessel where it is recycled by means of sorption materials. The process features that the whole process can be performed at low temperatures and without large pressure drops, while gives the food a gentle treatment at minimized energy costs. The sorption material can then off-line be regenerated.

To facilitate the process development a screening setup was designed and constructed to be used in further developments. Several sorption materials were investigated, which led to a concept of using liquid sorption media. Tests provided data on the use of such media in a continuous manner throughout the drying process. Such continuous processing mode allows for improved ease of operation and reduction of both energy costs (operation costs) and investment costs, since less equipment items are required. Data were used to provide information for scale-up, processing cost calculation and life-cycle-analysis (see below).

3.1.3 CO₂ pasteurisation

3.1.3.1 Introduction

Preservation under modified atmosphere is used to protect food products from microbial and enzymatic spoilage. Before packaging, the microbes and enzymes originally present on the product need to be inactivated, which is currently done by chlorine-based washing. However, the formation of carcinogenic chlorinated compounds (chloramines and trihalomethanes) calls the use of chlorine into question. Alternative developments are ultraviolet radiation, the use of microwaves, and hyperbatic pressure treatment. Alternatively to these, microbial inactivation by pressurized CO_2 is a preservation technique that has been increasingly investigated, predominantly for liquid food products (e.g. fruit juices and milk). Whereas CPT was before this project mostly investigated on liquid food products such as fruit juices, beer and milk, few reports had been published on CPT (CO_2 pasteurization technique) on solid foods (e.g. meat, shrimp, fruit, and vegetables).

Within the project, the applicability of CPT was broadened by studying the feasibility of the method for solid food stuffs (vegetables, meat and fruit). Including novel analytical methods, product quality, safety and kinetic data were obtained, for a range of three candidate products and to provide data for scale-up, process costs calculations and lifecycle analysis (see below)

These data will give input to the kinetics of microbial and enzymatic inactivation obtained by CPT. Furthermore, we will focus on maintaining the product quality (nutritional value, flavour and texture).

In the case of liquid food products, combining CPT with PEF treatment gave a synergistic effect between the two methods with respect to microbial inactivation. The synergistic action of CPT and PEF or ultrasound treatment will be studied for solid food stuffs.

3.1.3.2 Product quality and safety

Within the project, the applicability of CPT was broadened by studying the feasibility of the method for solid foodstuffs (vegetables, meat and fruit). First screening showed that for successful use such of technology a firm product consistency is needed for the sample to be treated without burst. Among possible ready-to-eat materials, three products were tested in more detail: coconut, carrot and dry cured ham surfaces, each one belonging to a different food category (fruits, vegetable and meat). Conventional and novel techniques (PCR and FCM) have been used for the determination of microbial load and enzymatic activity. Besides microbial load and enzymatic activity, quality indicators to assess the product quality included: texture, sensorial impact, chemical-physical attributes (pH, total acidity, and colour) shelf-life.

The main goal of the CO_2 pasteurization study was the identification of process conditions of temperature, pressure and treating time to assure a sample microbiologically stable and qualitative good. The process comprises then of inserting the material in a pressure vessel and pressurizing that in a controlled manner to the desired value. Results show that fairly low pressures, typically 120 bar, will bring down the microbial loads to safe levels, already within 15 min of processing. Such pressures are much lower than applied in hyperbaric treatments where pressures of thousands of bars are required. To speed up the process to such times, the temperature needs then to be increased to typically 40-45°C.

The natural microbial flora of coconut and carrot was analysed comprising the most important spoilage bacteria: mesophilic bacteria, coliforms, phycrophilic bacteria, lactic acid bacteria and yeast and molds. The CO₂ pasteurization particularly worked nicely for the candidate products. The bacterial load was reduced down to undetectable levels about 2-6 Log(cfu/g) depending on the initial natural content. The most resistant microbial strains were found to be the mesophilic and lactic acid bacteria, especially on the surface of carrot, while the most easily inactivated were coliforms, yeasts and moulds.

Other than the above bacteria mostly involved in the spoilage of the food, studies have focused also on microorganisms that may affect the food safety. To this extent *Escherichia coli, Salmonella enterica* and *Listeria monocytogenes* were spiked on coconut, carrot and ham, respectively. The project results showed that CPT was able to inactivate the spiked microorganisms to undetectable levels. In particular for ham, CPT allowed compliance with "zero tolerance" and with the limit of *L. monocytogenes* detection required for human safety, satisfying both the U.S. and European requirements and preserving the overall acceptability: pH, acidity, colour and sensorial attributes were similar to the untreated product. Satisfactory and promising results were also obtained for the other two candidate products in terms of quality and sensorial attributes, that carrot was affected in terms of colour and consistency modification

PCR and FCM shown to be valuable techniques in quantifying cells with permeabilized membrane and in distinguishing between viable and damaged microorganisms, giving precious insight to the inactivation mechanisms and important indications to how exploiting CPT to pasteurize safely foodstuffs assuring a long stability. The techniques also demonstrated the limits of conventional standard plate count methods, that do not take into account that, under environmental stress conditions, a number of bacterial cells enter into a so called "viable but not cultivable state" and become actually more resistant to stress.

For carrot and coconut, also the reduction of the enzymatic activity was investigated, which showed that a certain enzyme inactivation was achieved, and however the inactivation was not total, it was clearly better than the fresh product. In addition, a shelf life study of 4 weeks was performed to reveal the effects of the storage on the microbial content and quality attributes of the products. The study showed no re-growth phenomena of the food microbial content. Further, analyses of dry matter, fat content, total polyphenols, total flavonoids, antioxidant capacity, and phenolic acids were performed for coconut and phenolic content, antioxidant capacity, ascorbic acid content and total carotenoid content for carrot. CPT did not have considerable effects on coconut while some changes were observed for carrot.

Overall, CPT was demonstrated a promising technique, over thermal treatment to pasteurize solid food with a firm structure.

3.1.3.3 Process development

Process development focussed, apart from the above mentioned optimization of processing settings, on the use of CO₂ pasteurisation in combination with other processes.

CPT pasteurisation during CO₂ drying

Similar trends between CPT treatment and CO₂ drying were observed; overall, significant microbial reductions were found where aerobic mesophiles and lactic acid bacteria showed to be the most resistant. For all products the load of coliforms and yeast and moulds were reduced to none detectable limits while phycrophilic bacteria were only detected after short drying CO₂ drying of basil.

From the studied products it appeared that the CO_2 drying had a greater microbial inactivation effect over freeze drying, except for basil. For herbs and leafy vegetables, the drying time is very short. This indicates that the presence of 'free water' in the product is essential for using CO_2 as pasteurization method. By applying, an additional holding time before the drying process can decrease the microbial content further in products with a relatively short drying time.

Ultrasound enhanced CPT

Combining simultaneously CPT treatment with ultrasounds, a clear synergistic effect in terms of inactivation at milder process conditions was observed. It was shown that the treatment time for a certain degree of inactivation could be drastically reduced, keeping constant temperature and pressure. Additionally, all the main qualitative and sensorial attributes were maintained after the combined treatment. This finding has great potentiality in exploiting the technology at industrial level, where the treatment time is one of the main process parameter to consider. Results were particularly impressive for the inactivation of *Listeria monocytogenes* on ham surface.

3.2 Scale-up and demonstration results

To investigate scalability of processing, a range of actions were taken in the project, including trials at various processing scales (50 ml -10 litres), the manufacture of a pilot unit, the conceptual design of a semi-continuous process, and finally trials to provide samples to be shown to industrially interested partners and other stakeholders, among which though exposition at fairs and conferences.

Two of the three techniques qualified in time and technology readiness to be taken up as functionalities in a pilot unit that can be used for further development on issues that are critical for industrial implementation in a later stage, and for testing processes to provide larger batches for evaluation of product quality and application suitability. These processes were the CO_2 based drying and pasteurisation processes. Advantageous was that CO_2 base facilitated integration of both many of the principle processing items and auxiliary equipment.

After assessing the requirements, the equipment was constructed at Separex in Champigneulles (France) and after thorough testing, placed at project partner VNK in Biddinghuizen (NL). The capacity of the unit was designed for typically 40-70 kg batches input material. For the drying process, the output sample size is then normally 8-10%.

Movability of the equipment has been an important element, to allow for later demonstrations. Despite compromises were needed on this concept - for reasons of technical reasons, food grade operation and costs - the essence of the concept has remained.

The unit comprises a number of elements for evaluation to reduce processing costs at a larger scale. These include a single vessel design for drying, with a continuous in-process CO₂ flow cycle also for CPT and a liquid sorption recycle system.

Demonstration studies have been performed at 10 litre scale on a range of products:

- Dried tomato, basil, and raspberry as well as dried bell pepper and leek
- Pasteurised coconut

For drying, such trials revealed similar product quality results as for smaller scale trials. Also for pasteurised coconut a similar qualitative action could be observed. Further trials will be performed to further upgrade the technology readiness levels for both processes.

3.3 Product quality and safety assessment

3.3.1 Product quality indicators

Most preservation techniques modify the product properties, and as a result, the product is not regarded as fresh anymore. Therefore, the effect of the novel technologies on the product freshness has been investigated. Freshness is defined by a range of quality aspects: nutritional value, flavour, texture, microbial and enzymatic activity. For each product, freshness can be connected to different quality indicators that need to be chosen carefully to be relevant, measurable and workable.

For the candidate products, this led to the set of base quality attributes (Table 2) for which product specific indicators should be selected. Comparisons are possible more quantitatively using such indicators, even though for many of the sensory indicators, subjectivity is not easily avoided. Comparisons were made on two aspects: deviations between fresh and treated, and deviations between treated with novel process and treated with conventional processing. The former is particularly relevant for the novel pasteurisation and the prefreezing methods. In the pasteurisation, consumers expect little or no deviation from fresh, to justify the process for ready-to-eat products, and current technologies using chemicals approach that. In prefreezing processing, there is currently no alternative process, for spinach and strawberry, and approaching fresh quality is a large challenge. Conversely, in drying the comparison with alternatively dried material is somewhat more relevant. The benchmark in high-quality drying process is currently freezedrying both in terms of structure preservation and nutritional quality. The main objective was to relate the product quality with freeze-drying, and where it exceeded to compare with fresh. As a – generally lower quality – reference also (hot) air drying was taken as reference.

Table 2. Base quality indicators.

Appearance	Microbial activity
Texture	Enzyme activity
Colour	Sensory
Nutritional ingredient	contents

In interpreting the quality results it must also be taken into account that all most of the products are not eaten as a standalone product, but in combination with others, some dry and others hydrated or after another secondary kitchen processing setup.

The investigations and demonstrations led to a better insight in the width of the applicability of the processes, and the application of the products (Table 3). The results have been summarized into the table below.

Table 3. Applicability of the new techniques from a (product) technical perspective

Technology	Product cate	egories		Application
Freezing with freeze protection	Red fruits			General applications of fruits and
	Selected	leafy	vegetables	vegetables where appearance and

	(spinach) Selected roots (parsnip)	bite is relevant and e.g., toppings
CO₂ drying	Vegetables (leek, bell pepper, tomato, broccoli, onion) Fruits (Apple, strawberry, raspberry)	General applications such as instant soups, meals and sauces. Cereal ingredients (fruit) Healthy fruit/vegetables snacks
СРТ	Hard fruits (coconut) Hard vegetables Meat	Ready-to-eat applications

3.3.2 Process safety throughout the chain

Safety of food chains may improve as a consequence of using other preservation technologies. The impact can be assessed by means of a HACCP analysis. A HACCP (hazard analysis and critical control points) is a systematic approach to perform this kind of analysis. When developing new products and processes it is very important to assess potential hazards in an early phase, before commercialization. Such hazard analysis was performed. It included: 1) hazard identification (what may be present), 2) hazard assessment (which of the identified hazards require control), 3) control measure assessment (which control measures are effective and to what extent).

In terms microbial safety, the HACCP analysis for the different methods showed that:

- For CO₂-drying and drying in general it is naturally crucial to reduce the water content and thus the water activity (aw) to a level at which bacteria or moulds cannot grow and/or produce toxins. The results show that that is possible and in addition a microbial reduction of 2-5 log CFU/g is possible. The additional microbial reduction can be relevant in cases were the dried product is added under rehydration to products that are stored for a longer period, or in (unfortunate) cases that storage is improperly performed at too high aw levels.
- For freeze protection, a low temperature and good hygiene will be important to avoid growth and possible accumulation of bacteria or biofilm production in the treatment chambers. When the product has been frozen there is no microbial activity. Hence for freeze protection no CCPs have been identified.
- The CPT-treatment is the only method in the project originally constructed to reduce the number of microorganisms in the treated product. It is thus important to show a reduction of bacteria similar to a selected reference method. For some of the investigated products (i.e. carrot, coconut) the CPT treatment is a new/added step in the process introducing a possibility for reduction of bacteria generating safer products and products with prolonged shelf life. Calculations for an industrial food chain indicate that the shelf life can be increase by a week when using CPT.

Apart from microbial safety hazards, other hazards have been evaluated, but no increased risk is expected, considering additives and auxiliaries are food derived components.

3.4 Process sustainability assessment

3.4.1 Life Cycle Assessment (LCA):

An important aspect when developing new technologies is the environmental sustainability. The environmental suitability has been used as one of the parameters during the development of the new technologies and process.

In particular, the Global Warming Potential (GWP) and Cumulative Energy Demand (CED) have been used as indicators in the LCA analysis, showing that new food processing technologies can improve and reduce the environmental performance of a supply chain. Many factors can influence the environmental performance; the actual technology or that the new technology imposes upstream or downstream changes in the supply chain that affects the environmental impact. However, specifically, for freeze protection, the technology may allow for a reduction of the impact of transportation of fresh imported products during off season. For CO₂ drying, the lower energy consumption compared to freeze drying results in a substantial decrease of the carbon footprint. Calculation illustrate that whereas freeze-drying led to an emission of 30 kg CO₂-eq/kg product, for CO₂ drying those can be reduced to 5 kg CO₂-eq/kg dried product. The decrease amounted for the case of basil while CPT and CPT with high power ultrasound (HPU) could result in less consumer waste. The results were confirmed by full LCAs also covering eutrophication (terrestrial, marine and freshwater), POF (Photochemical Ozone Formation) and acidification for three of the novel food processes and their supply chains being CO₂ dried basil; freeze protected spinach and CO₂ pasteurization (CPT) combined with high power ultrasound (HPU) of coconut.

3.4.2 Use of LCA in SME decision making

The methodological approach in PRESERF takes its starting point in three important needs relating to the development of new sustainable food processing technologies: The need for a systemic approach when evaluating the sustainability benefits; The need to bring in the sustainability aspect early in the technology and product development phase; The need for a simple and affordable approach for SMEs for evaluation of the sustainability along with the process /product development phase. A three step "working model" consisting of a qualitative survey, a quantitative tool for screening for hot spots and a conventional LCA was found to be useful as developing the new food processes. Specifically the benefits of the qualitative survey, the quantitative tool for screening is that they are simple and cheap. These methods are in particular intended for the early process and product development phase. The conventional LCAs also recommended are generally more time consuming and costly to perform but are on the other hand more complete and can be used for the communication to customers.

3.5 Process cost and economic potential assessment

Processing costs have been estimated for the three processes investigated in the project, on the basis of a number of assumptions. Within the limits of the assumptions, the results of these calculations can be used to assess the economic potential of the processes, taking also market trends and demands into account. Generally, preservation makes products more expensive than fresh, so a benefit is needed through seasonal scarcity or convenience. Nevertheless, process cost analyses, as performed in a selection of the candidate products, illustrates that the novel processes can be economically viable.

For instance, preliminary calculations for freeze-protection of spinach show that using a base quality spinach that is normally used for frozen spinach can give retail price levels similar to that of in-season high quality fresh produce. The technology is less far developed that the other two, though and process applicability at a pilot scale is required to ascertain the such cost evaluation

For CO₂ drying, wholesale prices are estimated to be reduced by 26% and 63% for tomato and basil respectively, as compared to freeze-drying.

In the sense of overall preservation cost, the cost is least for CPT processing. Excess retail prices for treated, shelve-life extended vegetables are currently estimated at around 5 ct per portion of 100g.

3.6 Overall project results

Subject	Research indicators	Expected result	Baseline description	Realised result
Freeze	Number of food materials developed	3 products	successful tests freeze protection with spinach	3 developed
protection	Scientific publications	4 publications	publication tests freeze protection	4 publications
using VI and	Turgor loss	<40%	Substantial turgor loss due to freezing	<10%
PEF	Cell rupture	<30	Majority of cells ruptured	< 10%
	Drip loss on thawing	<2%	Noticeable drip loss immediately on thawing	< 5%
CO ₂ drying	Number of food materials developed	3 products	Tests have been done with broccoli and carrots	4 developed
food stuffs	Scientific publications	2 publications	Scientific publications	1 publication submitted
	Patents	1 patent	Patents	1 patents
	Water activity products CO ₂ drying	< 0.35	Water activity of dried products	< 0.30
	Rehydration capacity (RC)	RC > 60%	Poor rehydration capacity for air drying	Succeeded
	Energy cost CO ₂ drying	6 MJ/kg water	Energy costs freeze drying are high	3.5 MJ/kg water
CPT treatment	Number of food materials developed	6 products	Number of solid food materials screened	3 developed
(solid food products)	Scientific publications (solid food products)	3 publications	scientific publications (solids food products)	7 publications
	Microbial inactivation CPT treatment	>3Log	Microbial inactivation CPT treatment	2-6 Log
	Shelf-life ready-to-eat product after CPT treatment	2 weeks	Shelf-life ready-to-eat products	1-4 week
	Sourness/Off- flavour/Hardness/Colour	Similar to original	Sourness/Off- flavour/Hardness/Colour	Little softer
Life Cycle	Number of scientific	2	LCA strategy food processing and	3 publications
Assessment	publications	publications	production	(submitted)
	Number of process lines evaluated	3 process lines		3 process line
	Number of products evaluated	3 products		3 products

Screening for suitable sustainability indicators:		No standard procedure for evaluation of sustainability	
Number of alternatives to be considered	5 indicators	indicators for process exists	
Sets of sustainability indicators evaluated,	3 sets	No sustainability indicators for the	
1/process line		new technologies developed	

3.7 Project Conclusions

The project has shown that the three processes have potential for implementation in industry.

Freeze-protection using PEF/VI processing was confirmed to be technically feasible at lab scale for a leafy product such spinach, a soft fruit such a strawberry and a tuber product such as parsnip. Insight was gained on how the process affects different types of tissue and by doing so confined the multivariate window of operation when other products are to be investigated. Process modelling and metabolic research gave insight in how various tissues reaction on PEF and VI treatments. It further showed that the cultivar and grow conditions can be relevant for the applicability of the process to different species, where it identified some relevant indicators to be taken into account. For the candidate products suitable conditions were identified, which made the process ready for further upscaling investigations. Actual upscaling was not achieved, but relevant research questions for such stage have been identified.

CO₂ drying procedures and operating conditions were determined for a set of product categories, assessing a wider portfolio of use of this process: hard vegetables, soft vegetables, fruits and herbs. Quality assessments that were performed showed that on average the products could meet the quality of freezedried products. The appearance generally does not approach that of fresh handheld material, but comes close to freeze-dried material. In general, positive attributes are the firmer bite in dry and wet stage, the lower sogginess after rehydration and the good preservation of colour, flavour and nutrition value, which can be appealing to some applications. Inherently, the lower sogginess is also expressed in somewhat lower and slower rehydration capacities, which may be seen as a disadvantage for some other applications. The established in-situ pasteurisation (CPT) may be advantageous in securing food safety, as there is no need for immediate consumption after rehydration. Rather than temporal inactivation by water removal, a permanent inactivation is achieved.

Larger scale samples (bell pepper, tomato, basil) and applications (farmer's soup and dried red fruit for dessert applications) have been well received by potential interested parties, either as suppliers, as industrial manufacturers or as purchaser.

A novel CO_2 recycling process proved to be technically feasible in lab trials and subsequent process calculation showed promising results in terms of reduced energy costs and lower investments, enlarging the potential of industrial implementation. It was therefore decided to take this method up in the pilot equipment that further built on the basis of other process development results.

CPT was shown to be interesting during initial screening for firm vegetables, fruits and meat. Softer ingredients may be affected by collapse or otherwise. Further investigation revealed that microbial load could be reduced to safe levels for both spoilage bacteria and pathogens like listeria, without significantly affecting the product attributes (nutritional, colour, flavour and structure). In some cases also enzyme activity was reduced but it must be stated that the influence of CO₂ treatment differed for the various enzymes. Positive results were obtained for combining CPT treatment with ultrasound treatment, on pasteurization effect, sensorial impact, product integrity, and particularly required processing times. Finally, on the basis of the results obtained within the project, a continuous apparatus to be exploited at industrial level has been designed. A video has been made illustrating such processing line.

Pilot equipment was successfully constructed having two functionalities - CO₂ drying and CO₂ pasteurisation – for investigating scale-up issues and to provide larger samples for application trials, i.e. larger piece farmer's soup, non-soggy fruit pieces and safe ready-to-eat coconut pieces. The equipment has been functionally tested at Separex (France) and was later moved to VNK for further trials.

A sustainability assessment assay was successfully derived using LCA to be used to compare process unit operations, not only stand-alone but also in a chain, particularly to facilitate scenario development and decision making during process development from an environmental viewpoint. The 3-stage approach will allow the users to integrate the sustainability aspects early in the process development and to select the right level of detail required for the case of interest. LCA was applied for the novel processes investigated in this project. For CO₂ drying, the lower energy consumption compared to freeze drying results in a substantial decrease of the carbon footprint. The decrease amounted for the case of basil while CPT and CPT with high power ultrasound (HPU) could result in less consumer waste.

During the project contact has been made with a range of stakeholders, among which industrial partners interested in using or applying the technology. Having such contacts was perceived as valuable for industrial and societal feedback. On the basis of the developments two spin-off companies have been established for further exploitation of the freeze-protection technique and the CO₂ drying technology.

4 Impact and implications

The impact of the results of the project closely relate to the actual food chains involved. Below a short insight is reproduced on these markets.

EU countries are large suppliers of fruit and vegetables. One part is supplied as fresh products directly to consumers, while the other part is supplied as ingredient to the food processing industry. (EU Market Survey 2005, Preserved Fruit and Vegetables, 2005). A considerable part is related to fruit juice for which this project is only indirectly relevant. EU production of frozen vegetables is estimated at 2 million tonnes, of which around one quarter is supplied by Belgium. (EU Market Survey 2005, Preserved Fruit and Vegetables, 2005).

Dried products can be separated in fruits and vegetables. The only two countries in the EU supplying notable volumes of dried fruit are Greece (currants) and France (prunes). These examples are generally used in different applications as their respective fresh ingredients, and therefore do not apply for the current research.

Dried vegetables are mainly produced outside the EU, (EU Market Survey 2005, Preserved Fruit and Vegetables, 2005), as well as hence other dried fruits. In 2003, 17 million tonnes of selected preserved fruit and vegetables were imported into the EU-25, representing a value of € 13.9 billion of which 25 percent came from developing countries. (EU Market Survey 2005, Preserved Fruit and Vegetables, 2005).

4.1 Sustainability impact

From the LCA work it can be concluded that the drying process has significantly lower energy consumption compared to freeze drying results in a substantial decrease of the carbon footprint, while CPT could result in less consumer waste.

Direct primary energy savings are then anticipated at 9 PJ for dried fruits and vegetables, when applying CO₂ drying over freeze-drying for above volumes (at 10% freeze-drying). Whereas the drying process for tomato for example may double or triple the global warming potential as compared with the primary production of the tomato. Whereas for dried products, transport adds little to the energy costs, this is different for fresh material.

Cases where dried products can off-season directly replace fresh products, e.g. fruits like strawberry, seem still limited. Having freeze-protection operating at commercial scale, freeze-protected frozen strawberry

could be an ecologically sound alternative to fresh produce from e.g. Egypt. The primary energy use for this example can be reduced from 75 MJ/kg to 25 MJ/kg (air flight to Sweden). To be further noted, the CO₂ drying could provide an even 30% lower primary energy consumption than the freeze-protected freezing process after successful eco-cost effective scale-up and commercialisation to industrial level.

For the year 2004, Marriott (2005) found that 6 per cent of non-EU fresh produce to the UK was airfreighted; amounting ca. 140 kton fruits and vegetables. However, this small proportion of airfreighted produce accounted for 81 per cent of the CO₂ emissions associated with such non-EU produce imports. The paper further shows that most of these transport comes from countries much farther away than Egypt. Using the 50 MJ/kg as a benchmark value, the energy saving potential of using freeze-protected frozen fruits amounts 7 PJ/a for the UK alone. In addition, because of the perishability of fresh fruit the wasted material will be higher in the reference case, adding to the global warming potential.

The sustainability advantages of CPT result only in less waste at retail and consumer level. The process itself adds to the energy cost. For instance for coconut a direct increase of the global warming potential of 7% was calculated when introducing the CPT process. Losses throughout the food chain should therefore decrease by a similar percentage to make the process to add to the sustainability of the product. Considering that losses of 25% throughout the chain have been reported, the 7% improvement is not impossible, but more research is required to validate the effects of a process is compared with other measures to reduce food waste.

4.2 Impact on economics

The potential for freeze protection is high in terms current volume that are frozen. If and when the technology appears to be reproducible for a wide group of product candidates, the process could serve reasonable portion of the current frozen vegetable and fruit markets and could enlarge this when it concerns products that are now being frozen. The market could the typically be then 5-10% of the current frozen food market: 100-200 M€. Already for spinach the process could be interesting, provided that the process does not appear to be hampered by scale-up issues. The process is in that sense not yet well defined. If investment costs would be excessing that

Considering the lower costs, CO₂ drying could be a serious alternative to freeze-drying. When the energy costs prove in industrial practice as low as calculated, running costs will be comparable to air-drying processes and some of these applications become accessible as well. The novel process with only a single high pressure vessel assists in reduction of the investment costs. Assuming this market cannot be reached, the market potential is limited to the freeze-dried products. Assuming a 10% of the dried vegetables are freeze dried, and a similar volume for fruits, the CO₂ food dr ied products market potential will amount to ca. 100 M€ in Europe. Nowadays, most freeze-drying is done outside Europe for costs reasons. Having a cheaper process available in Europe could bring back this market to some extent. For reason such as higher traceability and the additional microbial inactivation feature can add up to that.

Note further that the technology here developed wider applicable than only for solid foods. It could in principle also be applied to other sensitive ingredients such as proteins, starches, various nutraceuticals, clinical foods and pharmaceuticals. The total global freeze drying machinery market was valued at €7 billion in 2012 and is expected to grow towards €14 billion in 2019³. In Europe, processing foods take only around 10% of the respective market, which comes down to 686 M€. The rise in demand indicates that there is not only a replacement market. Also for food processing high quality foods this market we expect the market to rise because of the growing demand for healthy convenient foods.

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³http://www.culrav.org/pr/global-freeze-drying-equipment-market-will-reach-usd-30-98-billion-by-2019-transparency-market-research.php

The RTE market which the CPT process could serve has grown enormously. Dutch households bought in 2010, for instance, in total 120 kton pre-cut vegetables.⁴ Estimating how much the novel technology could serve that market is difficult. Even if the percentage would be limited – as a consequence of the limitation of applicability to firmer materials – the size of the market could make the economic potential considerable.

4.3 Impact on food quality

There is two aspects related to the food quality impact: general nutritional value of the food and food safety.

4.3.1 General nutritional value

The impact of preservation technologies on food quality is all relative, since the benchmark is fresh food. Better than fresh it will not become. Ideally everybody eats enough fresh foods that have been subject of this research. Practice shows people do not. So then the question changes to will the technologies attribute to eating more vegetables and fruits as a consequence of the novel preservation technologies, now or in the future.

4.3.2 Food safety

Ready-to-eat products such as pre-cut vegetables and salads are becoming more and more popular. For instance an 8% increase was observed in Italy per just a quarter in Italy in 2010⁵. It combines the demand convenience and natural healthy living and minimal processing adds to that healthy perception. Natural means also that they are vulnerable to natural decay, by microbial or enzymatic activity. Pathogen contamination can occur in any step of the production chain. Since to current opinion using disinfectants in washing water should not be seen as a means of sanitation of the product itself, there remains to be a great demand for means that do. The health and economic effects for food chains have shown to be large as recent *E. coli O104:H4* outbreak has shown, for small farmers up to the European Committee. To illustrate, a compensation offer of € 210 million was made by the European Commissioner for Agriculture & Rural Development to farmers in 2011.⁶ A minimum processing technique attributing to safety can hence be very valuable, not only in Europe, but also in other parts of the world that have less moderate climates.

4.4 Target groups relevant

This research can be relevant for primary food suppliers, e.g. those with highly seasonal crops, food processors, final food manufacturers as well as retail, using such products. From all of these groups, companies and institutions have been contacted and consulted during the project.

⁴ A. Borgdorff, W.van den Berg, R. 'Vers gesneden groente routineaankoop, fruit impulsaankoop' Report: PT 2011 – 36. ProductschapTuinbouw, 2011

⁵ F. D'Acunzo, A. del Cimmuto, L. Marinelli, C. Aurigemma, M. de Giusti. Ready-to-eat vegetables production with low level waterchlorination. An evaluation of water quality and of its impact on end products.

⁶ "EU boosts E. coli compensation offer for farmers". Deutsche Welle. Retrieved 10 June 2011

5 Use and dissemination of foreground

5.1 Section A (public)

Dissemination of the foreground was performed by papers in scientific and technological journals, by presentations at conferences and exhibitions and via various other means of media coverage, among which a movie presented at a workshop to introduce Horizon 2020, later available via youtube and press releases, also as a video available available on youtube. Finally, PRESERF organized a workshop at VNK Biddinghuizen, displaying among others the new pilot machine.

#	Type of activity	Partner	Name exhibition/ conference/ fair	Date	Location	Audience	Market
2010							
1	Presentation	FEYECON D&I bv	Food and nutrition delta conference	1 June	Utrecht, NL	Scientific community (higher education, Research) - Industry - Medias	The Netherlands
2	Exhibition	FEYECON D&I bv	IntraFood	15 September	Kortrijk, BE	Industry	Benelux, France, UK, etc
2011							
3	Exhibition	FEYECON D&I bv	Foodtech	25 May	Rosmalen, NL	Industry	The Netherlands, Belgium, Germany
4	Presentation	Univ. TRENTO	FEMS 2011. 4th Congress of European Microbiologist.	26 June	Geneva, SU	Scientific community (higher education, Research)	Europe
5	Organisation of Conference	FEYECON D&I bv	SIMGBM 29th National meeting Pisa	21 September	Pisa, IT	Scientific community (higher education, Research)	Italy
6	Organization of Conference	FEYECON D&I bv	13th European Meeting On Supercritical Fluids The Hague, 9-12 October 2011	9 October	THe Hague, NL	Scientific community (higher education, Research)	Worldwide, focus Europe
7	Organisation of Conference	Univ. TRENTO	13th European Meeting On Supercritical Fluids	9 October	THe Hague, NL	Scientific community (higher education, Research)	Worldwide, focus Europe
8	Presentation	LUNDS Univ.	Innovation Food Conference Nonthermal processing division	12 October	Osnabruck, AU	Scientific community (higher education,	Worldwide, focus Europe

				workshop 2011			Research) - Industry -	
9	Organisation Conference	of	Univ. TRENTO	Aiche Annual Meeting	16 October	Minneapolis, MN, USA	Scientific community (higher education, Research)	Worldwide, especially USA
10	Presentation		FEYECON D&I bv	Dutch Drying Symposium	1 December	Utrecht, NL	Scientific community	The Netherlands
2012								
11	Exhibition		FEYECON D&I bv	ANUGA	27 March	Cologne, DE	Industry	Worldwide, focus Europe
12	Presentation		Univ. TRENTO	10th International Meeting on Supercritical Fluids	13 May	San Francisco, CA, USA	Scientific community (higher education, Research)	Worldwide
13	Presentation		FEYECON D&I bv	Food Factory	4 July	Laval, FR	Scientific community (higher education, Research) - Industry	Europe mainly
14	Presentation		LUNDS Univ.	16th World Congress of Food Science and Technology	5 August	Iguazu, Brasil	Scientific community (higher education, Research)	Worldwide
15	Exhibition		FEYECON D&I bv	IntraFood	12 September	Kortrijk, BE	Industry	Benelux, France, UK, etc
16	Presentation		LUNDS Univ.	International Conference Bio & Food Electrotechnologies	26 September	Salerno, IT	Scientific community (higher education, Research)	Worldwide, focus Europe
17	Presentation		SIK	8th International Conference on Life Cycle Assessment in the Agri-Food Sector (LCA Food 2012)	1 October	Saint Malo, FR	Scientific community	Europe mainly, USA, Asia
18	Presentation		FEYECON D&I bv	Food Valley Expo	25 October	Arnhem, NL	Industry	Europe
2013								
19	Presentation		Univ. TRENTO	ISASF: Conference of Supercritical Fluids and their Applications	29 April	Naples, IT	Scientific community (higher education, Research) - Industry - Civil society	Worldwide, focus Europe
20	Presentation		Univ. ZAGREB	EuroFoodChem	7 May	Istanbul, TU	Scientific community	Worldwide, focus Europe

21	Demonstration/ exhibition	FEYECON D&I bv	IFT Chicago	13 July	Chicago, USA	Industry, Scientific community	US
22	Presentation	Univ. TRENTO	European Congress of European Microbiologists	21 July	Leipzig, DE	Scientific community	Europe
2014							
23	Presentation	Univ. TRENTO	14th European Meeting on Supercritical Fluids	18 May	Marseille, FR	Scientific community (higher education, Research)	Europe
24	Organization of Workshop	VNK HERBS	PRESERF WORK SHOP	28 May	Biddinghuizen, NL	Industry	Europe
25	Presentation	LUND Univ.	XVIII Conference of the International Society for Isothermal Calorimetry	1 June	Lund, SE	Scientific community	Worldwide, focus Europe
26	Presentation	Univ. ZAGREB	8th International congress of food technologists, biotechnologists and nutritionists	21 October	Opatija, HR	Scientific community	Worldwide, focus Europe
27	Presentation	SIK	28th EFFoST International Conference	25-28 November	Uppsala, Sweden	Scientific community	Europe mainly

Media coverage

- Press release NL Agency (part of Dutch ministry of Economic Affairs). 13/December/2013
 https://www.youtube.com/watch?v=VZhnG1qqpRs&list=UUvGUBm9ACmPzgjuz4OebhmQ&index=9 and/or http://www.co2dry.com/news/agentschapnl-films-co2dry
- Swedish National Television SVT1 "Rapport" (National news). 13/May/2014
 - Press release Lund University. 13/May/2014

https://www.youtube.com/watch?v=udxQFUQL7EY&feature=youtu.be

• Swedish magazine "KemivärldenBiotech" 13/May/2014

http://www.svt.se/nyheter/vetenskap/ny-frysmetod-for-gronsaker

http://www.kemivarldenbiotech.se/nyheter/grundforskning-gav-ny-frysmetod/#.U3PZJ1ZyQph.facebook

Magazine "Prevention" U.S.A. 15/May/2014

http://www.prevention.com/food/healthy-eating-tips/new-way-freezing-makes-frozen-spinach-look-fresh

• Magazine "Teknisk Ukeblad" Norway. 16/May/2014

http://www.tu.no/industri/2014/05/14/holder-frukten-fersk-etter-frysing-med-elektrisk-stot

template A: list of scientific (peer reviewed) publications, starting with the most important ones

NO.	Title	Main author	Title of the periodical or the series	Number	Year of publication	Relevant pages	Permanent identifiers ⁷ (if available)	Is/Will open access ⁸ provided to this publication?
1	Supercritical CO2 induces marked changes in membrane phospholipids composition in Escherichia coli K12	S Tamburini	Journal of Membrane Biology.	Accepted for publicatio n	2014	-		yes/no
2	Validation of a mathematical model for predicting high pressurecarbon dioxide inactivation kinetics of Escherichia coli spikedon fresh cut carrot	G. Ferrentino	Journal of supercritical Fluids	85	2014	17-23		
3	Analysis and identification of mathematical models of bacterial inactivation through supercritical CO2 on solid food matrices	F. Galvanin	Journal of Food Engineering	120	2014	146-157		
4	Supercritical carbon dioxide pasteurization: quality attributes of fresh-cut coconut	G. Ferrentino	Journal of Chemistry	Article ID 703057	2013			

⁷ A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

⁸ Open Access is defined as free of charge access for anyone via the internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

5	Comparison of quantitative PCR and flow cytometry as cellular viability methods to study bacterial membrane permeabilization following supercritical CO2 treatment	S. Tamburini	Microbiology	159	2013	1056-1066
6	Effect of supercritical carbon dioxide pasteurization on natural microbiota, texture, and microstructure of fresh-cut coconut	G. Ferrentino	Journal of Food Science	77	2013	E137-143
7	On - line color monitoring of solid foods during supercritical CO2 Pasteurization	G. Ferrentino	Journal of Food Engineering	110	2012	80-85
8	Influence of Pulsed Electric Field Protocols on the Reversible Permeabilization of Rucola Leaves.	P. Dymek, K. Dejmek	Food and Bioprocess Technology	7.3	2014	761-773
9	High pressure carbon dioxide pasteurization of fresh-cut carrot	Spilimbergo	The Journal of Supercritical Fluids	79	2013	92-100
	Supercritical carbon dioxide processing of dry cured ham spiked with Listeria monocytogenes: inactivation kinetics, color, and sensory evaluations	G. Ferrentino	Food Bioprocess Technology	6	2012	1164-1174
9	Microscopic studies providing insight into the mechanisms of mass transfer in vacuum impregnation.	Valentina Panarese	Innovative Food Science and Emerging Technologies	18	2013	169-176
10	Comparative evaluation of CO2 drying as an alternative drying technique of basil (Ocimum basilicum L.) – the effect on bioactive and sensory properties	Arijana Bušić	Food Research International	To be published after corrections	2014	

5.2 Section B: Exploitation of foreground (confidential)

Template B1: List of applications for patents, trademarks, registered designs, etc.						
Type of IP Rights: Patents, Trademarks, Registered designs, Utility models, etc.	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)/Inventers			
Patent	EP 1354863	"Dehydration process that employs an ionic liquid choline salt." U.S. Patent Application 13/976,297.	FeyeCon/ Hofland, Gerard Willem, Tjerk Jan De Vries, and Maarten Stolk.			
Trademark		CO ₂ Dry	FeyeCon			

Please complete the table hereafter:

Template B2: Overview table with exploitable foreground							
Exploitable Foreground (description)	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable, commercial use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved		
Process for CO ₂ drying with continuous CO ₂ recycling	CO ₂ drying equipment made as such	 Food Cosmetic Pharmaceutical 	2016 2016 2019	Patent is an addition to others in the field and strengthens exploitation opportunities. Licencing possible after making a commercial unit	FeyeCon, to be exploited by CO2Dry by spin-off of FeyeCon		
Method for LCA for SME	Service/software/app	1. Food	2016	Protected by software	SIK		
Data on PEF/VI treatment	Equipment	Food Agriculture	2018	Protected by patent before start of the project. Further process development and scale-up needed for exploitation	Univ. Lund, to be exploited by Optifreeze, spin-off of Univ. Lund		
Data on CPT pasteurisation, giving assurance that the method works to the inactivation levels needed	Process based on those data	Food, medical, farmaceutical	2016	Know-how	Univ. Trento FeyeCon in terms of equipment		

6 Report on societal implications

Replies to the following questions will assist the European Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

Α	General Information (completed autor	matically when Grant Agreement number is	ente	red.
Gra	nt Agreement Number:	245280		
Title	e of Project:			
Nam	ne and Title of Coordinator:	erard Hofland, PhD		
В	Ethics	State Honaria, File		
1.	Did you have ethicists or others with specifi project?	ic experience of ethical issues involved in the	0	Yes
2.	Please indicate whether your project involved	any of the following issues (tick box):		YES
INF	ORMED CONSENT			
•	Did the project involve children?		No	
•	Did the project involve patients or persons not able	to give consent?	No	
•	Did the project involve adult healthy volunteers?		Yes	
Did the project involve Human Genetic Material?				
•	Did the project involve Human biological samples?			
•	Did the project involve Human data collection?		No	
RES	SEARCH ON HUMAN EMBRYO/FOETUS		<u> </u>	
•	Did the project involve Human Embryos?		No	
•	Did the project involve Human Foetal Tissue / Cells?	?	No	
•	Did the project involve Human Embryonic Stem Cell	ls?	No	
PRI	VACY			
	• Did the project involve processing of genetic lifestyle, ethnicity, political opinion, religious or	information or personal data (eg. health, sexual philosophical conviction)	No	
	Did the project involve tracking the location or contained.	observation of people?	No	
RES	SEARCH ON ANIMALS			
	• Did the project involve research on animals?		No	
	Were those animals transgenic small laboratory	animals?	-	
	• Were those animals transgenic farm animals?		-	

Were those animals cloning farm animals?				
Were those animals non-human primates?				
RESEARCH INVOLVING DEVELOPING COUNTRIES				
Use of local resources (genetic, animal, plant etc)			-	
Benefit to local community (capacity building ie access to	healthcare, education etc)		-	
DUAL USE				
Research having potential military / terrorist application			No	
C Workforce Statistics				
Workforce statistics for the project: Please indicate worked on the project (on a headcount basis).	e in the table below the	number of	people who	
Type of Position	Number of Women	Number of I	Men	
Scientific Coordinator		1		
Work package leader	5	5		
Experienced researcher (i.e. PhD holders)	11	6		
PhD Students	1			
Other	7	9		
4 How many additional researchers (in companies and universities) were recruited specifically for this project?				
Of which, indicate the number of men:				
Of which, indicate the number of women:			5	

D	Gender A	spects				
5	Did you carry out specific Gender Equality Actions under the project?			0	Yes	
						No
6	Which of	the following actions did you carry out and	d how effectiv	ve were they?		
					Very effective	
		Design and implement an equal opportunity	policy	0000	0	
		Set targets to achieve a gender balance in the	ne workforce	0000	0	
		Organise conferences and workshops on gen	nder	0000	0	
		Actions to improve work-life balance		0000	0	
	0	Other:				
7		e a gender dimension associated with the ch as, for example, consumers, users, patie? Yes- please specify No				
E	Synergi	es with Science Education				
8	-	r project involve working with students strivals and events, prizes/competitions or Yes- please specify No			n days, pa	rticipation in
9	Did the pr	roject generate any science education mat Yes- please specify	erial (e.g. kits Website	s, websites, explan	atory book	klets, DVDs)?
	0	No				
F	Interdis	ciplinarity				
10	O Which disciplines (see list below) are involved in your project? O Main discipline ⁹ : Food engineering, chemical engineering, food science, analytical chemistry					

⁹ Insert number from list below (Frascati Manual)

	0	Associated discipline ⁹ :	0	Associated discipline ⁹ :		
G	Engagin	g with Civil society and policy ma	akers			
11a		id your project engage with societ nity? (if 'No', go to Question 14)	al act	ors beyond the research	0	Yes No
11b	If yes, did groups et	I you engage with citizens (citizens' pan c.)? No	els / j	uries) or organised civil socie	ty (NGO:	s, patients'
	0	Yes- in determining what research should b	e perfo	ormed		
	0	Yes - in implementing the research				
	0	Yes, in communicating /disseminating / usin	ng the	results of the project		
11c	dialogue	so, did your project involve actors who with citizens and organised civil soccation company, science museums)?		, -	0	Yes No
12	Did you organisat	engage with government / public lions)	oodie	s or policy makers (includ	ling into	ernational
	•	No				
	0	Yes- in framing the research agenda				
	0	Yes - in implementing the research agenda				
	0	Yes, in communicating / disseminating / usin	ng the	results of the project		
13a	Will the p O O	roject generate outputs (expertise or scie Yes – as a primary objective (please indicate Yes – as a secondary objective (please indic No	e areas	below- multiple answers possible	e)	makers?
13b	13b If Yes, in which fields?					

<u>Agriculture</u>

Audiovisual and Media

Budget

Competition

Consumers

Culture

Customs

Development Economic

Monetary Affairs

Education, Training, Youth

Employment and Social Affairs

Energy

Enlargement

Enterprise

Environment

External Relations

External Trade

Fisheries and Maritime Affairs

Food Safety

Foreign and Security Policy

Fraud

Humanitarian aid

Human rights

Information Society

Institutional affairs

Internal Market

Justice, freedom and security

Public Health

Regional Policy

Research and Innovation

Space

Taxation

Transport

13c If Yes, at which level?

O Local / regional levels

 $\quad \text{and} \quad$

- National level
- European level
- International level

H Use and dissemination					
14 How many Articles were published/accepted for publication in peer-reviewed journals?					
To how many of these is open access ¹⁰ provided?				0	
How many of these are published in open access journals	s?				
How many of these are published in open repositories?					
To how many of these is open access not provided?					
Please check all applicable reasons for not providing ope	n access	S:			
☐ publisher's licensing agreement would not permit pub	lishing i	n a repo	ository		
☐ no suitable repository available					
☐ no suitable open access journal available					
no funds available to publish in an open access journa	l				
☐ lack of time and resources					
☐ lack of information on open access☐ other:					
15 How many new patent applications ('prio ("Technologically unique": multiple applications f jurisdictions should be counted as just one application	or the	same			1
16 Indicate how many of the following Intellectua	al Prope	erty -	Trademark		
Rights were applied for (give number in each box	().	ı	Registered design		1
		(Other		
17 How many spin-off companies were created / the project?	are pla	anned	as a direct resu	lt of	2
Indicate the approximate numbe	r of addi	itional j	obs in these compa	nies:	1 (now already)
18 Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:					
					rises
☐ Safeguard employment, or					
☐ Decrease in employment, ☐ None of the above / not relevant to the project					t to the project
☐ Difficult to estimate / not possible to quantify ☐					, ,
19 For your project partnership please estimate the employment effect resulting directly				ectlv	Indicate figure:
from your participation in Full Time Equivalent (FTE = one person working fulltime					7

 $^{^{\}rm 10}$ Open Access is defined as free of charge access for anyone via the internet.

	for	a year) jobs:			
Diff	ficult :	to estimate / not possible to quantify			
I	N	ledia and Communication to the	ger	neral public	
20	As	part of the project, were any of the beneficial O	(but t	ofessionals in communication of here were such professionals wor the project)	
21		part of the project, have any beneficiaries vice to improve communication with the gene	ral pu		nunication training /
22		hich of the following have been used to com	munic	ate information about your p	roject to the general
	×	Press Release		Coverage in specialist press	
		Media briefing		Coverage in general (non-specia	list) press
		TV coverage / report		Coverage in national press	
		Radio coverage / report		Coverage in international press	
	X	Brochures /posters / flyers	X	Website for the general public /	internet
	X	DVD /Film /Multimedia	X	Event targeting general public exhibition, science café)	(festival, conference,
23	In	which languages are the information products	for th	e general public produced?	
		Language of the coordinator	X	English	
		Other language(s)			

Question F-10: Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

- 1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]
- 1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)
- 1.3 Chemical sciences (chemistry, other allied subjects)
- 1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)
- 1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

- 2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)
- 2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]
- 2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

- 3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)
- 3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)
- 3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

- 4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)
- 4.2 Veterinary medicine

5. SOCIAL SCIENCES

- 5.1 Psychology
- 5.2 Economics
- 5.3 Educational sciences (education and training and other allied subjects)
- 5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary,

methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

- 6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)
- 6.2 Languages and literature (ancient and modern)
- 6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group].