FP6 Co-operative Research Projects

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UHPH

Development and Optimisation of a Continuous Ultra High Pressure Homogeniser for Application on Milks and Vegetable Milks

CRAFT

Final report

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MACHINE BUILDING AND TESTING

During the first period SFP have liaised with UAB, UCC and UMII to ensure equipment in the project maintained operation. Implementation of improved valve materials and design has been made to the systems at UMII and UCC (the pressure control of the homogeniser was reconditioned for an easier utilization: the hydraulic actuator already in place on the equipment was changed to a pneumatic one) and inter-stage cooling system added to UCC system.

At the first project meetings, RTD partners suggested several improvements to the design of 90 L/h units and to the benchtop sized unit (8 L/h). SFP developed a single intensifier model bench top machine as a proof of concept and then developed a twin intensifier model (BTH) with many additional features to optimise the ease of operation and functionality. The system incorporates a flow through high pressure transducer which can be flushed and has non dead legs. They also incorporate a new ranged of pilot air operated inlet and outlet control valves which are closed couple to the intensifier system. New two stage homogenising valve block has temperature control options. A new variable timer based intensifier overlap synchronisation software system has been developed, allowing operator simple control over the pressure balance and the ability to unbalance the system if required. New pressurised feed systems and feed pump systems have been developed for use with the systems to help with working with food products which can otherwise be difficult to pump. There has also been a practical commercial ‘spin off’, being the model developed in its own right for applications such as cell rupture and pharmaceutical emulsion systems where it is expected to find a market, offering superior performance to known established systems. On February 2006 the benchtop unit was delivered to the UAB. It was tested and conditioned in order to work in microbiology studies. On July 2006 it was sent to UMII where it was used to perform emulsion studies.

During the first period, SFP worked on the design and building of the new 90 L/h - 400 MPa homogeniser system which was delivered and installed at the UAB by the end of the second period. Improvements to the opening and closing system have been implemented for this system, to provide more reliable operation. Improvements have been made to the overlap timing operation to provide more reliable synchronisation of intensifier handover by user of modified software. Ceramic rams have been developed and tested on the new 90 L/hr system. These are expected to provide improved wear resistance and seals life. Material changes and geometry changes have been made to the seal gland housings to improve the reliability of these components. A new valve seat has been developed and implemented for evaluation in tests. Initial reports are that the performance of this valve seat is an improvement. An external inter-stage heat exchanger has been introduced. Additional thermocouples have been introduced into the product flow path so that the systems can be more fully instrumented. Operation and maintenance manuals for both benchtop and 90 L/h machines have been drafted.

Figure 1. UHPH 90 L/h prototype at the UAB.

Figure 2. UPHP Twin 8 L/h bench-top prototype.

MACHINE OPTIMISATION FOR MILK

In order to achieve the objectives of this workpackage as well as to assess equivalence among devices, SFP, UAB, UCC and UM II have adapted the different UHPH equipments. They all comprehend heating/cooling devices, thermocouples and pressure probes located at key control points.

UM II (FPG7400H, Stansted Fluid Power Ltd):

The pressure control of the homogeniser was reconditioned by SFP for an easier utilization: the hydraulic actuator already in place on the equipment was changed to a pneumatic one. The pneumatic actuator was easier to work compared to the previous hydraulic one, although the pressure was less stable. In this prototype, one operating cycle (20 s) comprises a first step of 8-9 seconds corresponding to filling of the single intensifier, and a second step of 11 seconds corresponding to pressure build-up followed by the intensifier discharge. The pulsed flow rate ranges between 16.0 l/h at 100 MPa and 13.6 l/h at 300 MPa. It has been equipped with T thermocouples and high pressure gauges, so that fluid temperature and pressure were measured at different positions and times during processing to follow the processing history of the fluid. Cooling devices with circulating water at 10°C were used to avoid fluid overheating during processing, at different places of the homogeniser: around the intensifier, at the immediate outlet of the HP valve and after the low pressure valve (LP).

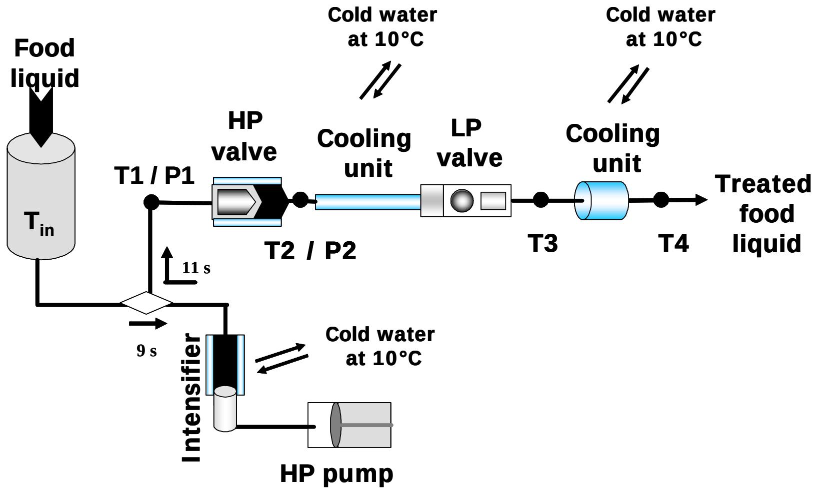

Figure 3. Scheme of the UHPH prototype from Montpellier.

Thanks to the T thermocouples installed on the HP homogeniser, the increase in the fluid temperature with the homogenisation pressure was precisely determined and the efficiency of the cooling device installed immediately after the HP valve was proved. T1, T2, T3, T4 P1 and P2 indicate the points where pressures and temperatures can be measured

<u>UAB (FPG11300, Stansted Fluid Power Ltd):</u>

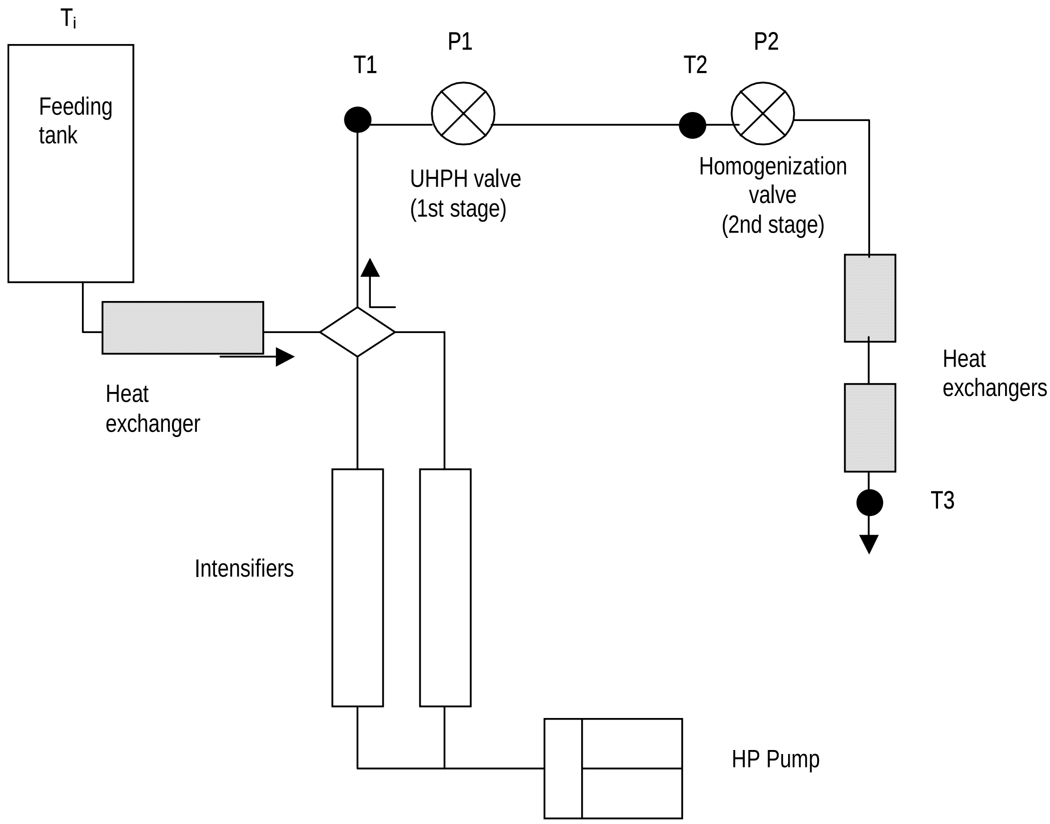

Figure 4. Schematic representation of ultra-high pressure homogenizer.

Significant time and efforts have been invested to install the first 90L/h UHPH equipment at the UAB. Heat holding sections were installed. They can be connected to the UHPH pilot unit by easy-to-handle hose connections, depending on the needs of each specific application. Also the possibility of selecting different residence time of milks and other fluids at different temperatures after the high pressure valves was considered and a device installed for this purpose. Sanitary connections between UHPH equipment, heating/cooling devices, an aseptic tank and an aseptic processed packaging unit have already been installed. T1, T2, T3, P1 and P2 indicate the points where pressures and temperatures can be measured.

In addition several experiments were carried out to adjust the conditions of cleaning and disinfection in the UHPH machine. A specific sanitation programme developed for the UHPH machine was applied each time before and immediately after submitting the milk samples to UHPH treatment. A mixture of peracetic acid and hydrogen peroxide was used as a disinfectant agent, whose bactericidal efficacy was previously evaluated using a quantitative test of suspension.

UCC (nm-GEN' 7400H, Stansted Fluid Power Ltd):

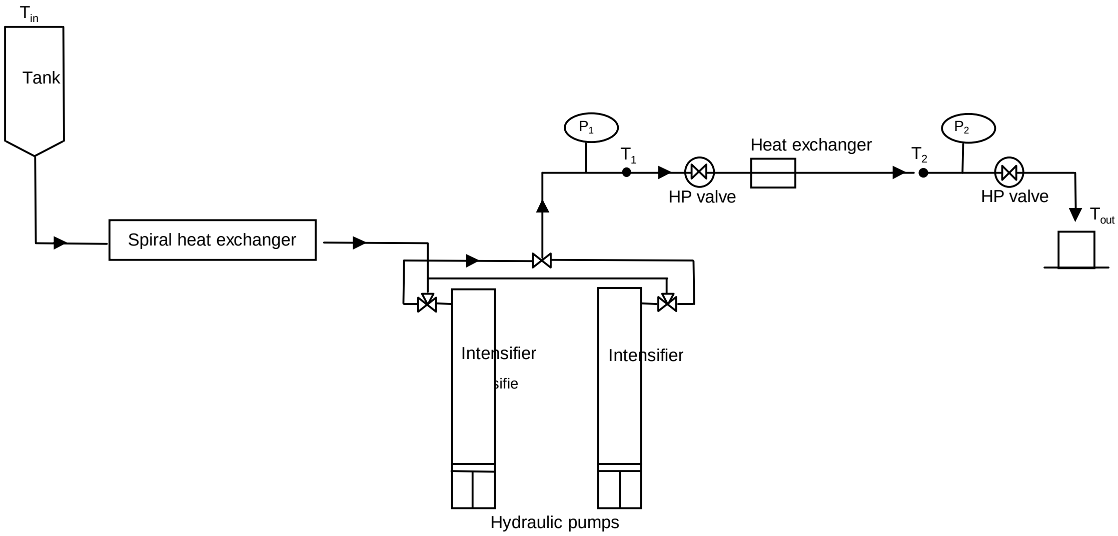

Figure 5. Flow diagram of UCC UHPH homogeniser.

This is a twin-intensifier, two-stage 12 L/h high pressure homogeniser. T1, T2, P1 and P2 indicate the points at which pressure (P) and temperature (T) can be monitored. This unit was also updated at the beginning of the project. In addition, high efficiency refrigerating devices were installed

Benchtop unit (FPG 12600.2, Stansted Fluid Power Ltd) at UMII:
This prototype 8L/h capacity was shared among partners of the project. The UAB has received this unit on February 2006 and conditioned it in order to work with milk samples inoculated with different microorganisms. It was placed inside a Class II bio-safety laboratory. UMII received the machine on July 2006 and used it in order to study the effects of UHPH on emulsion systems. A complete disinfection and sanitising procedure was performed before sending the UHPH unit to UMII on July

2006. This Benchtop UHPH-Prototype developed by Stansted for the project was studied at University Montpellier II for the period of September 2006 to January 2007. Whey protein dispersions and emulsions were processed using the Benchtop prototype, and compared to the FPG7400H homogeniser. The Benchtop prototype comprised a high pressure valve (HP valve; first stage) with ceramic needle and seat (400 MPa maximum) and a low pressure valve (LP valve; second stage) with a steel ball and a ceramic seat (40 MPa maximum). The LP valve (second stage) was located immediately after the HP valve (first stage) without cooling device between the two stages. Needle and seat located in the HP valve were of a different design in the Benchtop prototype compared to the UMII homogeniser. At UMII, The distribution of residence times (RTD) in the whole Benchtop prototype has been determined at 200 MPa (P1) with the HP valve operating only (one-stage, single-pass) for 2 independent essays of homogenisation using bromophenol blue as a tracer during an emulsion production (emulsion containing, w/w, 4.3% protein and 15% peanut oil), and measuring the recovery of the dye at the homogeniser outlet.

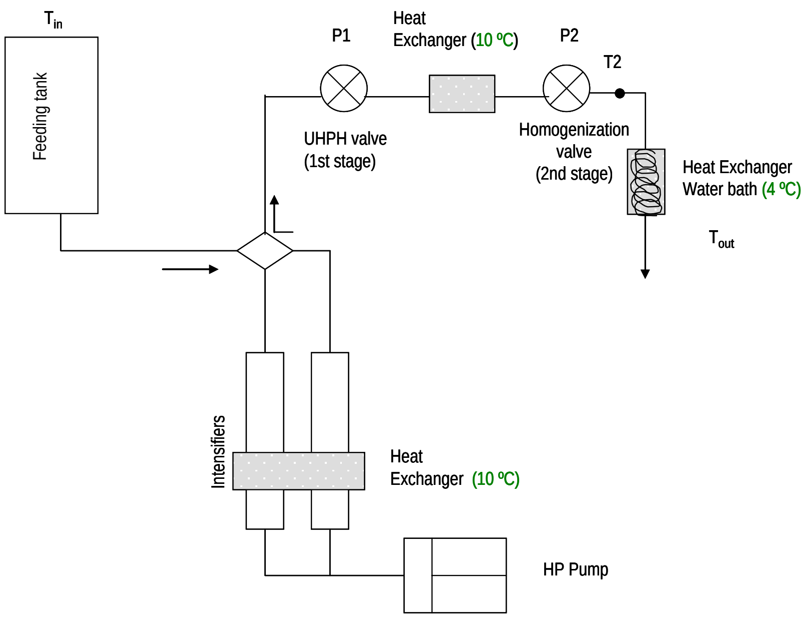

Figure 6. Flow diagram of benchtop homogeniser.

UAB (FPG11300, Stansted Fluid Power Ltd):

Following delivery, SFP and UAB teams were in charge of testing and adapting the connections of this new system. Like the former unit, this machine was connected to feeding tank system and to heat exchangers able to warm up or refrigerate the fluid that needs to be processed and the fluid which was already processed. The UHPH unit was also connected to the aseptic tank and the aseptic packaging machine. It was elected to add additional temperature monitoring equipment and the two teams integrated two additional temperature monitoring thermocouples. With pre-existing and additional thermocouples installed temperature readings and logging is now possible at the following locations in the UHPH unit: before the high pressure valve; after the high pressure valve; after the refrigerating system; after the second valve; before the inlet of the aseptic tank.

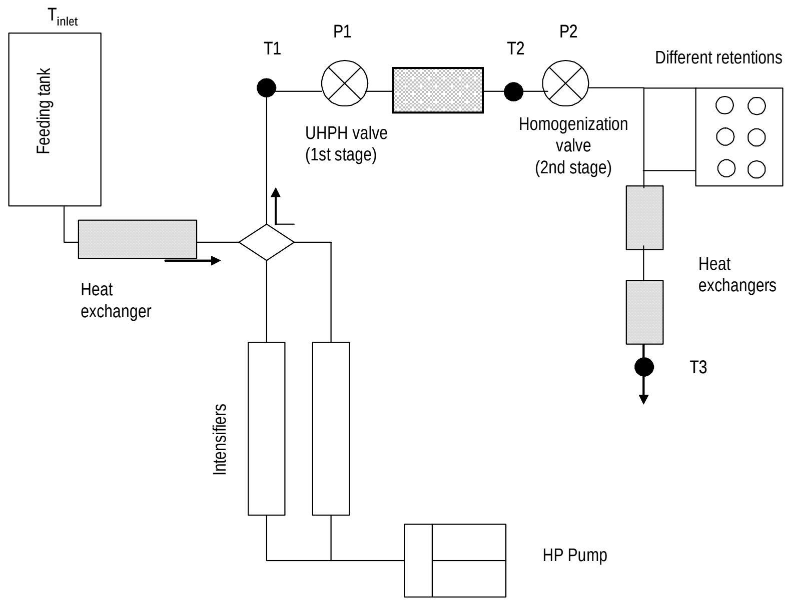

Figure 7. Schematic representation of ultra-high pressure homogenizer (model FPG11300, Stansted Fluid Power Ltd) at UAB.

The mean fluid flow of this machine without pressure is 120 L/h; nevertheless, when the pressure is increased up to 350 MPa, a flow reduction inferior to 20% might be encountered. The residence time of the fluids was assessed. This machine comprises also a device able to increase the residence time of the treated fluid at the temperatures reached after the high pressure valve. The different holding time possibilities are 2, 4, 6, 15, 30 and 45 s before entering in the cooling exchanges.

To allow complete cleaning and disinfection of the whole circuit, flexible connection pipes have been used to connect the UHPH unit. The hose that connects the feeding tank with the inlet of the unit is made of silicone and steel, whereas those pipes associated with the connection of the UHPH with the inter-stage cooling system, holding time tubes and outlet to the aseptic tank are made of coiled steel tube. The coiled steel was used to give flexibility to the system connections so that the essential features of the easy opening and closing for maintenance and cleaning are not lost. Thus they permit easy opening and closure of the unit in order to clean and refurbish the intensifiers without damaging the UHPH components. It is also possible to use steam to finish disinfection procedures by connecting it to the connection already present in every intensifier. Cleaning and disinfection protocol was reviewed.

EFFECT ON MICROORGANISMS AND PHYSICOCHEMICAL CHARACTERISTICS OF MILK

Effects on micro-organisms in milk

Effect of UHPH on pathogen indicators using UHPH homogeniser model DRG FPG7400H:350 (Stansted Fluid Power Ltd., Essex, UK). The UAB evaluated the use of UHPH in inactivating different strains of pathogen or pathogen surrogates of interest in milk and milk products, such as *Escherichia coli* (strains ATCC 10536 and CCUG 44857 *(*O157:H7), *Listeria innocua (*ATCC 33090), *Staphylococcus aureus* (ATCC 13565) and *Staphylococcus carnosus* (CECT 4491). Initial concentration of cells in inoculated UHT milk was approximately 7.0 log cfu/ml^{-}. These experiments were performed inside a Class III biosafety laboratory. The type of milk significantly influenced ($P < 0.05$) the degree of inactivation reached in both strains of *E. coli,* being higher in whole milk. The level of inactivation was similar for *E. coli* ATCC 10536 and *E. coli* O157:H7 CCUG 44857, reaching lethality values of 4.30 and 3.94 $\log_{10}$ cfu mL^{-1} respectively, at an inlet temperature of 20 ºC. No sublethal injuries were detected after treatments. Slight decreases of viable counts during storage at 4 ºC were observed. Inactivation of *Listeria innocua* was higher at 20 ºC inlet temperature causing few or no sublethal injuries in *L. innocua*. During storage at 4 ºC after treatments, counts increased by approximately two logarithmic units from days 0 to 9 in whole milk. For *S. aureus* and *S. carnosus* the strain influenced significantly ($P <$ 0.05) the lethality level, which was higher for *S. aureus* in whole milk. The level of inactivation was similar between mediums and no sublethal injuries were detected after treatments. At low inlet temperatures viable counts showed greater resistance.

UHPH inactivation of pathogenic and spoilage microorganisms using UCC unit. Active cells (10^7-10^8 CFU mL^{-1}) from bacterial strains *Stapylococcus carnosus* ATCC 51365 and *Pseudomonas fluorescens*, suspended separately in reconstituted sterilised skim milk were homogenised at pressures of 100, 150, 200 and 250 MPa and at an inlet temperature of 30°C. These homogenised suspensions were then plated both on nutritive media and on nutritive media supplemented with NaCl (6%). UHPH homogenization in the pressure range of 100 and 150 MPa had a significant effect on inactivation of *Pseudomonas fluorescens.* However, further increasing the process pressure up to 200 MPa did not cause additional microbial inactivation. The storage of homogenized samples at 4˚C for 7 days resulted in a complete recovery of *Pseudomonas fluorescens*. This indicates the existence of bacteria sub-lethally injured by UHPH treatment and thus able of recovering in the relatively nutritionally-rich medium of milk. The inactivation of *Stapylococcus carnosus* ATCC 51365 was also studied in reconstituted skim milk, and shown to increase with increasing homogenization pressure from 100 to 250 MPa; at 250 MPa complete inactivation was obtained.

UHPH inactivation of pathogenic and spoilage microorganisms in milk using the benchtop unit. The studies performed with the benchtop comprised *Listeria monocytogenes* and *Mycobacterium smegmantis* (intending to be a surrogate for *Mycobacterium avium* subsp. *paratuberculosis;* in skim and 3.5% fat). The machine was refrigerated using heat-exchangers with water at 10 ºC between valves and 4 ºC after the outlet. Direct epifluorescent micrographs and transmission electronic micrographies provided further information. For *Listeria monocytogenes***:** cells were suspended in milk at two different loads of approximately 3 and 6.5 log cfu/mL. Strain used was 15520 from Culture Collection of Gotenberg University. *L. monocytogenes* cells were inoculated into 0.3, 3.5, 10 & 15% fat milk and the evolution of the counts during storage at 6 ºC was assessed.

<u>Effects of UHPH on natural-occurring micro-organisms in bovine milk:</u> At the UAB, milk obtained from a local farm was deliberately deficiently managed in order to obtain high microbial counts. Ultra-high pressure homogenization of raw milk was performed with a Stansted high-pressure homogeniser (90 L/h, model FPG11300, Stansted Fluid Power Ltd., Essex, UK). Milk was UHPH-treated under the following conditions: 100, 200 and 300 MPa (single-stage) with inlet temperature of 40ºC and at 200 and 300 MPa (single-stage) with Ti = 30ºC. To obtain high-pasteurized milk (PA), raw milk was subjected to a two-stage homogenization (18 MPa plus 2 MPa) and subsequently heat pasteurized by using a heat exchanger at 90ºC for 15 s. Microbial and physico-chemical analysis of treated samples were conducted after treatment (day 0) and at 7, 14, 18 and 21 days of storage. The microbiological quality of the different milks, including raw milk, was assessed by enumerating the following micro-organisms: total bacteria counts, psychrotrophic bacteria, coliforms, *Lactobacilli*, *Lactococci*, *Enterococci*, *Pseudomonas* spp., total spore count, search for *L. monocytogenes* and *Salmonella* spp. Microbiology results concerning UHPH treated bovine milks and comparison with raw and pasteurized milk were displayed (total counts, psycrhotrophs, *Enterococci, Lactococci, Lactobacilli*, coliforms) including the evolution of the counts in a three-week storage period at 4ºC. Total bacteria counts were reduced to 1.5 log cfu/mL, similar to a 90 ºC, 15 s pasteurisation treatment. The evolution of the counts was also similar to those of pasteurised milk up to 14-days. The same behaviour was observed for psychrotrophic bacteria. *Lactococci* were able to recover more rapidly in UHPH treated samples than in pasteurised ones. The major drawback of UHPH treatments seems to be spore counts in milk which suffered a clear rise after 14 days.

Effect on the physicochemical characteristics of cow's milk

At the UAB, UHPH treatments were mainly performed with the 90 L/h UHPH machine. Inlet temperatures were 30 and 40ºC. Experiments with only one high pressure valve active used: 100, 200 and 300 MPa. When the second valve was used 30 MPa was the pressure selected, and the treatments are referred to as 130, 230 and 330 MPa, respectively. Different physicochemical analyses were performed: particle size, pH, acidity, viscosity and microstructure.

<u>Particle size assessment.</u> The effect of UHPH on reduction in particle size in whole raw bovine milk was studied by the UAB and the UCC. Raw whole milk was compared to traditionally homogenized milk. A Beckman Coulter laser diffraction particle size analyzer (LS 13 320 series, Beckman Coulter, Fullerton CA, USA) was used by UAB while a Malvern Zetasiser instrument (Malvern Instruments S.A.) was used by UCC and UM II.

In UAB studies, size distribution changed markedly for all UHPH treatments. Increasing the pressure significantly decreased $D_v0.5$ except for two-stage homogenised samples treated at 230 and 330. Considering $D_v0.9$ values, two-stage homogenisation did not seem to affect except for the treatment at 330 MPa, which was in fact significantly higher than the one of raw milk. The size distribution of samples treated at 330 MPa showed a second smaller peak. This phenomenon could be explained by the formation of aggregates produced by the interaction of small fat globules and whey proteins. In UCC studies, UHPH treatment resulted in a much narrower fat globule size distribution and smaller mean globule size than conventional homogenisation, and there was little difference between the effects of the three pressures studied. This may reflect a limit to the fat globule size that can be achieved, either due to the geometry of the homogeniser, or to the amount of surface-active (emulsifying) material present in milk. The distribution width, a parameter characterizing polidispersity, was however, found to be lower at higher pressures.

pH and acidity: The pH of milk samples was monitored during storage using a pHmeter and total acidity was determined by titration with 0.1 N NaOH. Only UHPH treatments at 100 and 130 MPa showed different pH, lower than the other treatments and control milk. This behaviour coincided with the higher acidity of the UHPH treatments at 100 and 130 MPa as a consequence of higher content of free fatty acids (data not shown. Immediately after treatment, small but significant differences were detected in the pH value of milk treated at 200 MPa 30ºC (~ 6.72) in comparison to raw milk and to the other treated milks (~6.74). For each day of sampling, the pH of milks treated at 200 MPa 30ºC was always below the values of the other milks. The reduction in pH was accompanied by an increase in acidity.

Viscosity: Viscosity of milk samples was measured in duplicate at 20ºC with a rotational rheometer (Haake Rheo Stress 1, Thermo Electron Corporation, Karlsruhe, Germany)**.** Viscosity values measured after treatment of milks treated at 200 MPa were significantly different from those of raw, PA, and UHPH milks treated at 300 MPa. At each sampling time during the storage study, milks treated at 200 MPa always had the lowest viscosity value. The formation of these clusters could explain the higher viscosity values from milks treated at 300 MPa.

Lactoperoxidase and alkaline phosphatase determination: Lactoperoxidase (LP) activity was determined spectrophotometrically at 413 nm and 20ºC using 1 mM ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid. Alkaline phosphatase (ALP) activity was determined spectrophotometrically at 400 nm using *p*-nitrophenyl di-sodiumphosphate (15.8 mM in 0.9 M 2-amino-2-methyl-1-propanol buffer at pH 10.45) and 37ºC . Enzyme units were expressed in units/ml, where one unit (U) is defined as the amount of enzyme that catalyzes the production of 1 µmol of product/min Heat treatment performed at 90ºC for 15 s inactivated completely the enzyme as occurs with high-pasteurized milks. Temperature achieved during UHPH treatments at 300 MPa also produced total enzyme inactivation. Milk treated at 200 MPa 40ºC had a residual activity less than 1%; however, only milk treated at 200 MPa 30ºC showed significant differences to the other UHPH milk samples. This treatment was able to reduce LP, but a residual activity of 35% was maintained. Both, heat and UHPH treatments reached a complete inactivation of phosphatase.

Colour: Significant differences ($P < 0.05$) in L* values were observed between raw, PA and UHPH milks at day 0. Pasteurized milk was whiter than UHPH milks and raw milk; nevertheless, differences in instrumental colour measurements were not visually obvious. Treated milks were whiter than raw milk probably due to the increase of fat globules which diffract light more efficiently. On the other hand, differences between UHPH treated milks and conventional homogenized milk could be related to the state of the casein micelles. During storage, PA milk always had a higher L* value compared to UHPH milks. Over time, the lightness (L*) of all milks did not show a defined tendency but in general an increase in this parameter was observed between day 0 and day 21. The a* value remained largely unchanged during storage, while b* showed a tendency to a lineal increase.

SET YOGHURT

Several experiments were carried out at the UAB food plant in order to study the acid coagulation process of milk and most relevant functional properties of yoghurts. The manufacture of these yoghurts was previously standardised at RINY facilities and the UAB respected the procedure. Also once optimum UHPH processing conditions were selected, they were validated at RINY facilities. UHPH treatments were initially performed at 100, 200, 300 MPa with bovine whole milk. The influence of the second stage at 30 MPa (120, 230, and 330 samples) was also evaluated. Inlet temperatures of milk were fixed at 30 and 40ºC. Controls were: conventionally homogenised and pasteurised milk (P) and the same fortified with 3% (w/w) skim milk powder (SMP). Coagulation took place at 43ºC. The starter used was a mixture of *Streptococcus termophilus* and *Lactobacillus delbruekii* sbsp. *bulgaricus*. The onset of gelation increased in all UHPH treated milks compared to controls (P and PSM), being also higher for inlet temperatures of 40ºC. However, the total times needed to get pH 4,6 (final point), were shorter than in Control samples (except for 100/130 MPa samples). Therefore, UHPH increased the rate of aggregation. Yoghurt firmness at pH 4,6 increased from 100 to 300 MPa but the use of the second 30 MPa stage caused a slight decrease of firmness. After 24 h of refrigeration, forced syneresis by centrifugation and puncture tests were performed to set yoghurts to estimate their water retention capacity and gel strength. Treatments of 100 and 130 MPa at the two temperatures tested did not improve these characteristics of yoghurts compared to yoghurts made from pasteurised milk. On the contrary, UHPH treatments of 200/230 MPa showed approximately 10% less whey expulsion. Mean particle size decrease occurred as expected, without noticing differences between both inlet temperatures. Cluster formation was observed at higher pressures than 200 MPa. Milk treated at 200 or 300 MPa showed higher gel strengths on their coagulation, higher gel firmness in texture analysis, less syneresis and lower titrable acidity compared to conventionally-treated milk fortified with 3% SMP. In terms of all parameters analysed, yoghurts prepared from UHPH-treated milk over 200 MPa presented better quality attributes (less syneresis and higher gel firmness) than yoghurts prepared from heat-treated milk supplemented with 3% of SMP. The use of the second stage generated some clusters that might have hindered aggregation rate during coagulation process and reduced gel strength. The two inlet temperatures did not alter the grouping of the different treatments for any of the parameters analysed, so in order to minimize thermal effects during the UHPH treatment, 30ºC is likely to be the most appropriate inlet temperature.

The effects of ultra-high pressure homogenization (UHPH) on skim milk destined to yoghurt production were also investigated. UHPH-treated milk was compared to thermally treated milk as described for whole milk experiments. Results of the present study showed that UHPH (inlet temperatures 30 ºC) at two stages caused formation of clusters (related to the passage/impact through the second valve at high velocity), increased viscosity and changed colour due to casein disintegration. Decrease in total nitrogen in whey and whey proteins denaturation was observed in UHPH-treated samples (β-lg more denaturated than α-la) but to a lesser extent than in thermally processed samples. Nevertheless, results involving coagulation properties and yoghurt characteristics reflected that milk subjected to heat-treatment, especially when enriched with SMP, was better in terms of obtaining good quality skim milk yoghurts (higher gel strength, increased water retention, higher firmness) compared to all UHPH conditions assayed.

To study the stability and the evolution of yoghurts from whole UHPH-treated milk, yoghurts were prepared from milks treated at 200 and 300 MPa at 30 ºC. These conditions were chosen considering the results obtained in the previous studies of acid coagulation and functional properties of UHPH-treated milks. Yoghurts prepared from heat-treated milk at 90 ºC, 90s, homogenised at 15 MPa and enriched with 3% of skim milk powder were used as controls. In order to follow the evolution during storage at refrigerating conditions (4 - 6 ºC) the analysis were carried-out weekly (days 1, 7, 14, 21 and 28). This studies included microbiological analysis, determination of titrable acidity, determination of syneresis (spontaneous and by centrifugation) and textural and rheological evaluation. *S. termophilus* counts were very similar between the treatments studied, and cell loads were almost constant during the storage while *L. bulgarcius* counts decreased at the end of the shelf-life. Yoghurts prepared from heat-treated milk had higher titrable acidity values than yoghurts prepared from both UHPH treatments at 200 and 300 MPa (probably due to increased lactose content from SMP). However, the amount of lactic acid/100 g of milk was almost constant along the storage in all samples. Either spontaneous or syneresis forced by centrifugation studies reflected that UHPH yoghurts gave better results, especially those treated at 300 MPa, than yoghurts from heat-treated milk enriched with SMP. Firmness values were almost constant during storage in all treatments, but yoghurts prepared from UHPH-treated milk at 300 MPa were the firmest during storage. Rheological measurements showed that G' and the yield point increased until day 14 of storage, and were similar between the treatments.

CHEESE

In the first stages of workpackage 6, UAB and UCC assessed rennet coagulation properties of bovine milk from selected UHPH treatments: rennet coagulation time (RCT), rate of curd firming (RCF) and curd firmness (CF). Also cheese yield was estimated by centrifugation after milk coagulation.
Afterwards, several cheese productions were made from UHPH treated milk by different partners (UAB, UCC, MGLA, KOLL and RINY), studying the quality of these preliminary cheese productions. In the second period rennet coagulation studies

were finished and different rheological, textural and physicochemical properties of the cheese produced were assessed.

Effects of UHPH treatment on rennet coagulation properties:
UCC evaluated coagulating properties by observation of the visual coagulation time in a rocking water bath (the classical Berridge method) and by dynamic rheological measurements (using a Bohlin rheomoeter); rate of gel formation and curd firmness of milk was also investigated in detail during this period. Reconstituted skim milk and raw whole milk (4 % fat) was treated at 150, 200, 250 and 300 MPa at an inlet temperature of 4˚C. UHPH was found to not affect the average diameter of casein micelles, which was further supported by the experimental results of turbidity measurements on UHPH-treated skim milk. Nevertheless, UHPH of skim milk resulted in a shorter rennet coagulation time (RCT), higher gel formation rate (*dG´/dt*) and final storage modulus (*G´*) of rennet-induced gels. UHPH treatment also resulted in shorter rennet coagulation time of raw whole milk, without apparently affecting the rate of gel formation and final firmness values reached, which is very important for cheese manufacturers.

During the second period further coagulation studies followed. Skim milk and standardised whole milk (0.9 prot/fat ration) were batch pasteurised (63.5 ºC, 30 min) and then UHPH treated at inlet temperature of 55 ºC and pressures of 100, 200 or 300 MPa. For coagulation assays, milk was adjusted to pH 6.6 with lactic acid, warmed to 32 ºC and the rennet added. For skim milk, treatment at 300 MPa reduced gelation time and increased elastic modulus compared to control samples or those homogenised at 100 or 200 MPa. Apparent yield stress of milk gels also increased with the pressure. To study whether UHPH affected the size of casein micelles, these were suspended in synthetic milk ultrafiltrate (SMUF); mean micelle apparent diameter diminished from 190 nm to 160 nm after treatment at 200 MPa, suggesting a reduction in micelle size following UHPH. Particle size of fat globules in UHPH-treated standardised whole milk also decreased with pressure. Values of the size parameters $d_{3.2}$ and $d_{4.3}$ decreased on treatment at 300 MPa. Gelation time also diminished with increasing homogenisation pressure. Elastic modulus was strongly affected by the fat content of milk. By comparing effect of UHPH on 3.7, 0.2 and <0.2% fat milk, it could be observed that, for 3.7% fat milk, the increase in elastic modulus following UHPH was higher than the increase due to fat content itself. UHPH treatment also increased the apparent yield stress of whole milk gels and the shear stress necessary to fracture rennet-induced milk gels.

At the UAB rennet coagulation properties were assessed by the Optigraph (Ysebaert Inc, Frepillon, France). Inlet temperature of raw bovine milk was approximately 30ºC. Rennet coagulation times (RCT) were very much dependant on the treatment. Samples treated at 100 and 200 MPa (one and two stages) had significantly lower RCT than raw milk. On the other hand, the RCT of the samples treated at 300 MPa were similar to the one obtained with raw milk. Two-stage homogenisation did not seem to affect RCT, but, it significantly diminished both the rate of curd firmness (RCF) and curd firmness at 30 min (CF30) to values similar or below the ones of raw milk. One stage UHPH treatments at 200 and 300 MPa produced values significantly higher than those of raw milk. The UAB estimated the Curd yield of UHPH treated milks by centrifugation. Wet yields of the curds obtained from the UHPH-treated milks were in general, significantly higher than the ones of raw milk. Increased wet curd yield reflects primarily greater moisture retention, but also possibly incorporation of denatured whey protein. Both factors justified further studies carried out during the second period. It was observed that 30 ºC inlet temperatures and 100 MPa treatments caused rennet clotting times inferior to those of raw and of pasteurised milk, but for higher pressures clotting time was similar. The rate of curd firming was higher for 200 and 300 MPa treatments than that of pasteurised and raw milk. Nevertheless when the second stage was applied, the rate was reduced for all pressure treatments. Curd firmness was superior for 200 and 300 MPa treatments and similar to firmness reached with raw and pasteurised milk when the second stage was applied. For milk inlet temperatures of 40 ºC, the results were similar but 200 MPa rate of curd firming superseded those of raw and pasteurised milk.

Physicochemical parameters such as: particle size measures, confocal microscopy of the curds, pH, and acidity and viscosity variations were also assayed. Particle size distribution patterns of pasteurised and raw bovine milk showed two peaks (approximately at 0.2 and 4.0 µm) corresponding to casein and fat globule sizes. For 100 and 200 UHPH treatments at 30 ºC inlet temperature, the profiles showed one wider peak (0.15 µm approximately) with a small shoulder of higher size. Remarkably, when the second stage at 30 MPa was applied, several peaks with average particle size up to 30 µm appeared showing that aggregation of particles might have been favoured. This was confirmed by $d_{4.3}$ values and by transmission electronic microscopy.

<u>Effects of UHPH treatment on cheese production:</u>

<u>Cheddar cheese:</u> UCC developed a model for Cheddar cheeses (200 mL scale). Cheddar miniature cheeses will be manufactured to screen a range of possible UHPH treatment conditions which may prove to be interesting. Criteria for screening will include cheese-making efficiency, cheese composition, and cheese texture. Based on these studies, larger-scale (20 L) batches of cheese will be made from milk treated under the most promising conditions, and biochemical and microbiological changes during ripening in such cheeses will be studied. Cheddar cheese was manufactured from standardised milk, whole milk UHPH treated at 100 MPa (55 ºC inlet temperature) and UHPH-treated (100 or 200 MPa at 55 º C inlet temperature) 10% fat cream standardised by mixing after homogenisation with unhomogenised skim milk. Particle size of UHPH-treated 10% fat cream decreased with pressure; $D_{3.2}$ values decreased from control to 200 MPa and $D_{4.3}$ from control to 100 and 200 MPa 10% fat cream samples; for milk samples treated at 100 MPa, $D_{4.3}$ showed lower amounts of aggregates. Overall, changes in cheese composition were apparent due to UHPH treatment; cheese made from homogenised milk showed increased salt content, pH and yield compared to control samples. Chemical composition of the whey from cheese-making showed reduced amounts of fat and protein (for both cream and milk homogenised at 100 MPa). The curds obtained during cheese-making also varied, being softer and more disperse than in traditional manufacture of Cheddar cheese. After one month of ripening, cheese samples made from milk UHPH treated at 100 or 200 MPa presented similar resistance to fracture than control cheese; in contrast, treatment of whole milk at 100 MPa increased this parameter. The strain at fracture was also influenced by UHPH but only on the first month; the differences between homogenised and control samples decreased after 3 months.

Goat milk cheese: RINY partner performed three batch productions of 2000 L in order to standardise the goat's cheese model for the project. Other three productions involving UHPH treated goat milk were carried out in the Pilot Plant of the UAB. One experiment was successfully assayed (250 MPa, 30ºC inlet temperature). Preliminary analysis of milk and cheeses were carried out by RINY and UAB, and samples of milk, whey and cheeses were frozen for further analysis. Milk was obtained from a herd of Murciano-Granadina goats (Valencia, Spain). The milk batch was divided into three portions, pasteurized (PA) at 72ºC for 15s, pasteurized and homogenized (PA+H) at 72ºC for 15 s and 18+2MPa, and UHPH treated at 200 MPa and 30ºC of inlet temperature. In relation to the microbiology of milks, the UHPH treatment applied was almost as efficient as pasteurization treatment in the reduction of total bacteria and Coliform counts. Cheeses made from UHPH milk showed the most elevated cheese yield. It was then decided to screen several combinations of milk inlet temperatures, pressures (first stage plus second stage), refrigerating conditions, etc, in order to determine optimum cheese-making conditions by means of UHPH processing. This task consumed a significant amount of time. After selecting UHPH ideal conditions, rennet clotting time for UHPH milk was shorter than for PA and PH meaning coagulation began earlier. Once started, the rate of curd firming for UHPH milk was significantly higher than for PA and PH. Curd firmness at 30 and 45 were two times higher for UHPH cheeses evidencing differences in the coagulation process. It was observed that for UHPH samples, initial yield increased approximately 10. $L*$ values (lightning) were similar for the three sorts of cheese. Nevertheless, $b*$ values were initially lower for UHPH cheese (less yellowish) but after 30 days they reached PA values; $a*$ values initially higher than PA and PH decreased less during ripening of UHPH cheeses (less reddish).

Skim milk curd: In the industrial production plan of MGLA fresh skim milk cheese process, the skim milk is pasteurised (72ºC, 15s) and then cooled until 42ºC (after separating the cream). At this temperature a 6.5% tank culture is mixed. The pH normally reaches 4.6 in about 1 h, and casein flocculates form a soft structure that is agitated to obtain small grains. Via a special drum with holes the whey is drained and the dry matter can be adjusted before a mill cuts the curd in smaller equal pieces. The first UHPH experiments were held at the UAB, but the results were not encouraging. In this trial powder culture was added. pH 4.6 was reached only after 5 h, but the curd was agitated at pH 5,49. The soft curd formed did not get harder through stirring. Dry matter of the final product was 21.67% and wet cheese yield 30.99%. After 12 h in the forms, the whey drained and cheese dry matter reached 28.20% (near the desired 32%). After these trials it was evident that cheese-making procedure should be adjusted (due to changes in water retention of the curd and casein losses). It was then decided to study milk coagulation properties and to optimise cheese-making procedure from UHPH treated skim milk (100-250 MPa, 30 ºC). No differences were found in coagulation time; on the contrary firmness of the curds decreased with the pressure applied. The yield increased, but the high amount of water built a soft casein particle, which are not usual for the consumer. This fact was seen as a possibility of creating a new type of curd cheese. Curd cheeses were successfully manufactured and MGLA tested the standard curd product in comparison to a product treated with 1000 as well as one with 2000 bar with volunteer consumers. The volunteers clearly preferred the curd produced under standard conditions (taste, after taste, appearance, smell, colour, consistency). As a general conclusion, UHPH cannot be considered the method of choice for the production of curd. Both, the technological and the sensory properties are decreased by the pressure treatment of the skimmed milk. UHPH technology might be very useful for the production of drinks on milk or even on cereal basis where a homogeneous, soft consistency is desirable. This possibility could open up various possibilities for organic drink products where these kinds of additives are not wanted.

Tilsiter cheese: Tilsiter cheese is made from whole milk pasteurised (72ºC, 15s). After heat treatment the milk is inoculated with *Lactobacillus* microorganisms. After 1.5 h of fermentation 20 % of washing water is added. In order to perform the first UHPH experience the UAB asked KOLL partner to make it in its food plant. Regarding the UHPH outlet, temperature increased until 73 °C and was cooled until 33 °C. After adding the starter, 70 min. later both batches reached the desired pH of 4,6 and obtained strong curd consistency. However, after cutting the curd, in the UHPH batch, very small particles of unusual whiteness were observed. The dry matter directly after the filling should be at 55.88 %, but it was only at 38.39 %. Cheeses were brined and stored for ripening. Normally, after 4 weeks the Tilsiter is ready but this time with UHPH cheese it did not occur. After these trials it was evident that cheese-making procedure should be adjusted (due to changes in colour and structure of the curd, unsatisfactory ripening). It was then decided to separate cream from milk and treat 10% fat milk (200 MPa, inlet temperature 50 ºC) in order to further reconstitute skim milk up to 3.5% fat and protein contents. UHPH treatment diminished mean particle size of milk. Time to reach coagulation at pH 4.6 was similar to standard processed milk though the texture of the curd was softer and whiter. After cheese-making, the dry matter of optimised UHPH Tilsiter diminished in 10% but the yield increased from 10.85 to 13.09%. Ripening process was successful. Despite the fact that texture of UHPH Tilsiter was preferred by the consumers, taste and after taste were considered better in standard cheeses.

Figure 8. Samples of UHPH cheeses: goat milk cheese (left), fresh milk (centre) and Tilsiter (right).

Fresh cheese: Fresh cheese from bovine milk was manufactured according to an adaptation of the traditional protocol. Fresh cheeses were made from pasteurised (PA, 15s at 72 ºC); homogenised and pasteurised (HP, 15+3 MPa at 60 ºC and 15 s at 72 ºC) and UHPH (200 MPa, inlet 40 ºC) treated milk. It was noticed that UHPH treated milk coagulated faster (shorter rennet clotting times) than HP and PA milk. UHPH treated milk also produced a firmer curd compared to the softest with HP milk. Theoretical yield of fresh cheeses was assessed by centrifugation (12500 *g*, 75 min at 25 ºC) on days 1 and 2. This yield was clearly increased by UHPH (day 1). Forced centrifugation studies on day 2 showed that eventually there would not be great differences between HP and UHPH cheeses. Nevertheless, the evolution of spontaneous whey drainage followed during 13 days of storage at 4 ºC, resulted in UHPH cheeses holding more water than HP and PA samples. It can be concluded from the data that UHPH cheeses retain higher amounts of water. For UHPH cheeses the pH was lower than for PA and HP cheeses. Furthermore, the tendency of UHPH cheese pH was to decrease with time. On the contrary, for PA and HP cheeses pH remained stable for 13 days. Microbiology assays were performed in milk and cheeses. For UHPH milk it can be said that the values obtained immediately after UHPH treatment were equal or better than pasteurisation treatment. UHPH cheeses showed encouraging results. No pathogenic microorganisms were found in either cheese. Reduced chromaticity can be observed in UHPH cheese samples as well as lightness value (L*) found between HP and PA samples and. *b** values (yellowness) were similar for HP and UHPH, while *a** values (greenness) was increased for UHPH samples. From rheological studies it can be said that UHPH cheeses are similar to HP cheese in terms of being more elastic and softer than PA cheeses.

WHEY MILK PROTEIN ISOLATES

Whey proteins were selected among whey protein concentrates and isolates industrially produced, on the basis of their solubility and composition. A whey protein isolate (WPI) with a highly native state and representative of whey proteins (containing all whey proteins) from Lactalis (Retiers, France) has been selected for the study. The operating conditions for ultra-high pressure homogenisation up to 300 MPa were established. The homogeniser prototype FPG7400H (from Stansted Fluid Power Ltd.) and property of UMII was used for the study. The effects of UHPH treatments on whey protein dispersions were studied for protein concentrations of 3, 6 or 10% (w/w) and at the spontaneous pH of the dispersion (~ 6.5 ± 0.1) for different pressure of homogenisation between 100 and 300 MPa, and at an initial fluid temperature (Tin) between 4°C and 24°C (Tin of 24°C was chosen for most of the experiments). The method used to evaluate protein solubility at the spontaneous pH of the dispersion (close to 6.5) or after pH adjustment to 4.7, was conducted by centrifugation of the soluble constituents, followed by their quantitative determination by the BCA procedure. PAGE electrophoresis were carried out with or without dissociating/reducing agents (Sodium dodecyle sulphate, SDS; β-mercaptoethanol, MSH) on aliquots of control or UHPH-processed dispersions. Whey protein dispersions were also treated by short time thermal treatments (STTT of ~ 4 seconds) for comparison: the protein dispersions were heated by passing through a stainless steel tubing (2.10 mm inner ∅ and 3.18 mm outer ∅) immersed in a water bath previously equilibrated between 58 and 82°C. Protein aggregation was followed in details by Photon Correlation Spectroscopy (PCS), using the "Malvern Zetasiser 3000 HS" instrument (Malvern Instruments S.A., Orsay, France). The parameters of measurement by light scattering have been set and optimised for a precise determination of aggregate distribution in intensity frequency and in number frequency. Measurements were carried out for the non treated WPI dispersion, and for the processed dispersions (UHPH or STTT). Protein aggregation was accompanied by whiting of the WPI dispersion (initially yellow) in the case of UHPH processing as well as heat-treatments. These colour changes were quantified, as well as the dispersion viscosity to complete the PCS study.

The study of model emulsions was carried out with the homogeniser prototype FPG7400H (Stansted) and property of UMII. Model O/W emulsions stabilised with whey proteins from Lactalis (Retiers, France) were studied for both oil contents (15 and 30%, w/w, of the final emulsion) and one protein concentration of 4.3% (w/w, of the final emulsion) at close to pH 6.5. Pre-emulsions were prepared with a Silverson machine, and then processed through the homogeniser up to 300 MPa (Tin of 24°C). Emulsion temperature and pressure were measured at different positions and times during processing to follow the processing history of the fluid. Emulsions were characterised by the distribution of the droplets sizes and viscosity. Effect of recycling the emulsion up to three passes through the homogeniser was determined by droplet size measurements, to check the performance of the machine. Addition of xanthan gum to the emulsion was also studied. The stability of O/W emulsions upon storage at 5°C for 9 days or at − 30°C for 13 days was assessed by droplet size determination (Mastersizer 2000, Malvern) after dilution in deionised water ± 1 g SDS/L before diffractometry analysis, to check for the possible presence of coalesced or aggregated oil droplets. Frozen emulsions were analysed after thawing in a water bath at 25°C ± 0.5°C for 35 ± 5 min. Whey protein dispersions and emulsions have also been processed using the Benchtop prototype, comparing with the FPG7400H homogeniser of UMII. The performances of the two machines could be therefore compared using the same whey protein dispersions and emulsions. WPI dispersions were processed on the Benchtop prototype at an initial temperature of 24 or 40°C to evaluate the effect of short-live heating phenomena accompanying homogenisation processing, on protein denaturation.

LUPINE MILK

The aim of TTZ-Bremerhaven in this project was to develop a vegetable drink and derived product out of lupine seeds keeping nutritional advantages and getting good organic quality, without excessive bitter taste. Also it was aimed at studying the effects of UHPH processing on the lupine milk matrix developed. Lupines are legumes extremely rich in protein and traditionally used in brewery, sauces and as a titbit. The protein is gained from the dry-milled seeds (meat-cutter) by cold extraction with water or diluted alkali. Afterward the protein solution can be used for a drink with different flavour or to develop further curd-products. In the first period of the project, it was aimed at standardising manufacturing process of lupine milk. Soaking, milling, dilution, cold extraction, filtration and homogenisation steps were adjusted in order to optimise the formulation and the process.

The protein suspension from lupine seeds can be also used to develop protein curds. Aiming at producing derived products from lupine milk, three methods were assayed to develop these curds: *heat precipitation* (100ºC); *saline precipitation* (50ºC, 1.5 $MgCl_2$); *acid precipitation*. Further separation steps were also adjusted. The accomplished experiments demonstrated that lupine milk and lupine curd by heat precipitation will be the most economically interesting method, reaching also the best results in sensory evaluations.

During the second period, lupine milk formulation already standardised was UHPH processed at 2000-3500 bar and inlet temperature of 15 ºC. The product was compared to pasteurised/homogenised lupine milk (15s at 72 ºC/240 bar). Mean particle size of homogenised and UHPH lupine milk decreased to similar levels. Viscosity and viscidity of UHPH lupine milk samples showed a slight increase up to 2000 bar (2000-3500 bar samples did not differ) though viscosity decreased if a shear stress over time was applied. Typical lupine milk yellow colour decreased with the pressure applied. In terms of stability, treatments of 2000, 3000 and 3500 bar showed less sedimentation than pasteurised/homogenised samples being 2000 bar the most stable samples. Samples treated at 3500 bar had the most important decrease in bacteria and yeast counts. Nutritional characterisation of lupine milk was performed. Sensory analysis with consumers stated that UHPH treatment in comparison to the ordinary method does not have a significant impact on mouth feeling and bitter taste of the product. Lupine milk drinks (vanilla, chocolate and rusk tastes) and lupine tofu were developed. Specific consumer tests were designed for them. Tofu produced form basic lupine achieved a texture twice softer than ordinary soy tofu. For tofu, paprika-caraway appeared to bet he best product in appearance, taste, mouth-feeling and overall impression. For lupine drinks, the rusk flavoured one was the whith higher particle sizes. UHPH treatment reduced mean particle size from 36-40 µm to approximately 30 µm. Stability was also increased (no signs of sedimentation before 30 h). For lupine drinks, rusk was the best in appearance, taste, smell and overall impression even overcoming bitter taste of lupines.

Physical, nutritional, microbial and sensory parameters were intensively studied. During physical analysis particle size, viscosity and colour of the different samples was examined. Particle size and measurements of viscosity did not show any significant difference between the samples. The intensity of the yellow colour decreased from 14.2 (240/past.) to 9,39 (3500 bar). Nutritional properties have shown that lupine represents a high source of protein, vitamins and trace elements. Microbial changes do show a slight decrease of bacterial number after applying 3500 bar. The sensory analysis did not show any significant difference in mouth feeling and bitter taste according to different treated samples. In general it can be said that the characteristic quality parameters of the lupine milk is not influenced by the UHPH treatment. In conclusion, this means that UHPH treatment is a suitable method for processing lupine milk. For development of the lupine dinks it was difficult to overcome the normal bitter and pea like taste of the lupine milk. The milk for developing the lupine drinks was treated with 2000 bar. For the rusk (zwieback) drink the UHPH treatment was also applied after production. Here a decrease in particle size from 36-40 to 28-31 µm was noticed, which is better for the mouth feeling. The viscosity and stability of the rusk drink was also improved after UHPH treatment, which is also important for the mouth feeling and storage of the products.

For development of the tofu heat precipitation using a ratio of 1:5 lupine:water with a pH of 5,9 yielded the highest rate of curd. In comparison to the tofu developed with untreated lupine milk the one developed from 2000 bar treated milk was too soft in texture and did not form a proper structure. This can be due to the smaller particles and increased water holding capacity caused by UHPH treatment. Furthermore, it was found that the development of tofu from the spray dried protein concentrate did not work out due to similar problems mentioned above. Therefore for tofu production certain steps from milling the seeds over soaking and extraction had to be repeated and the tofu products were developed without UHPH treatment.

SOY MILK

The first tasks in carried out involving the studies of UHPH technology on soy milk were destined to standardize the manufacture process of the base soymilk. This base soy milk was afterwards processed by UHPH. UAB and NECT worked together in order to optimise all the relevant parameters. Compositional analysis, fine tuning of several analytical methods and preliminary UHPH tests were performed in the first period. The process which has been selected to obtain this base product consisted of dehulling soybeans, grinding with water at 90ºC in a colloidal mill (necessary to inactivate lipoxigenase) and filtration of the product. Samples UHPH treated at different pressures and inlet temperatures have been sent to BFEL in order to perform sensory analysis. The general aspects of soymilk in preliminary test of UHPH treatment led to the decision of working at 200 and 300 MPa and 40ºC inlet temperature to get temperatures in the pressure valve between 80 and 100ºC. That combination of pressure and temperature is thought to reduce trypsin inhibitors considerably. Samples of raw, UHT and UHPH treated soy milk were sent to FRCN in order to perform preliminary sensory analyses.

In the second period to assess microbial and physical stability of UHPH soy milk, forced conditions of incubation were used. Storage at 37 ºC was performed with no preservative (for microbial stability determination) and sodium azide (for physical stability determination). In parallel, samples of UHPH and UHT soy milk were stored in refrigeration at 4ºC to be analyzed on days 1, 15, 30, 45 and 60. Following to these preliminary trials, UHPH treatments were performed at 40ºC inlet temperature and 200 and 300 MPa.

Immediately after UHPH treatment, for 200-300 MPa treated samples absence of *Enterobacteriaceae* was achieved. An important reduction of aerobic total mesophilic bacteria was observed, remaining only sporulated forms. The exponential grow of bacteria during the stability study is prolonged without arriving to product coagulation at the end of the study (30-60 days, respectively). On the other hand, all treatments performed and stored at 37ºC coagulated before seven days except UHT treatment. The reduction of particle size was higher in UHPH than in conventional homogenisation. In general, 200 MPa was the treatment which gave the narrower range in the size distribution of particles. At 300 MPa, although particle size was quite similar to 200 MPa, the presence of aggregates was observed. UHPH treatment at 300

MPa caused a further diminution in lightness than 200 MPa; L* and b* values for all samples during storage for 60 days remained stable. The evolution of a* values presented differences between treatments applied to soy milk and also in the evolution during storage. However, the observed differences were not important for consumer terms of quality. Physical stability of soy milk was assessed by means of measuring the % (w/w) of total sediment solids after centrifugation of samples which were stored at 37ºC. The sedimentation index increased in 300 MPa treated soy milk during the storage which probably is related to the higher particles observed in this samples which could act as nuclei for aggregation formation for smaller particles during the forced sedimentation. The peak from 200 MPa samples thermograms had a smaller total calorimetric enthalpy than the peak from the base product, suggesting partial denaturation of the protein. No endothermic peaks were observed in 300 MPa and UHT samples, indicating total denaturation in both treatments. Only treatments of 300 MPa could diminish the oxidation degree compared to UHT and base product. Physical stability has shown to be excellent during 60 days in forced conditions of storage. Physical stability of UHPH soymilk was excellent. However, microbial analysis, has only pointed that UHPH in the conditions applied in this study, can be compared to a pasteurisation process. To increase the microbial inactivation, further studies of this product is needed.

Microbial quality of UHPH soymilk was similar to a pasteurized product which is suitable to produce "soghurt", as it is a low pH product. UHPH treatment conditions assayed were: 200 and 300 MPa at 40 and 50 ºC inlet temperature of the product. In addition, experiments were also performed at single and double stage homogenization. Coagulation properties of UHPH treated soy-milks, pH curve variation, firmness of the curd formed at pH 4.6, and water holding capacity of the gels (spontaneous and forced by centrifugation). In any case spontaneous syneresis was detected in UHPH soghurts. Puncture test proved that UHPH treated soymilk rendered a considerably better consistency of soghurt which was maintained during commercial life. Soghurt samples were then evaluated by sensory trials with consumers.

ALMOND MILK

Standardisation of the almond milk product was the first worry of NECT and UAB partners because regarding almond milk production, at the beginning of the project important problems that affected the stability of the final product were detected such as: phases separation after autoclave process, cooked colour, smell and taste. These problems were supposed to be overcome both by improving the drink formulation and by the application of UHPH process instead of severe heat treatments. Almond milk productions were planned and carried out by NECT and UAB partners in order to detect causes, modify defects and improve stability. The experiments were made changing the concentration and nature of emulsifying agents, the concentration of the stabilizer agent, the amount of almond flour, cooling/heating while mixing the ingredients. Finally, an improved formulation was obtained. With this standardized almond milk formulation, UHPH experiments were planned and carried out at the UAB food plant. In all of these trials, three different products were to be tested:

pasteurized, pasteurized and autoclaved (traditional product) and pasteurized and UHPH (250 MPa, inlet temperature 30ºC) treated almond milk. Organoleptic, physicochemical and microbiological analyses were performed. The UHPH product obtained was whiter than both the pasteurized and the autoclaved ones. Stability of phases as well as homogeneity achieved was also better. Palatability was improved, taste was near to natural but smell slightly faded. Microbiological results were good enough for a product to be kept under refrigerating temperatures but insufficient for a product destined to be stored at room temperature. During the second period, NECT and UAB continued the study the effects of UHPH on almond milk; flavoured almond milks and almond milk yoghurt were developed. UHPH treatments (200-300 MPa, 30-50 ºC inlet temperature, 0-15 s retention time) produced no significant changes in almond milk composition and were selected for designing refrigerated product. Viscosity of the product decreased with the pressure applied, its stability improved and its colour was whiter.

Figure 9. UHPH almond milk samples.

Microbiological and physicochemical characteristics were followed during refrigerating storage. Almond milk drinks with different tastes and sweeteners added were assessed. Almond milk yoghurts were also developed. Acidification characteristics, microbiological aspects and stability during storage were assessed. The strength of the gel formed was highest for 300 MPa samples; on the contrary, gels from pasteurised almond milk were softer with higher spontaneous or forced syneresis. UHPH almond milks and yoghurts were preferred to thermally treated samples by the consumers mostly due to its improved texture and flavour.

STUDY OF FOOD COMPONENTS

For preliminary research on food components, at FRCN facilities it was first installed a simple laboratory UHPH apparatus for producing own samples until partners were able to deliver UHPH treated samples. Starting material was commercial pasteurised and homogenised milk and untreated milk from a local farmer. As a second step, simple continuously working laboratory UHPH equipment was assembled for the production of larger samples that could be used for sensory testing. For this purpose a new sensory panel according to DIN (German Institute for Standardization) 10961

"Training of assessors for sensory analysis" was established and trained. The course included basic tests for training the sense of taste and smell. Besides, the assessors learnt to detect differences in characteristics for the sensory properties under investigation (such as taste, fragrance, flavour, skin feeling or texture) and the ability to describe those characteristics using verbal descriptors for the characteristics and scaling methods for the differences in intensity. The Institute was further engaged in the validation of several screening tests for possible UHPH induced changes (mutagenicity and toxicity in bacterial systems) and also started to work with UHPH vegetable milk treated samples. Metal content analysis was also carried at TTZ facilities and routine compositional analysis at UAB food plant.

During the second period UAB, NECT, TTZ and RINY partners sent to FRCN: 22 samples of bovine milk, 12 samples of soybean milk, 40 samples of almond milk, 5 samples of lupine milk, 3 samples of yoghurt from bovine milk and 3 samples of yoghurt from soybean milk. 'Sample' in this context means a certain amount of material that had been produced at the same time under same conditions, as shown by the sample codes given by FRCN. Considering the analyses have been performed at least twice, a total of about 4250 analyses have been performed by FRCN during that period. These samples were generally analysed for about 25 different properties including possible undesirable and desirable effects due to processing. (protein content; vitamins B_1, B_2, C; carotenoids; SDS-Page patterns; free sulphydryl groups; covalent bonds; peroxidase activity; surface hydrophobicity; antioxidant potential; antimutagenic potential, mutagenicity in *Salmonella* spp.; toxicity in *Salmonella*, acute toxicity (*Vibrio fischerii*) and antigenicity). No significant changes were found in the protein contents of the milks assayed. Regarding vitamin content, values were mostly stable for bovine and soy milk but a slight reduction in vitamin B_2 content was observed. Overall, electrophoretic patterns of the UHPH samples were similar to that of the control ones. Peroxidase activity decreased mostly after 200-300 MPa treatments for bovine and almond milk samples, whereas it remain unaltered for soy milk. The number of free sulphydryl groups decreased with the pressure treatment applied; on the contrary the formation of di-sulphyde bonds was increased in the three kinds of samples as well as surface hydrophobicity. The formation of aggregates might explain these results. Antioxidant potential was slightly altered in soy milk samples but showed no changes in almond and bovine milk samples. Antimutagenic potential was reduced for soy milk samples; no changes were observed in mutagenicity and toxicity in *Salmonella* spp. tests due to UHPH treatments. Neither was observed positive reactions in the acute toxicity tests (except for some raw almond milk samples). Antigenicity values were reduced for soy bean and soy bean allergens. The study of possible undesirable compounds formed on milk and vegetable milks components by UHPH treatment found neither negative effects nor severe changes in the assays applied. Changes were mainly detected in macromolecular systems like e.g. particle sizes, surface hydrophobicity of proteins, free sulfhydryl groups, aggregations. The possible consequences of these changes, negative or positive, regarding human nutritional physiology will be subject of further research.

CONSUMER EVALUATION

The consumer acceptance of the food and processes developed in the project were carried out by partners UAB, NECT, RINY, TTZ, MGLA, KOLL and UCC. The different foods assayed and compared to traditionally manufactured foods were as follows: bovine whole milk; bovine milk yoghurt; fresh cheese; skim milk fresh cheese; Tilsiter cheese; goat milk cheese; lupine milk and derived products; soy milk and soghurt; almond milk and almond milk yoghurt. The main results were as follows:

- *UHPH bovine milk*: Twenty four consumers participated in several trials made at UCC facilities. Irish consumers did not find anything notable or objectionable about UHPH treated milk.
- *UHPH bovine yoghurt:* Consumer sensory acceptance of yogurt was assessed based on the opinion of 40 potential consumers (men and women, from 23 to 55 years old). 55 % of consumers coincided in grading the 300 MPa UHPH yoghurt to be the best, (especially because of texture) followed by the 200 MPa UHPH treatment. Finally, traditional yoghurt was the worst graded.
- *UHPH Cheese*: Sensory analysis of fresh cheese prepared from UHPH and traditionally processed bovine milk was performed by a panel of 14 volunteers. Samples of fresh cheese from UHPH-treated milk at 200 MPa at 40ºC and conventionally homogenised-pasteurised milk were compared to control cheeses (from pasteurised milk). Considering the textural parameters, all the panellists agreed on the fact that UHPH fresh cheese was softer than the control. In addition, a great majority (93%) described UHPH fresh cheese as less springy. Such characteristics were positively appreciated. On the other hand, UHPH cheeses were found to be grainier (79%), pastier (79%) and wetter (57%) than control cheeses. 43% of the panellists found both the flavour and the aroma of UHPH fresh cheeses greater than the control, and 36% found no differences between these cheeses. Sensory analysis of Tilsiter cheese prepared under UHPH and standard conditions was performed with the UHPH cheese using 200 MPa optimised process and standard Tilsiter produced the same day (composition, consistency and sensory). For the sensory analysis, KOLL worked together with their existing customers who compared the two products without knowing what they were testing. The only preferred property of the UHPH cheese was the softer consistency which was not typical for this kind of cheese. The general evaluation of the UHPH Tilsiter was not as good as the one for the standard product. Sensory analysis of curd cheese prepared under UHPH and standard conditions was carried out by MGLA with their employees as well as customers who were willing to act as volunteers for the anonymous sensory test. MGLA tested the appearance, the smell, the consistency, the taste, the aftertaste and the general evaluation of an UHPH treated curd in comparison to a standard curd produced on the same day. The curd product produced under standard conditions was preferred by the volunteers. The decreased dry matter had a significant effect on the appearance and on the consistency of the curd.
- *Lupine milk and derived products:* For sensory analysis of the basic lupine milk 26 panellists were asked to examine the samples in terms of mouth feeling and bitter taste. All UHPH treated samples (200, 250, 300, 350 MPa) were tested against the sample prepared with the standard method (240 bar/past.). For this triangular test, it can be concluded with a confidence level

of 95% that there is no significant difference in mouth feeling and bitter taste between the lupine milk treated with standard method and 200, 250, 300 and 350 MPa. For lupine drink the following products were assessed by consumer analysis: Lupine milk drink (basic lupine milk treated with 2000 bar; Rusk (zwieback); Chocolate; Vanilla). The consumers were asked to put a mark at a seven scale for appearance, taste, smell, mouth feeling and overall impression. The flavour with rusk had the best appearance, taste, and smell and also got the best mark in overall impression. The drink with rusk (zwieback) flavour was seen as being excellent in sweetness and bitterness for consumers.

- *Soghurt:* The test was performed twice with 41 adult volunteers. In general they were not familiar with soy products. Soghurt samples were made from UHT (ultra high temperature treatment) treated soy milk, and soy milk UHPH treated at 200 and 300 MPa with 40ºC product inlet temperature. The most preferred soghurt sample was the one made from soy milk UHPH treated at 300 MPa; soghurt samples made from soy milk UHPH treated at 200 MPa was less appreciated by the consumers whereas UHT samples were found completely disagreeable.
- *Almond milk and almond milk yoghurt:* In consumer tests with 40 people three samples of almond milk (pasteurized and UHPH samples treated at 300MPa and 30 or 50ºC of inlet temperature) were randomly codified and offered to the panel for a test of preference. The favourite samples were the UHPH treated almond milks, showing more than 65% of acceptance. More than 52% of people would buy this product, if available in the market. Samples of almond milk yoghurt were randomly codified and presented for a triangular test. An 82% of the consumers recognized the right different sample in the triangular test. The preferences were about half-half for yoghurt made from pasteurized or UHPH almond milk, the texture of the samples being recognized as clearly different; texture of UHPH samples was preferred by the majority.

Overall, regarding sensory trials, most of the UHPH products (except for some cheese varieties) were more appreciated than the traditional products; in other occasions, it was not possible to tell the difference between the UHPH products and the traditional products (thermally processed products by high pasteurisation or ultra-high temperature treatments) by both trained panellists and consumers.

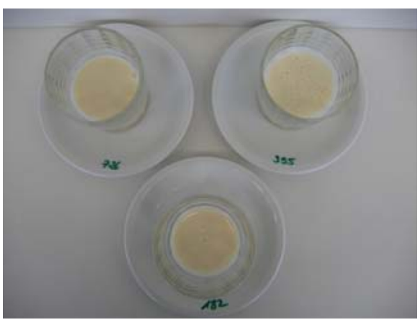

Figure 10. Image of three coded samples as an example for the triangle lupine milk sensory test

NOVEL FOOD REGULATION

In this project it was also intended to trace and integrate relevant information related UHPH treated foods and aspects of Novel Food regulation. For this purpose collaborative work between the partners UAB, UMII, FRCN, UCC and TTZ was necessary. From 1997 when Novel Food regulation (EC Num, 258/97 January 1997) was launched it was stated that all foods shall be allowed in the EU market unless it is known that toxic, carcinogenic, mutagenic or nutritional issues can be found in these foods. New foods may require a pre-market authorisation; in the case of foods receiving a novel processing that clearly affects its final state EU authorisation seems mandatory. The main results involving bovine milk, bovine milk yoghurt, cheese, lupine milk, soy milk, almond milk and derived products were discussed and analysed. To begin with, it must be stated that UHPH-products are very moderately pressure-treated with the aim of homogenisation, pasteurisation or both homogenisation and pasteurisation. Homogenisation using moderate pressure up to 100 MPa is a worldwide accepted and broadly applied process. The only novelty of UHPH would be the higher pressure level (300-400 MPa) with possible effects caused by the high pressure itself and/or the pressure drop (mainly mechanical effects or dynamic high pressure effects). Compared to isostatic High Pressure treatment which is an established process for ready-to-eat foods in Japan, Spain, Australia, New Zealand and USA, the process parameters regarding pressure level and holding time are clearly much more moderate for UHPH: 300-350 MPa for seconds instead of 500-600 MPa for minutes. It must be considered that by measuring temperature increases suffered by liquid samples travelling through the HP valve during UHPH treatment, and controlling the fluid inlet temperature and the efficiency of the cooling devices installed immediately after the HP valve on every machine, it is possible to characterise the whole process, limiting thus fluid overheating. Typical minimum and maximum residence times in the whole Stansted Fluid Power homogenisers may vary between 8 and 160 seconds, respectively, with mean residence times between 23 and 53 seconds depending on the homogeniser. Nevertheless, the residence time the fluid brought at the highest temperature (through and at the outlet of the HP valve) before efficient cooling is in most of the cases inferior to 1 second, which is an important advantage in terms of alteration of the processed food. In addition, in the cases where homogenisers are fed with fluids at moderate temperatures (30-50°C), UHPH treatment must be considered as a combined processing including UHPH plus a short-time-thermal treatment (maximal temperatures for the products hardly surpassed 110ºC for a few seconds). Under these premises, substantial equivalence with conventionally homogenised and/or pasteurised foods is very likely because of the especially moderate conditions compared to isostatic high pressure treatments (“pascalisation”) or ultra-high temperature treatments (UHT treatments up to 140°C for a few seconds).

Taking into consideration all the results for the foods involved in this project with a ***potential successful development*** we can state that from sensory, physicochemical, safety and allergenicity points of view:

- UHPH bovine milk is as good as UHT or pasteurised milks
- Yoghurt made from UHPH treated bovine milk is as good as industrial yoghurts prepared from pasteurised bovine milk. Both are prepared with the aid of traditional starter cultures (*Streptococcus termophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*); in the case of UHPH yoghurts

they are produced more efficiently (good texture and less syneresis without protein enrichment) with acceptable sensory quality.
- Cheese made from UHPH bovine or goat milk present high quality values (microbiologically, technological, and organoleptical attributes) once cheese-making process is optimised.
- Almond milk is a Spanish product commercialised for more than 30 years. UHPH almond milk presents similar microbiological quality to pasteurised almond milk but increased stability during shelf life and improved consumer acceptance.
- Almond milk yoghurt is prepared from the same almond milk using traditional starters taken from the dairy industry (*Streptococcus termophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*), and thus should not be considered as a novel food.
- UHPH treated lupine milk presents enhanced stability and rheological properties while it is completely similar to the traditional product for both consumer and trained panelists.
- Soy milk and soghurt products are currently being commercialised throughout the EU. The main advantages regarding the use of UHPH rely on the improvements achieved in texture, taste and stability properties.

From the results described above, it can be concluded that UHPH products are in most aspects similar to traditional products processed by heating. This questions whether UHPH foods should demand authorisation to be commercialised as novel foods. Nevertheless, for industrial development and commercialisation of the UHPH products involved in this project, it will be of every firm responsibility to prepare a report with the most relevant information supplied by RTD partners, comparing UHPH products with traditionally pasteurised treatments. This report should be delivered to the Food Safety Agency of the country where commercialisation is intended. Consultation to local government food safety office is advisable from the beginning. The European Food Safety Agency (EFSA) could be asked for a definitive opinion in the case where arbitration is necessary or product exportation envisaged.

DISSEMINATION AND USE OF THE KNOWLEDGE

Exploitable knowledge generated by the project are: *Development of improved UHPH equipment for food plants; Development of improved UHPH equipment for laboratories; Development of new high pressure valve systems; Validation of UHPH procedures to treat liquid products; UHPH processing of almond milk; UHPH processing of lupine milk; UHPH processing of soy milk; UHPH processing of milk; UHPH processing of milk destined to make yogurt; UHPH processing of milk destined to make cheese; UHPH processing of whey milk proteins; UHPH processing service.* A patent on high pressure concepts has been obtained. Patents on cheese, yoghurt and vegetable milk products/processes are being written. UHPH machines have been exhibited worldwide and orders for UHPH machines have already been secured by SFP partner. The main activities carried out were: press/radio releases, TV interviews addressed to general public; conference addressed to technical public and probable users of the technology (Dairy symposium); meetings with enterprises to raise interest in UHPH and exhibit the prototypes (Barcelona); poster and conference presentations in congress (Burgos, III Congreso Nacional de Ciencia y Tecnología de los Alimentos; Montpellier, Trends in High Pressure Proteins Science Congress; Non Thermal Food EFFoST Workshop, Cork; Nantes "13th IUFOST World Congress of Food Science and Technology, Food is life" 17-21 September 2006; etc.); a special workshop held at Barcelona in order to disseminate the knowledge generated by the project (AFE-Tech workshop); prototypes have been exhibited and promoted at various trade shows and symposiums; INTERPHEX (New York, USA, March 2006); IFT (Orlando, USA, July 2006); AAPS (San Antonio, USA, November 2006); NTP (Belfast, UK, September 2006); LIPOSOMES DAYS (London, UK, December 2006); SFP sales have also visited customers in USA, India, Singapore and Australia to promote the use of the new SFP homogenising system for food, pharmaceutical and cosmetic applications; SFP updated its web site to show the new range of models; UAB has updated the project website (http://quiro.uab.es/_v_tecno_aliments/uhph/); publication of articles technical/industrial magazines and in scientific journals (so far:

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- Inactivation of *Staphylococcus* spp. strains in whole milk and orange juice using ultra high pressure homogenisation at inlet temperatures of 6 and 20°C. 2007. W.J. Briñez, A.X. Roig-Sagués, M.M. Hernández-Herrero, B. Guamis-López. Food Control, in press.

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- Effects of Ultra-High Pressure Homogenization on the Cheese-Making Properties of Milk. 2007. A. Zamora, V. Ferragut, P. D. Jaramillo, B. Guamis, and A. J. Trujillo. J. Dairy Sci.,

- Acid coagulation properties and suitability for yogurt production of cows' milk treated by high-pressure homogenisation. M. Serra, A.J. Trujillo, J.M. Quevedo, B. Guamis and V. Ferragut. Int. Dairy J., in press.

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