Executive summary:

FUNENTECH Project followed on from two previous projects which explored different possibilities with respect to the applications of Ultra High Pressure Homogenization (UHPH): COOP-CT-2004-512626, "Development and Optimization of a Continuous UHPH for Application on Milks and Vegetable Milks", and the FAIR-CT96-1113, "High pressure treatment of liquid foods and derivates". The UHPH use and application in the treatment of milk for making cheese and yogurt with improving performance (reducing syneresis, increase yield, microbial safety, etc.) was investigated.

The challenges faced in FUNENTECH project were to achieve the sterilization of liquids foods, to improve stability, to reduce additives and to explore possible new applications such as the protection of bioactive components. At the same time it was very important to carry out toxicological and alergenicity studies to demonstrate that the materials and the process used were harmless to the consumer and also that some beneficial effects could be obtained.

The problems which had appeared in previous projects were mainly due to the low resistance to the materials used above all in the components where the high pressures are applied. The cleaning and disinfecting of the machines presented certain difficulties and it was necessary to establish maintenance routines. The best design of the valves was very important with regard to the method and materials used, and it was also very important to find a solution to the aseptic packaging of the processed products.

In this project we have managed to solve the majority of the problems, but not without suffering serious problems with the maintenance of the equipment used and resistance of the materials.

A great effort has been made to convert this technology to an industrial level. The most important achievements so far have been the following:
-Development of a prototype of a sterilization system using ultra high pressure homogenization that permits the commercial sterilization and stabilization of several diverse products such as juices (apple, orange) and vegetable milks (soya and almond).
-Confirmation of stability and long-term preservation at room temperature
-Confirmation of the non- toxicity of the products obtained and also possible nutritional properties
-Confirmation of the low thermal damage produced
-Establishment of protocols for maintenance, cleaning, disinfecting and use.
-Obtaining of stable cosmetic products by using nano-emulsions that are produced by UHPH processing
-Development of analytical techniques for determining particle size, stabilizing the obtained emulsions and controlling the products.
- The companies that have participated in this project have clearly seen the benefits of this technology with openings to new processes, new markets and new products.
- Several approaches have been made to search for new applications with the intention of protecting bioactive components within macromolecules and obtaining nano and submicrocapsules.

The design of equipment with greater production capacity, improved materials, development of functional foods and the protection of bioactive components are all planned in the near future. For this, the new Spin-off company YPSICON S.L. has been created with the aim of continuing with further investigation and development, opening this technology to the scientific community

Project Context and Objectives:

SUMMARY DESCRIPTION OF PROJECT CONTEXT AND OBJECTIVES
With the purpose of increasing the competitiveness of the SME involved, obtaining safe foods without losses of nutritional value, create natural cosmetics of high added value, and develop delivery systems able to stabilize bioactive compounds, the FUNENTECH project OBJECTIVE is to optimize continuous Ultra High Pressure Homogenizer (UHPH) for application on fluid food preparations (such as vegetable milks, juices and formulations designed for specific populations) cosmetics and biotechnology.

The Consortium of the FUNENTECH project is formed by four SMEs (Stansted Fluid Power Ltd.-Engineering firm, NECTINA S.A.-food producer, NAHO Cosmetics, Ab-biotics- biotechnology), two Universities (Universitat Autònoma de Barcelona and Université Montpellier 2) and one Research Centre (Max Rubner Institute-Federal Research Centre for Nutrition and Food) which participate in this project creating a multidisciplinary team. The diversity of this Consortium is basic to guarantee a good quality work in terms of the understanding of the phenomena taking place in food components, high pressure valve designs, suitability for biotechnological applications and improvement of cosmetic formulations. All is addressed for generating appropriate development and incorporation of innovative actions to EU SME's.

European SMEs will work together in areas of mutual but not conflicting interest, achieving the development of new products. SMEs from France and United Kingdom will participate to develop a new processing technology with SMEs of Spain, helping the social and economical cohesion of the European Community. In the food domain, if the project can show the advantages of the UHPH technology compared to the thermal treatment, it may ultimately result in the incorporation of UHPH machines to most of the liquid food industry in Europe. Such change means the creation of a very large market for the partners with capacity to manufacture machines. The success of the project is very important for the cosmetic and the biotechnology SME proposers since they are working on the production of cosmetics to which UHPH technology can be applied in order to improve quality and this offers them the opportunity to develop new concept products based on the investigation of the overall changes produced by UHPH.

The MAIN OBJECTIVE of the FUNENTECH Project was "to study the techno- and biological functionality of UHPH-processed foods and cosmetics in order to design functional foods of high safety and nutritional values, and to target specific industrial adaptation of the prototypes for food and cosmetic production".

To achieve this objective established a series of SPECIFIC OBJECTIVES:
1. To study the techno- and biological functionality of UHPH-processed foods
2. To design functional foods of high safety and nutritional values
3. To design innovative and optimised UHPH valves and spares for specific applications
4. To target specific industrial adaptation and optimisation of UHPH machines for food, cosmetic and biotechnology productions
5. To assess investment costs, environmental impact and energy consumption

6. To design functional biotechnology strategies (without involving genetics) to be used for food, cosmetic and pharmaceutical applications
7. To design clever cosmetics for specific targeted end users

DESCRIPTION OF WORK PERFORMED AND MAIN RESULTS

The work was focus on the development of vegetable milks (soy, almond, hazelnut, and walnut), fruit juices and liquid food preparation destined to populations with specific nutritional needs. Research was carried out to ensure the safety of the process (destruction of pathogenic micro-organisms, absence of toxic and carcinogenic substances), improvement of functional properties and nutritional characteristics. Different studies were conducted on the consumer acceptability of the developed processes and the products treated by UHPH. A plan for exploitation of the technology and developed products was done.

UHPH machines optimization involved the study of the effects caused by the use of different valve geometries and the design of new high pressure valves, using new materials in order to increase system control, robustness and wear. In a near future, this system is expected to substitute high temperature short time pasteurisation and ultra high temperature processing of fluids with less energy consumption and less contamination.

Three types of UHPH machines prototypes were used in the project. They are able to provide different flow fluids 8, 15 and 90 L/h. All the machines are equipped with systems to control the treatment temperature (30-150°C). During the first stages of this project SFP provided three different valve geometries to perform studies with vegetable milks, fruit juices, and emulsions and microbiological assays. The data obtained was studied in order to use expensive materials such diamond for the new high pressure valves designed. The machinery described above was adapted in such a way that allows its use to treat the different products involved in the project, in order to guarantee the self life and stability of foods or emulsions during long periods of time.

Combinations of inlet/outlet temperatures and pressures (single/two stages) to reach optimum treatment conditions in accordance with the final product were also evaluated. Improved nutritional and organoleptic value was targeted. The effects of UHPH on microbiological (studies so far involve the use of pathogens, spoilage flora, virus and spores) and physicochemical characteristics of food fluids was studied and to compared to traditional methods such as conventional homogenisation, pasteurisation, and sterilisation. The aim of the experiment was to obtain high quality products with better organoleptic and functional properties, no additives and increased stability and food safety. Optimum treatment conditions were established.

Evaluation of nutritional and toxicological characteristics of the products developed using UHPH (especially vegetable milks and derived products) was carried out. It was aimed to establish the absence of substantial differences between the UHPH product and the corresponding conventionally homogenized/thermal treated product using sum-parameters like sensory assessment, antioxidative potential, mutagenicity, antigenicity, antimutagenic potential, acute toxicity, HPLC-patterns, electrophoretic patterns, UV/VIS/NIR spectral changes.

The project team studied the use of UHPH technology in order to produce oil in water (O/W) emulsions stabilized by proteins at the oil/water interface instead of low molecular weight surfactants. Such emulsions can be proposed as oral supplement/complement or addressed to medical nutrition. Submicro emulsions and other systems such as encapsulating systems was also subject of investigation in order to stabilise bioactive compounds incorporated into cosmetics or functional foods.

EXPECTED FINAL RESULTS AND POTENTIAL IMPACTS

It is expected that UHPH technology substantially complement the activities of these SMEs, and allow them to grow in employees, in the turn over and size and market share. It is expected that the project will have a positive impact on employment, with the creation of specialised jobs cause extensive product consumption. Both factors have great impact on employment in different European countries. Moreover, it can lead to further applications of the technology in different research areas such as pharmaceutical and chemical. The application of this technology seeks to increase competitiveness of European companies, especially SMEs, inside the European Community, and around the world (exportation of machines and processed food).

Project Results:

Main S&T results are summarized in the next sections:

A. EFFECTS OF UHPH TREATMENTS ON PURE STRAINS OF MICROORGANISM INOCULATED IN DIFFERENT FOODSTUFFS
B. STATEMENTS ON UHPH BIOCHEMICAL AND POSSIBLY TOXICOLOGICAL EFFECTS
C. VEGETABLE BEVERAGES (ALMOND AND SOY MILK)
D. EMULSIONS FOR COSMETICS
E. NANOENCAPSULATION

A. EFFECT OF UHPH TREATMENTS ON PURE STRAINS OF MICROORGANISMS INOCULATED IN DIFFERENT FOODSTUFFS

Results from the assays with vegetative bacterial cells

Listeria monocytogenes CCUG 15526 was inoculated at a concentration of approximately 7.0 log 10 cfu/mL in milk samples with 0.3, 3.6, 10, and 15% fat contents in order to assess fat content or viscosity effects on lethality values. Milk samples with 0.3 and 3.6% fat content were also inoculated with a lower load of approximately 3.0 log 10 cfu/mL. Microbiological analyses were performed up to 15 days of storage at 4°C. Maximum lethality values were observed in samples treated at 400 MPa with 15 and 10% fat (7.95 and 7.46 log10 cfu/mL), respectively. However, in skimmed and 3.6% fat milk samples, complete inactivation was not achieved and, during the subsequent 15 d of storage at 4°C, L. monocytogenes was able to recover achieving initial counts. For 10 and 15% fat milk samples, L. monocytogenes treated at 300 and 400 MPa maintained the reductions achieved. Fat content increased the maximum temperature reached during UHPH treatment; this could have contributed to the lethal effect achieved (Roig Sagués et al. 2009).

In whole egg inoculated (3 and 7 log CFU/ml) of Salmonella enterica serovar Senftenberg 775W and submitted to UHPH treatments at 150, 200, and 250 MPa. In samples with an initial load of approximately 7 log CFU/ml, the highest lethality value of 3.2 log CFU/ml was obtained at 250 MPa, and it is similar to those values reported in other surveys for thermal pasteurization with this same Salmonella strain. For initial loads of 3 log CFU/ml total inactivation was apparently obtained even after 10 days of storage at 4° C. Nevertheless, viable Salmonella cells were detected with an immunoassay method during the entire storage period (Velázquez-Estrada et al., 2008).

In fruit juices (grape and orange) the effect of UHPH on Listeria monocytogenes and Salmonella enterica serovar Senftenberg 775 W was also assessed. Pressure level had a significant impact on the lethal effect of UHPH and complete inactivation of Salmonella enterica serovar Senftenberg 775 W was achieved at 400 MPa. L. monocytogenes proved to be more resistant to UHPH treatments and no significant differences were observed between 300 and 400 MPa treatments in both juices. Sublethal injuries were not detected in any case. During the storage at 4°C viable counts of both strains showed a decreasing trend. L. monocytogenes viable counts became undetectable in UHPH treated and also in control samples of grape juice which could be attributed to the presence of natural compounds with antilisterial effect (phenolic compounds such as: flavan-3-ols, anthocyanins or hydroxycinnamate) (Velázquez Estrada et al., 2010).

Results from the assays with bacterial spores

Spores from strains of Bacillus cereus, B. subtilis, Paenibacillus taichungensis and Lysinbacillus sp., isolated from soymilk and almond milk were used to evaluate the effect of UHPH treatments. A benchtop UHPH model/DRG FPG12500 from Stansted Fluid Power Ltd. (Essex, UK) was used for these assays. Valve B was used in this experiment. The spores (105-106 spores/mL) suspended in sterilized soy and almond milk (4%) were subjected to a single cycle. The pressure applied was 300 MPa with different inlet temperatures (55, 65, 75 and 85°C).

When a single UHPH treatment at 300 MPa was applied with inlet temperatures of 55, 65, 75 or 85°C, a high reduction of the colony counts was observed. In fact, in all strains tested the inlet temperature applied influenced significantly (Pless than0.05) in the lethality, obtaining the highest when the inlet temperature applied was 85°C (greater than 5 log cfu/ml) and the lowest when the inlet temperature was 55°C (between 1 and 4 log cfu/ml). In some UHPH treatments, especially when the inlet temperature applied was 85°C, was achieved a complete microbial inactivation. After incubation at 30°C for 10 days, neither coagulation and nor bacterial growth was observed.

A B. cereus strain obtained from soymilk showed to be the most resistant to UHPH, while the strain of Lysinobacillus isolated from almond milk was the most sensible. For UHPH treatments with inlet temperatures of 55, 65, 75 and 85°C, the maximal temperatures achieved after valve were around 110°C, 120°C, 130°C and 136°C, respectively, which can explain why lethality of spores was higher in samples treated at 85°C. It is well known that dormant spores are highly resistant to many physical and chemical agents. UHPH treatment exerts sporicidal effects at when inlet temperatures of 75°C and specially 85°C are applied, when the maximal temperature achieved after valve was around 136°C during a retention time less than0.3 s, resulting in a minimal heat damage, respect to the usual UHT treatments applied for food industries. UHPH could be a useful and innovative tool for Bacillus spores control in fluid foods.

Results from the assays with viruses

In this study, the bacteriophages of Escherichia coli MS2 and Qß were used like a surrogates of NoV and HAV. These phages are used in survival, disinfection and inactivation studies (Allwood et al., 2004; Black et al., 2010). MS2 has been widely used as a surrogate for human enteric viruses in many studies on virus transport, inactivation, disinfection, and fate (Dreier et al., 2005; Black et al., 2010). Qß virus is similar to MS2 and it also displays morphological and structural resemblances to enteric viruses and it too shows good potential as a virus indicator (Feng et al., 2003).

The lethality values observed for MS2 and Qß viruses after UHPH treatments can be regarded as extremely positive considering that it is accepted a reduction of the initial phage levels by at least 99.9% because of the difficulty of demonstrating a 5 log reduction in viruses (Gulati et al., 2001, Anonymous, 2005).

It was also aimed at assessing different valve designs using flat or wedged end needles in the valve assembly. Unfortunately conclusions cannot still be drawn because either valves design or strains of viruses shown contrary effects .

B. STATEMENTS ON UHPH BIOCHEMICAL AND POSSIBLY TOXICOLOGICAL EFFECTS

Materials under Investigation

In the progress of FUNENTECH project the MRI group received or produced and analysed and screened a total of about 90 vegetable milk samples of different composition and treatments: 42 almond samples produced and treated at UAB, 12 almond samples produced and treated at MRI, as well as 19 soybean, 10 hazelnut and 7 walnut milk samples produced and treated at UAB. The MRI produced samples were either untreated or UHPH treated at 300 MPa and 85°C inlet temperature. The purpose of that was to gain a number of standardised independent samples with same origin, same production procedure and same UHPH treatments to make possible statistical significance tests.

Objectives and experimental methods

Work package 3 demanded experimental procedures to enable statements on UHPH biochemical and possibly toxicological effects, including cytotoxicity and genotoxicity in human intestinal cells.

In the progress of work package 3, MRI executed about 19 different analytical or screening protocols, respectively (nutritionally valuable ingredients, biochemistry, and toxicology) on UHPH treated vegetable milk samples received mainly from UAB.

A Chroma-Meter CR-300 (Minolta, Japan) was employed to allow qualitative and quantitative statements about possible changes in colours and their intensities through remission photometry.

Liquid chromatographic patterns are e.g. required for characterization and confirmation of processed foods in support of new marketing applications providing data on identity, homogeneity and purity and naturally also about alterations due to processing. Reverse-phase liquid chromatography (RP-HPLC) using a dual pump Shimadzu Liquid Chromatograph LC-10 AD connected to a Merck Hitachi Diode array detector L 7455 via a Hitachi interface D 7000 was employed for that purpose.

Measuring changes in hydrophobicity of a proteinous solution or emulsion, respectively may prove useful in understanding and predicting structural or functional effects due to UHPH treatment. In FUNENTECH the interaction of the fluorescent dye ANS (A-1028, SIGMA) with UHPH milks was analysed with a Shimadzu RF-1502 fluorescence spectrophotometer and conducted in quartz optical cells of 10 mm path length.

SDS-PAGE detected electrophoretic patterns may give information on UHPH effects on the samples' macromolecular compounds in the range of 12 to 78 kDa.

Dityrosine formation is a significant biomarker of oxidative stress and may be induced by processing. Because of its intense fluorescence it is useful as marker in biological media including foods like proteinous emulsions. Samples were analysed using a Shimadzu RF-1502 fluorescence spectrophotometer (EX 315/EM420nm) and conducted in quartz optical cells of 10 mm path length.

Tryptophan fluorescence is widely used as a tool to monitor changes in proteins and to make inferences regarding local structure and dynamics introduced e.g. by energy input due to processing. Trp lambdamax is quite sensitive to its local environment, ranging from 308 nm (azurin) to 355 nm (e.g., glucagon) and roughly correlates with the degree of solvent exposure of the chromophore.

Some food processing treatments are likely to result in transformation between –SH and –S–S– groups. Thus, the content and changes of -SH and –S–S– groups are often assayed when exploring process related changes of properties of proteins in foods. MRI applied the most widely used method, which is Ellman's method, in which 5,5-dithio-2-nitrobenzoate (DTNB) is used to react with SH groups to produce a yellow substance with a maximum absorbance at 412 nm.

For the determination of the essential vitamins thiamine and riboflavin, Vitamin B1 and B2, in UHPH treated milks of the FUNENTECH project an appropriate sample solution after dephosphorylation using 0.1 M hydrochloric acid and enzymatic was measured by HPLC and subsequent fluorimetric detection.

The Ames Test, also called Salmonella Reversion Test, is used to determine the mutagenicity of complex environmental and biological mixtures. A considerable number of mutagens first detected by the Salmonella test have been shown subsequently to be carcinogenic in animal tests including several of the protein pyrolysis products produced by cooking foods, like the food-borne mutagen 2-amino-3-methyl-imidazo[4,5-f]quinoline (IQ), which was used as mutagen in the FUNENTECH project. Samples were assayed for mutagenic activity using standard plate incorporation and preincubation methods, in accordance with OECD Test Guideline 471. Salmonella typhimorium tester strains TA98 and TA100 were used in presence and also in absence of S9 metabolic activation. Changes in mutagenic activity were evaluated on basis of the grade of inhibition of revertant colonies. This test allows also for determination of ANTI-mutagenicity and of toxicity in bacterial cell cultures.

The BioFix Lumi luminous bacteria test, used for the FUNENTECH samples, allows the determination of acute biotoxicity in accordance to EN ISO 11348. Luminous bacteria tests are able to evaluate the toxicity of a whole sample. As an internationally standardised method it is one of the most important biological tests for toxicity analysis beside the also standardised fish-, daphnia- and algae-tests.

Allergens are substances that, even present in low concentrations, cause allergic reactions for certain people and can be life-threatening. Labeling is obligatory for food ingredients classified as being possible allergens or possibly intolerant, e.g. peanuts, hazelnuts, eggs, gluten/gliadin, almonds, soya, sulphite, milk and lactose. MRI used commercially available allergen tests (ELISAs) that are aimed at detecting traces of those allergens in raw and processed foods.

In vitro tests for cytotoxicity and genotoxicity allow to investigate whether new compounds with cytotoxic and genotoxic properties at considerable concentration are generated by UHPH treatment of food. Caco-2 cells were used as a model of human intestinal epithelial cells. Differentiated Caco-2 cells show morphological and biochemical similarity to normal epithelium. The cytotoxicity was investigated using two bioassays: WST-1 and calcein-acetoxymethyl ester (Calcein-AM) tests. WST-1 assay measures the metabolic activity of the cells as an indicator for the vitality. Calcein-AM assay tests cellular esterase activity and plasma membrane integrity. Genotoxicity (DNA strand breaks) was determined by the comet assay in Caco-2 cells.

UHPH treatment can reduce the concentration of bioactive constituents. The possible changes in antioxidant activity of plant milks caused by

UHPH treatment was investigated in Caco-2 cells. Oxidative stress was induced by iron ions which are known to be able to induce lipid peroxidation in cells and are proposed to be responsible for the increased risk of colon cancer caused by the high consumption of red meat, rich in iron. Lipid peroxidation was assessed by measuring malondialdehyde using HPLC with fluorescence detection. Antigenotoxic activity was investigated in Caco-2 cells using oxidants/prooxidants such as hydrogen peroxide or iron ions and comet assay.

Cellular uptake and transepithelial transport: Processing of liquid food, like soy or almond milk, using ultra high-pressure homogenisation technique is a mechanical food processing process which includes disruption of food matrix with subsequent particle size reduction. However, food processing can also lead to destruction of food bioactive compounds and lower their concentration in food. These factors could be crucial factors leading to modified bioavailability of health beneficial food compounds.

In vitro cell culture models make it possible to investigate the absorption of food components by intestinal cells and to avoid the effects of some factors which are difficult to control: efficacy of food matrix disruption, solubilisation of food bioactive compounds and incorporation into mixed micelles. Caco-2 cell monolayers grown on permeable supports mimic intestinal absorptive epithelium and show good correlation between the drug transport through cell monolayer and that seen across the human intestine in vivo. The integrity of the monolayer was determined by measurement of transepithelial resistance and fluorescein permeation across the monolayer. Concentrations of carotenoids were estimated in the apical and basolateral compartment as well as in the cells. Carotenoids were extracted and determined by reversed-phase HPLC.

Most important results

Work packages 3 and 4 demanded experimental procedures to enable statements on UHPH biochemical and possibly toxicological effects including cytotoxicity and genotoxicity in human intestinal cells. In the progress of work MRI executed about 30 different analytical or screening protocols, respectively on UHPH treated vegetable milk samples received mainly from UAB.

While samples treated at MRI showed a significant increase in brightness (p less than 0.05), colour values of samples received from UAB showed no unified, standard response to UHPH treatment, independent of source (walnut, hazelnut, almond) or treatment.

All HPLC patterns of UHPH showed a unified, standard response to UHPH treatment, independent of source (walnut, hazelnut, and almond). For low molecular weight compounds virtually no changes were discernible, all peaks were preserved and not changed in their relative height, no new peaks (=substances) appeared. A clear indication of substantial equivalence! But there were changes in the higher molecular structures.

Surface hydrophobicity was generally increased in all UHPH samples. That of hazelnut milk was also increased similarly, but interestingly, in hazelnut and also walnut milk the pasteurised samples showed even higher values. Surface hydrophobicity of walnut milk was not changed significantly due to UHPH, but the number of walnut milk samples was insufficient for final statement.

In SDS-PAGE gels of almond and soybean milks no differences in the electrophoretic patterns were to be discerned, independent of the UHPH treatments applied. That means no effect on macromolecular compounds in the range of 12 to 78 kDa (SDS 4 marker). This result corresponds to the HPLC patterns where also no changes were detected in the more hydrophilic compound range.

All UHPH samples showed a significant increase in tyrosine linkage (p less than 0.05). Tyrosine linkage values of almond milk samples received from UAB unanimously showed similar response to UHPH treatment. Tyrosine linkage in hazelnut milk and also in walnut milk was much less distinct.

Tryptophan fluorescence assays showed, that during the UHPH induced conformational changes the solvent exposure of tryptophan residues was not much affected. Also the fluorescence intensities at peak maximum remained nearly identical (358 and 361). Almond milk samples delivered by UAB showed similar results independent of the intensity of treatment. Hazelnut milk (UAB) showed no significant differences in peak maximum and height for UHPH and sterilisation.

Reduction of free sulfhydryl groups after UHPH was a common feature of all samples. This can be explained by the oxidative conditions in combination with a relative high energy input during UHPH which leads to cross linking via – S – S - bridges. Hazelnut and walnut milks showed similar, but less pronounced behaviour.

In almond, walnut and hazelnut milks vitamin B1 was absolutely stable in UHPH treatments, vitamin B2 also showed no significant changes, but values had more fluctuation. The comparison with sterilised samples demonstrates the clear advantage of UHPH in retention of Vitamin B1.

During the samples over all compositions and treatments were assayed for mutagenic activity and in all tests revertant colonies were completely inhibited, thus not any mutagenic activity was detected. In not one single test addition of 500µl food resulted at least in a 50% reduction of mutant growth, thus also not any antimutagenic activity was detected. There was also no toxic effect on the mutant bacteria.

In the acute toxicity tests all samples showed the common feature that in all cases UHPH leads to a reduction of the luminescence inhibition compared to that of the raw material. Some raw and also sterilised walnut milk samples, but not hazelnut or almond or soybean, showed enhanced inhibition, possibly due their high amount of acids (raw) or due to maillard products (sterilised).

In all UHPH almond samples more than 98 % (!) of the almond antigens are no more detected by the commercial ELISA test for traces of raw and processed almond protein (no significance test necessary). In hazelnut milk (350 MPa, 75 °C, from UAB) about 900 to 3000 ppm antigenic protein was detected which is according to the range of total protein while in corresponding sterilized hazelnut milk samples no signal was found. In UHPH treated walnut milk (350 MPa, 75 °C, from UAB) about 100 to 400 ppm antigenic protein was detected which is below the range of total protein while in corresponding sterilized walnut milk samples only about 10 to 100 ppm were found.

Cytotoxicity, genotoxicity, antioxidant/antigenotoxic activity: No differences in cytotoxic and genotoxic potential between UHPH-treated (up to 350 MPa, 85°C) and treated by conventional methods almond, hazelnut and soy milk were observed. Almond and hazelnut milk (UHPH-treated and non-treated) are similarly effective in protecting intestinal cells against iron induced lipid peroxidation. Almond and hazelnut milk (UHPH-treated and conventionally treated (sterilized)) are similarly effective in protecting intestinal cells against iron induced DNA strand break formation. Both UHPH-treated and non-treated almond milk do not protect intestinal cells against hydrogen peroxide induced DNA strand break formation. Thus, in in vitro cell models used, UHPH-treatment of almond, hazelnut and soy milk shows similar cytotoxic, genotoxic potential and antioxidant/ antigenotoxic activity as compared with milks treated by conventional methods (raw product, UHT-treatment or sterilization) indicating that observed changes in plant milk physico-chemical properties induced by UHPH have not a relevant role for the effects investigated on human intestinal cells.

Cellular uptake and transepithelial transport: Soy milk can increase ß-carotene, a provitamin A carotenoid, cellular uptake from micelles, but there was no statistically significant effect of UHPH treatment of either almond or soy milk on the ß-carotene cellular uptake and transepithelial transport. Astaxanthin, which is more polar than ß-carotene, is also more efficiently taken up than ß-carotene by the intestinal Caco-2 cells. There was no effect of UHPH-treatment of almond and soy milk on the transepithelial transport of astaxanthin. But, UHPH-treatment of almond milk lowered the cellular uptake of astaxanthin indicating the possible modification of bioavailability of bioactive food constituents by UHPH treatment of almond milk. Cellular uptake and transport of water soluble compounds, such as soy isoflavones, are not affected by UHPH treatment of soy milk.

Thus, UHPH-treatment of liquid food mostly did not affect cellular uptake and transepithelial transport of bioactive compounds. However, in the case of astaxanthin UHPH treatment of almond milk modified astaxanthin cellular uptake, indicating that there is no general conclusion and bioavailability of a bioactive compound can be changed depending on the kind of food treated by UHPH.

The ELISA results with almond milk is a really important finding, that UHPH induced conformational changes of the proteins lead to no more being recognized by specific anti bodies.

C. VEGETABLE BEVERAGES (ALMOND AND SOY MILKS)

Vegetable beverages are widely consumed in European as alternatives to cow milk, although they are not really comparable foods. They are particularly useful for people with problems of lactose intolerance or with allergy to cow milk proteins. Moreover, vegetable milks are considered as healthy foods, mainly as protectors against cardiovascular diseases, due to their high content of unsaturated fatty acids. They also contain other beneficial bioactive compounds such as antioxidants (flavonoids), vitamin E and polyamines, fibre and phytosterols, which reduces cholesterol absorption.

Vegetable beverages available on the market are processed by the application of the heat treatments, especially UHT sterilisation.

Ultra high pressure technology (UHPH) could prevent some of the undesirable effects of heat application on their sensorial, nutritional and biofunctional properties. The potential minor impact of UHPH on foods micro compounds compared with heat treatments can be a good argument for placing UHPH treated vegetable beverages into the market.

Aim of the study

Vegetable milks face several quality aspects to be solved. From a hygienic point of view, the treatment applied must be capable to destroy the spores usually common in vegetable products as part of its microbiota. These, are resistant forms of microorganisms difficult to inactivate. Moreover, vegetable milks are colloidal systems formed by dispersed particles such as oil droplets, solid particles from raw materials, proteins and starch granules in some extent. This complexity make difficult to obtain a stable product to be stored, even for a not very long time. Commonly, in UHT-treated vegetable milks, as soymilk, sedimentable particles separate from the continuous phase causing lost of quality.

UHPH increases the physical stability and reduce microbial inactivation since it causes a breakdown of particles, including microorganism. From a physical point of view, causes reduction of fat globules and facilitates de interaction with aqueous phase of macromolecules leading a better dispersion capacity.

Study design

Comparison of UHPH treatment of vegetable milks (soy and almond) with conventional treatments (Pasteurisation and UHT) was performed. At the same time, different combinations of pressure and inlet temperature of the product were applied to establish the best UHPH treatment for obtaining good hygienic and physical quality of vegetables milks.

UHPH treatments (pressure and inlet temperature of the product) were the following:
300 MPa, 55°C; 300 MPa, 65°C; 300 MPa, 75°C; 200 MPa, 55°C; 200 MPa, 65°C; 200 MPa, 75° C.

Analyses on vegetable milks were conducted to determine microbial destruction (total counts, spores, enterobacterial counts, Micrococaceae, yeast and moulds and Bacillus cereus), physical stability (% w/w of sedimentation after centrifugation of vegetable milks and particle size distribution) and oxidation (hydroperoxides content the days 1 and 15 after production). On the other hand, protein content almond milk (aprox. 1%) low compared to soy milk. This factor may cause difficulties of milk stabilisation, especially during storage of the product. This is why the almond milk was obtained without the addition of any ingredient, just made from raw almond, and with the addition of 0.03 % of lecithin (quantity previously tested).

Main results

From the microbial analysis it was observed that only UHPH conditions at 300 MPa with 65 and 75°C of inlet temperatures were capable of producing vegetable milks without any bacterial or spores grown after incubation at 30°C for a week. This UHPH conditions, in principle, were similar to UHT. Other UHPH treatments milder that those above mentioned produced total destruction on day 1 but were no capable to resist the incubation of the vegetable milk for one week. So, the latter were considered as pasteurized foods.

Physical stability of vegetable beverages was assessed by measuring the % (w/w) of total sedimentable solids after centrifugation of samples. The determination was performed on days 1 and 15 after treatment. The higher the value of this parameter, the lower the stability of milks as a consequence of particle sedimentation. This index may also be related to the graininess perceived in the mouth. In addition to this evaluation, visual observation of the presence of a spontaneous settled particle layer on the bottom of the bottle during storage was made. UHT soymilk and almond milk presented this thin layer after 7 days of storage, while in UHPH it was not observed in any case. On the other hand, the measured index agreed with this observation. Vegetable milks processed by UHT, in general, showed approximately double values of stability index than UHPH samples. On the other hand, the sedimentation index increased after 15 days of storage in 300 MPa treated soymilk. This is probably related with the presence of the higher frequency of big particles or aggregates detected in the particle size determinations. At the same time, those particles or aggregates in this sample could act as nuclei for aggregations for smaller particles during the sedimentation forced by centrifugation.

Parameters obtained from the analysis of particle size distribution curves give different information about the particle size of the disperse phase of vegetable diluted emulsions. Therefore, it is difficult to distinguish what kind of disperse particles this analysis is giving information about, as the detection range of the equipment used is between 0.04 and 2000 nm. In this range it is possible that the particle size analysis is including not only fat globules but also some protein aggregates and even protein-fat globule and globule aggregates. Raw vegetable milk was the sample which presented the highest values of particle size, closely followed by pasteurized and UHT, indicating that the conventional homogenization applied to the UHT vegetable milks did not produce an additional reduction of particle size compared to that obtained by the colloidal mill. UHPH treated milks exhibited a considerable reduction in particle size compared to raw and heat treated milks but increasing pressure from 200 to 300 MPa did not produce a further reduction of particle size. On the contrary, when comparing the magnitude of pressure applied, soymilk and almond milk treated at 300 MPa presented the highest values of particle size parameters. The mean diameter (d3.2) and Dv 0.5 experienced a slight but significantly higher value when the UHPH treatment was increased from 200 to 300 MPa, indicating that to some extent coalescence phenomenon could take place under these UHPH conditions.

In almond milk without lecithin, particle size distribution curves presented a multimodal distribution compared with those almond milk with lecithin added. Probably in the former, aggregation phenomena are responsible of the big particle size observed.

Almond, and specially, soy milk contains polyunsaturated fatty acids (mainly linoleic and linolenic acids) which are subtracts for further oxidation. Lipid oxidation refers to the reaction of unsaturated fatty acids with molecular oxygen. This process results in the formation of free radicals and odourless and tasteless intermediary products known as fatty acid hydroperoxides; however, these compounds are unstable and can degrade yielding volatile flavour products such as aldehydes, acids, alcohol and ketones.

The development of this process depends on the fatty acid composition, but also external factors such as light exposure, temperature, oxygen, transition metal ions and presence of pro or antioxidant compounds are necessary for the initiation of the reaction. Moreover, oxidation may take place by lipoxidase (LOX) enzymatic catalyzed pathway.

After applying pasteurization, UHT and UHPH treatments, LOX enzyme was not detected, thus indicating that there was a total inactivation. However, there was formation of primary oxidation product (lipid hydroperoxides).

In general, the UHPH treated milks only presented a slightly higher content of hidroperoxides than those milks proceses conventionally.

D. EMULSIONS FOR COSMETICS

O/W emulsions stabilized by small molecular weight surfactants are developed for cosmetic purposes in a large range of oil contents. In both cases, the small size of oil droplets obtained in the submicron range allows an excellent stability of the UHPH-processed emulsions vs. the storage time.

The behaviour of submicron emulsions was studied on model of cell monolayers. Because of their small size, submicron emulsions are expected to display excellent properties of transport to ensure delivery of active compounds. Submicron emulsions may be used to incorporate functional components within the droplets or at the O/W interface, with interesting biological activity such as antimicrobial, antioxidant, metabolic, hydration or nutritional properties. A vitamin was chosen by UMII, as a biological marker incorporated into oil droplets to monitor the behaviour of submicron emulsion droplets through in vitro cell monolayers. It was found that the molecule was transported and metabolized into the cells, and that submicron emulsion droplets did not impair cell metabolic activity and did not cause membrane cell injury, which demonstrated the benefit of submicron droplets as molecule delivery systems.

E. NANOENCAPSULATION

The purpose of the research was to explore the possibility to encapsulate ß-carotene as bioactive component within casein. The ß-carotene was specifically chosen for being deeply colored and very lipophilic component. Increasing UHPH pressure triggered higher reduction of particle size (meausred by dynamic light scattering using a Zetasizer Nano-ZS90) with a concomitant increase of the stability of the nanodispersion (evaluated using a Turbiscan Lab). Differences between electrodensity could confirm the possible nanoencapsulation of this component. However, the encapsulation of ß-carotene cannot be confirmed by the micrographs obtained by Cryo-TEM. According to the obtained results, it is possible to extrapolate to similar components of ABB products. These products were extracted from mechanically disruption bacterial cells and to be used to recover internal metabolites (in particular bioactive compounds) aiming at its further incorporation into delivery systems for food use.

Potential Impact:

The potential impact is summarized in the next sections:
A. POTENTIAL IMPACT OF THE IMPROVEMENTS ON THE PROTOTYPES

B. POTENTIAL IMPACT OF UHPH ANTIMICROBIAL EFFECTS
C. POTENTIAL IMPACT OF UHPH BIOCHEMICAL AND POSSIBLY TOXICOLOGICAL EFFECTS
D. POTENTIAL IMPACT UHPH VEGETABLE BEVERAGES (ALMOND AND SOY MILKS)
E. POTENTIAL IMPACT UHPH EMULSIONS
F. POTENTIAL IMPACT UHPH NANOENCAPSULATION

A. POTENTIAL IMPACT OF THE IMPROVEMENTS ON THE PROTOTYPES

SFP currently trades machines within the EU, primarily UK, France and Germany and globally outside the EU in USA, Far East (Malaysia, Singapore, Taiwan, etc.). The target market areas for the new and improved ranges of high pressure homogenisers will allow SFP to develop improved sales in existing markets in the EU and outside the EU through resale distributor networks. The target is to increase sales by being the leading technology for the new emergent applications of high pressure homogenisation as well as increasing substantially market share against traditional high pressure homogenisation equipment suppliers, with 70% of the current world market being supplied by USA and Canadian based manufacturers. By increasing sales into new applications and increasing general market share SFP will create new jobs within SFP in the EU and amongst our EU suppliers.

UHPH machines will be publicised stressing positive improvements to capital costs, environmental and energy impacts of high pressure homogenisation by the process optimisation will reduce the cost of process implementation making the technology more readily usable by industry, and reduce energy and environmental impacts associated with traditional inefficient high pressure homogenisation processes.

It is important to highlight that the concept of UHP-homogenisers and their adaptations to new valve designs has promoted and agreement between SPF and YPSICON S.L. The later is a Spin-off company of new creation, dedicated to the design, assembly, sale and consultancy of special High Pressure Systems (High Hydrostatic Pressure and UHPH) for industrial use. That youth company will be on charge of scaling up the UHP-homogenisers for food and cosmetic application.

Furthermore, SFP is considering if the new interaction chamber device can be patented. Moreover, the small system developed within the project has been marketed as some cell rupture and small scale homogenisation applications and about 25 systems have already been sold.

EXPLOITABLE RESULTS OBTAINED DURING THE PROJECT: Valves with customised design for food, cosmetic, and biotech applications, with easy removal and putting in place facilities as well as decreasing cost. In order to increase both performance and reliability of UHPH equipments, different configurations on the valve design were tested. Their geometrical changes due to thermal expansion during operation were assessed. New materials were tested and have replaced old materials, e.g. aluminia oxide by zirconia oxide. Concerning seals design, their redesign increased their life considerably. Microbiological studies showed that one of the valve designs resulted in a much higher destruction of spores, thus validating the technical changes and higher UHPH performance.

B. POTENTIAL IMPACT OF UHPH ANTIMICROBIAL EFFECTS
The lethality of various microorganisms, viruses and spores was evaluated using different designs of high-pressure valves and UHPH process

parameters (pressure and product inlet temperature). The results obtained have shown that in some cases, and depending on the food matrix evaluated, could be obtained aseptically packed product, stable for several months at room temperature.

Dissemination:

Publications - Articles:
- Suárez-Jacobo, Á. Gervilla, R.; Guamis, B.; Roig-Sagués, A.X.; and Saldo, J. (2009). Microbial inactivation by ultra high-pressure homogenisation on fresh apple juice. High Pressure Research. (29), 46-51.
- Suarez-Jacobo, A.; Gervilla, R.; Guamis, B.; Roig-Sagues, A.X.; Saldo, J. (2010). Effect of UHPH on indigenous microbiota of apple juice. A preliminary study of microbial shelf life. International Journal of Food Microbiology. (136), 261-267.
- R.M. Velázquez-Estrada, M.M. Hernández-Herrero, T.J. López-Pedemonte, W.J. Briñez-Zambrano, B. Guamis-López, A.X. Roig-Sagués (2011). Inactivation of Listeria monocytogenes and Salmonella enterica serovar Senftenberg 775W inoculated into fruit juice by means of ultra high pressure homogenisation. Food Control. (22), 313-317.
- Suárez-Jacobo, A.; Saldo, J.; Rüfer, C.E.; Guamis, B.; Roig-Sagués, A.X. and Gervilla, R. (2012). Aseptically packaged UHPH-treated apple juice: safety and quality parameters during storage. Journal of Food Engineering. (109), 291-300.

Publications - Abstracts:
- Velazquez-Estrada, R.M.; M.M. Hernandez-Herrero, T.J. López-Pedemonte, B. Guamis and A.X. Roig-Sagués (2009). Inactivation Of Salmonella Enterica Serovar Senftenberg 775w Inoculated Into Fruit Juice By Means Of Ultra High Pressure Homogenisation. Second SAFE Consortium International Congress on Food Safety. Girona, Spain. 27-29 April 2009.
- Velazquez-Estrada, R.M.; C. Torres-Alemán, K. Valero-Leal, M.M. Hernandez-Herrero, B. Guamis and A.X. Roig-Sagués (2009). High hydrostatic pressure inactivation of acid-adapted Listeria monocytogenes and Escherichia coli O157:H7 in orange juice. EFFoST "The European Federation of Food Science & Technology". Budapest, Hungary. 11-13 November.
- Suarez-Jacobo, A.; Gervilla, R.; Guamis, B.; Roig-Sagues, A.X.; Saldo, J. (2010). Shelf-life of ultra-high pressure homogenization (UHPH) treated apple juice; EFFoST annual meeting. Food and health. Dublin, Irlanda. 10-12 November.

C. POTENTIAL IMPACT OF UHPH BIOCHEMICAL AND POSSIBLY TOXICOLOGICAL EFFECTS

Data are valuable information for policy makers. There is strong evidence that UHPH causes no adverse effects to human nutrition. Also the evidently strong effects on macromolecular structures seem not to disturb human nutritional physiology.

Dissemination:

Conferences:
- Butz, P. Sceening assays to investigate quality and safety aspects of food preparations processed by high pressure homogenisation (UHPH). Workshop FUNENTECH. Advances in UHPH Processes, Barcelona, 15 December 2011.

- Briviba, K. In vitro cellular assays to investigate nutritional and safety aspects of food preparations processed by high pressure homogenisation, UHPH. Workshop FUNENTECH. Advances in UHPH Processes, Barcelona, 15 December 2011.

Publications - Abstracts:
- Nanotechnologies in Oncology. 30 October 2010. Cellular uptake and transcellular transport of beneficial food compounds from submicro/nanometer scale formulations. K. Briviba, S. Unser, C.E. Rüfer, P. Butz.

EXPLOITABLE RESULTS OBTAINED DURING THE PROJECT: Preparation of food drinks with high nutritional value and bioactive properties using submicro-encapusulation or emulsification. Submicron emulsions obtained by UHPH did not impair but favoured TC7 metabolic activity. The efficiency of the delivery system was proven by the presence of by-products. The formulation of the emulsion had a clear effect on both speed and residence time of the delivery system. Moreover, UHPH treatment can lead to formation of particles in the nanometer range in which components can be very effectively absorbed. On one hand, the cellular uptake and transport of water soluble compounds, such as soy isoflavones, is not affected by UHPH treatment of soy milk. On the other hand, UHPH of liquid food can modify the bioavailability of poor water soluble compounds, which can result in beneficial or adverse effects depending on the compound with increased bioavailability.

D. POTENTIAL IMPACT UHPH VEGETABLE BEVERAGES (ALMOND AND SOY MILKS)

UHPH has demonstrated its suitability to the production of high quality vegetable beverages, which can be summarized as high hygienic and high physical stability products by enhancing both physicochemical and bacteriological stability of vegetable milks throughout their shelf life. Furthermore, since UHPH improves the bioavailability by enhancing the delivery system of bioactive compounds, NECT is evaluating the enrichment or fortification of their products traditionally manufactured by means of the UHPH technology.

Dissemination:

Conferences:
- Poliseli, F. H., Hernández, M., Guamis, B. and Ferragut, V. (2009). An approach to sterilization of soymilk by ultra high-pressure homogenization. EFFoST "The European Federation of Food Science & Technology". Budapest, Hungary. 11-13 November.
- Ferragut, V.; Hernández-Herrero, M.; Poliseli, F.; Valencia, D.; Guamis, B. (2011). Ultra High Pressure Homogenization (UHPH) treatment of vegetable milks. 11 th International Congress on Engineering of Food. Athens, Greece. May 22-26.
- Valencia, D., Poliseli, F., Hernández-Herrero, M., Guamis, B., Ferragut, V. (2011). Influencia de la adición de lecitina en el tamaño de partícula de licuado de almendra tratado por ultra alta presión de homogeneización (UHPH); VI Congreso Nacional de Ciencia y Tecnología de los Alimentos. Valencia, Spain. 8-10 June.
- Poliseli, F.; Valencia, D.; Hernández-Herrero, M.; Guamis, B.; Ferragut, V. (2011). Shelf life of refrigerated Ultra High Pressure Homogenizad-treated vegetable beverages. 2nd International ISEKY_Food Conference. Bridging Training and Research for Industry and the Wider Community. Milan, Italy. 31 August-3 September.

Publications - Abstracts:
- Poliseli, F. H., Hernández, M., Guamis, B. and Ferragut, V. (2009). An approach to sterilization of soymilk by ultra high-pressure homogenization. EFFoST "The European Federation of Food Science & Technology". Budapest, Hungary. 11-13 November.
- Ferragut, V.; Hernández-Herrero, M.; Poliseli, F.; Valencia, D.; Guamis, B. (2011). Ultra High Pressure Homogenization (UHPH) treatment of vegetable milks. 11 th International Congress on Engineering of Food. Athens, Greece. May 22-26.
- Poliseli, F.; Valencia, D.; Hernández-Herrero, M.; Guamis, B.; Ferragut, V. (2011). Shelf life of refrigerated Ultra High Pressure Homogenizad-treated vegetable beverages. 2nd International ISEKY_Food Conference. Bridging Training and Research for Industry and the Wider Community. Milan, Italy. 31 August-3 September.

EXPLOITABLE RESULTS OBTAINED DURING THE PROJECT: Formulation improvement of beverages with high-nutrionally added-value targeted to specific consumer needs and improved stability at ambient or cold temperature or low levels of preservative agents or reduced potential allergenicity. UHPH treatment exerts sporicidal effects at inlet temperatures of 75 °C and specially 85 °C, when the maximal temperature achieved after valve was around 136 °C with a retention time of less than0.3 s, resulting in a minimal heat damage, respect to the usual UHT treatments applied in food processing.

In the case of almond milk, UHPH treatments were effective in reducing completely the counts of Enterobacteriaceae, Micrococaceaes and molds & yeast. However, with soy milk, UHPH at 200 MPa resulted in growth of Bacillus cereus.

UHPH treated almond milk samples showed higher stability than conventional treatments such as pasteurization and ultra-high temperature treatment by inhibiting both creaming and spontaneous sedimentation.

E. POTENTIAL IMPACT UHPH EMULSIONS

Results indicated that monomodal emulsions can be obtained by UHPH up to 200 MPa using the model emulsions formulated by NAHO. UHPH-processed emulsions displayed droplet sizes well under the micron and an excellent stability against creaming and coalescence during storage for 2-3 weeks at 50°C which is equivalent to ~ 2 years storage at ambient temperature for cosmetics. Such emulsions remained fluid. However, spray-drying of submicron emulsions induced an increase of the droplet sizes after emulsion rehydration with deionised water. Thus, further studies are necessary to improve drying by modifying the formulation.

The results obtained on double W/O/W emulsions allowed the optimisation of the emulsified systems and confirmed the feasibility of double emulsions for cosmetic issues.

Addition of starch or protein in the aqueous phase allowed obtaining nice double globules of higher size (up to 60 µm) in diameter at atmospheric pressure. The viscosity of the emulsion was found to be crucial in order to resist the elongational forces in the HP-valve; high viscosities of the initial fluid developed too high elongational forces in the HP-valve and destroyed initial globules into smaller ones.

The final objective for NAHO is to continue to innovate. NAHO Cosmetics is thinking for a patent to be taken for emulsions prepared by high-pressure homogenisation and emulsions processed by UHPH. At the moment, several ways are studied, with an economical feasibility approach too.

Dissemination:

Conferences:

- E. Hebishy, M. Buffa, V. Ferragut, B. Guamis and A.J. Trujillo (2011). Physical and oxidative stability of whey protein oil-in-water emulsions stabilized by ultra high pressure homogenization: effect of pressure and protein concentration on emulsion characteristics. 2nd international ISEKI_Food conference. Milan, Italia. August 31- 2 September.
- E. Hebishy, M. Buffa, B. Guamis and A.J. Trujillo (2011). Obtención de emulsiones submicrónicas con aceites vegetales por ultra alta presión homogenizacion; Congreso Nacional de Ciencia y Tecnología de los Alimentos. Valencia, Spain. 8-10 June.
- E. Hebishy, M. Buffa, B. Guamis and A.J. Trujillo (2011). Obtención de emulsiones submicrónicas con aceites vegetales por ultra alta presión homogenización. Escuela de altas presiones. Tenerife. Spain. 27 June-1 July.
- Dumay, E. (2010). Potentially of dynamic high-pressure for manufacture of submicron emulsions. Workshop AgroParisTech, Massy: Novel Structures & Delivery Systems. Paris, 28-29 October 2010.
- Dumay, E. (2011). Use of biological tools to investigate effects of high-pressure treatments on food components. EHPRG 49th. Budapest, 28 August-2 September 2011.
- Dumay, E. (2011). Homogenization. EFFoST 2011. Berlin, 9-11 November 2011.
- E. Hebishy, M. Buffa, B. Guamis and A.J. Trujillo. Effects of protein concentration and oil volume fraction on the physico-chemical stability of whey protein oil-in-water emulsions stabilized by ultra high pressure homogenization. 2011 EFFoST Annual Meeting. Berlin, 9-11 November 2011.
- Hebishy, E. Effects of protein concentration and oil volume fraction on the physico-chemical stability of whey protein oil-in-water emulsions stabilized by ultra high pressure homogenization. Workshop FUNENTECH. Advances in UHPH Processes, Barcelona, 15 December 2011.

Publications - Abstracts:

- Submicron emulsions processed by ultra-high pressure homogenisation. (2009). D. Chevalier-Lucia, M. Cortés-Muñoz and E. Dumay. Book of abstracts. XLVII EHPRG Meeting. Paris, France. 6-11 September.
- Dumay, E. (2010). Potentially of dynamic high-pressure for manufacture of submicron emulsions. Workshop AgroParisTech, Massy: Novel Structures & Delivery Systems. Book of abstracts. Paris, 28-29 October 2010.
- Dumay, E. (2011). Use of biological tools to investigate effects of high-pressure treatments on food components. Book of abstracts. EHPRG 49th. Budapest, 28 August-2 September 2011.
- Dumay, E. (2011). Homogenization. Book of abstracts. EFFoST 2011. Berlin, 9-11 November 2011.
- E. Hebishy, M. Buffa, B. Guamis and A.J. Trujillo (2011). Effects of protein concentration and oil volume fraction on the physico-chemical stability of whey protein oil-in-water emulsions stabilized by ultra high pressure homogenization. Book of abstracts. 2011 EFFoST Annual Meeting. Berlin, Alemania, 9-11 November.
- Cell cultures as a biological tool to study effects of high-pressure processing on food components (2009). Benzaria, M. René-Trouillefou, B.

Caporiccio and E. Dumay. Book of abstracts. XLVII EHPRG Meeting. Paris, France. 6-11 September.
- Potential applications of Ultra-High Pressure Homogenisation. A. Gracià-Julià, D. Chevalier-Lucia, L. Picart-Palmade, A. Benzaria and E. Dumay. Book of abstracts. 48th EHPRG International Conference, Uppsala (Sweden), 25-29 July 2010.

EXPLOITABLE RESULTS OBTAINED DURING THE PROJECT: Preparation and characterisation of O/W nanoemulsions by UHPH for cosmetic formulations. Feasibility of emulsions dried by spray-drying. Monomodal emulsions can be obtained by UHPH up to 200 MPa using the model emulsions formulated by NAHO. UHPH-processed emulsions displayed droplet sizes well under the micron and an excellent stability against creaming and coalescence. Spray-drying of submicron emulsions unfortunately induced an increase of the droplet sizes after emulsion rehydration with deionised water. Thus, further studies are necessary to improve drying by modifying the formulation.

EXPLOITABLE RESULTS OBTAINED DURING THE PROJECT: Optimisation of emulsified systems and feasibility of double emulsions for cosmetic applications. UHPH double emulsions for cosmetic issues. The results obtained on double W/O/W emulsions allowed the optimisation of the emulsified systems and confirmed the feasibility of double emulsions for cosmetic issues. The double W/O/W emulsions resisted quite well to the elongational forces in the HP-valve of the homogeniser. Addition of starch or protein in the aqueous phase allowed obtaining nice double globules of higher size (up to 60 µm) in diameter at atmospheric pressure. The viscosity of the emulsion was found to be crucial in order to resist the elongational forces in the HP-valve.

F. POTENTIAL IMPACT UHPH NANOENCAPSULATION

The purpose of the ABB research was orientated to solve their needing for new protective devices and target delivery systems, designing safe delivery systems that can be incorporated into food products (nano-emulsions, encapsulated nano-particles). Vitamins A, D, E, K and carotenoids are liposoluble compounds which are naturally present in food. However, their poor water solubility has made their use problematic for food formulations. Nanotechnology provides a good opportunity to improve the solubility of such active ingredients and to increase bioavailability. Nanoencapsulation is defined as a technology to pack substances in miniature and refers to bioactive packing at the nanoscale. Nanoencapsulation could be efficient for the development of functional products and may help to solve difficulties such as loss of functionality during processing or in storage, incompatibilities between core and shell materials, generation of bad odors and flavors, deterioration of texture, and in the case of enzymes, lose activity, etc.

FUNENTECH project allow the creation of high added value foods, and with a certain economical benefit but also taking into consideration food safety and nutritional aspects. The main economic impacts are likely to take part to the smart food sectors; healthy and natural food sectors; specialized diet and nutrition sectors; foods for hospitals rehabilitation and elderly centers; design for particular countries or ethnics with nutritional deficiencies or intolerances.

Conferences:

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- Escriu, R. Encapsulation by UHPH. Workshop FUNENTECH. Advances in UHPH Processes, Barcelona, 15 December 2011.

EXPLOITABLE RESULTS OBTAINED DURING THE PROJECT: Encapsulation of biomolecules or bioactive compounds as food additives. ?-carotene was used as a model for evaluating the feasibility of biomolecules encapsulation by means of UHPH technology. It must be emphasized that at the moment, there is no direct technique able to confirm the encapsulation of a compound. UHPH has proven to increase the stability of nanoemulsions by reducing extremely the size of the particles. Differences between electrodensity could confirm the possible nanoencapsulation of this component.

List of Websites:

http://grupsderecerca.uab.cat/FUNENTECH