Universitat Autònoma de Barcelona

Facultat de Veterinària

Departament de Ciència Animal i dels Aliments.

Official Master's Degree in Quality of Food of Animal Origin

Thesis presented to overcome the 15 credits of module Master's Thesis of Master in Quality Food of Animal Origin

Physical and oxidative stability of UHT milk-based product enriched with conjugated linoleic acid emulsified by Ultra-high Pressure Homogenization

CRISTINA GUTIERREZ-MERIDA

Bellaterra, 7th July 2016

Antonio José Trujillo Mesa Profesor Titular del área de Tecnología de los Alimentos de la Universitat Autònoma de Barcelona,

INFORMA

Que el trabajo de investigación titulado: "PHYSICAL AND OXIDATIVE STABILITY OF UHT MILK-BASED PRODUCT ENRICHED WITH CONJUGATED LINOLEIC ACID EMULSIFIED BY ULTRA-HIGH PRESSURE HOMOGENIZATION" ha sido realizado bajo mi supervisión dentro del módulo Trabajo Fin de Máster del Máster Oficial de Calidad de Alimentos de Origen Animal de la Universitat Autònoma de Barcelona.

Bellaterra, 7 julio de 2016

Dr. A.J. Trujillo

Acknowledgement

I would like to express my deepest appreciation to all those who provided me the possibility to complete this master´s thesis. First of all I would like to give a special gratitude to my project supervisor Toni Trujillo for giving me the opportunity to work on this project and for their constant guidance, support, advices and patient. Moreover I would like to acknowledge with much appreciation to Cristina for sharing with me her knowledge on the methodology and instruments and her contribution in stimulating suggestions.

Furthermore, I would like to thank people from Ciència Animal i dels Aliments departament for their always kind and useful advices and for their amiability. Special thanks go for Sonia Llorens for her constant useful advices.

Likewise I am really glad to have met all the fellows whom I learn a lot for their always useful advices and also I enjoyed a lot inside and outside work.

Finally, but not less important I would like to acknowledge my lovely family and friends for all the help given, advice, support and love. Without them this master´s thesis would not have been possible.

Abbreviations key

ANOVA	Analysis variance
Asp	Aspartic acid
BS	Backscattering
CH	Conventional homogenization
CH-E	Emulsion treated by conventional homogenization
CH-M	Mixing of the emulsions and the skim milk before UHT treatment treated by conventional homogenization
CH-P	UHT milk-based product containing CLA emulsion treated by conventional homogenization.
CLA	Conjugated linoleic acid
$d_{3,2}$	Surface mean diameter
$d_{4,3}$	Volume mean diameter
FAA	Free amino acids
FFA	Free fatty acid
Glu	Glutamic acid
T	Transmission
UHPH	Ultra-high Pressure Homogenization
UHPH-E	Emulsion treated by Ultra-high Pressure Homogenization
UHPH-M	Mixing of the emulsions and the skim milk before UHT treatment treated by Ultra-high Pressure Homogenization
UHPH-P	UHT milk-based product containing CLA emulsion treated by Ultra-high Pressure Homogenization emulsion

Table of content

Abstract

The effect of Ultra-high Pressure Homogenization (UHPH; 200 MPa) on the physical and the oxidative stability of an UHT milk-based product containing a soybean oil emulsion enriched with conjugated linoleic acid (CLA; 6%, v/v) in a free fatty acid form was evaluated and compared with a conventional homogenization (CH; 15 MPa). Fresh emulsions (CH-E and UHPH-E), the mixing of the emulsions and the skim milk before UHT treatment (CH-M and UHPH-M) and the final milk-based product samples (CH-P and UHPH-P) were characterized by particle size distribution. Physico-chemical composition of the fresh milk-based products was also evaluated as well as rheological properties, colour, pH, physical stability, proteolysis and lipid oxidation during storage (4 months at 21ºC). UHPH-P exhibited similar rheological characteristics but greater physical stability compared to CH-P due to the lower particle size value ($d_{3,2}$ and $d_{4,3}$) of UHPH-P, which also presented lower content of hydroperoxides.
These results address the physical and the oxidative stability improvement of an UHT milk-based product containing a soybean oil emulsion enriched with conjugated linoleic acid produced by using the emerging technology of UHPH.

Key words: UHT milk-based product, conjugated linoleic acid, physical and oxidative stability, Ultra-high Pressure Homogenization, functional food.

Resumen

Se ha estudiado la estabilidad física y oxidativa de un producto de base láctea UHT conteniendo una emulsión de aceite de soja enriquecida con ácido linoleico conjugado (CLA; 6%, v/v) en forma de ácido graso libre, estabilizada por homogenización a ultra alta presión (UHPH; 200 MPa) en comparación al proceso de homogenización convencional (CH; 15 MPa). Se caracterizaron las emulsiones (CH-E y UHPH-E), la mezcla de las emulsiones con la leche desnatada antes del tratamiento UHT (CH-M y UHPH-M) y el producto final de base láctea (CH-P y UHPH-P) por su distribución y tamaño de partícula. También se evaluó la composición fisicoquímica del producto fresco así como sus propiedades reológicas, color, pH, estabilidad física, proteólisis y oxidación lipídica durante su almacenamiento (4 meses a 21 ºC). El UHPH-P mostró similares características reológicas pero mejor estabilidad física en comparación al CH-P debido al menor tamaño de partícula ($d_{3,2}$ y $d_{4,3}$) del UHPH-P, el cual también presentó contenidos menores de hydroperóxidos. Estos resultados sugieren una mejora en la estabilidad física y oxidativa de un producto UHT de base láctea enriquecido con una emulsión de aceite de soja conteniendo CLA utilizando la tecnología emergente de UHPH.

Palabras clave: producto UHT de base láctea, ácido linoleico conjugado, estabilidad física y oxidativa, homogenización a ultra alta presión, alimento funcional.

1. Introduction

There is a growing public interest in functional food products that includes the daily intake of required micronutrients, such as vitamins, essential fatty acids and antioxidant, due to their role in maintenance of health and in the prevention of diseases (Gao et al., 2014). In the past decade, there has been an increasing interest in the incorporation of lipophilic bioactives, such as conjugated linoleic acid (CLA) into various food products, the most common being dairy products (Campbell et al., 2003; Gao et al., 2014; Yao et al., 2013).

CLA is a mixture of positional and geometric isomers of octadecadienoic acid (18:2) and the double bonds are adjacent with no interceding methylene group as in linoleic acid (18:2n-6). It is naturally found in dairy and beef products from ruminant animals (Kim et al., 2016) at levels of 0.3-0.8% (w/w) fat (Griinari & Bauman, 1999). The position of conjugated double bonds may vary from 7 to 14 carbon atom by including all possible geometric configuration of cis-trans, trans-cis, cis-cis, and trans-trans isomers which may have different effects (EFSA Panel on Dietetic Product, 2010; Ha et al., 1987). Among the CLA isomers, the most representative are c9,t11-18:2 (rumenic acid) and t10,c12-18:2 (EFSA Panel on Dietetic Product, 2010; Fernandez-Avila et al., 2016; Yao et al., 2013). However, rumenic acid, is the main isomer constituting 90% of the total CLA found in dairy and beef lipids (Ferreiro et al., 2015; Sonja & Jan, 1998). CLA has been found to confer many potential health benefits, such as anticarcinogenic, antiobese, antidiabetic, antihypertensive properties, atherosclerosis reduction and immune response enhancement with a daily dietary intake of 3 g (Fernandez-Avila et al., 2015; Gao et al., 2014; Khaskheli et al., 2013; Koba & Yanagita, 2014; Yao et al., 2013; Yettella et al., 2011).

However, CLA exhibits very poor water solubility and chemical stability presenting autoxidation and isomerization during thermal processing (i.e. UHT). This phenomena decreases its nutritional value and may cause off-flavour development (Fernandez-Avila et al., 2016; Xiang et al., 2015; Yang et al., 2000; Yao et al., 2013; Zhang & Chen, 1997). To overcome these problems, it is important to develop effective delivery systems to encapsulate and protect CLA. Among the methods used to protect CLA, different strategies have been used, such as nanocapsules (Gao et al., 2014; Heo et al., 2016; Jimenez et al., 2008; Lalush et al., 2005), spray-drying (Choi et al., 2010;

Costa et al., 2015; He et al., 2016), and emulsions produced by conventional homogenization (Fernandez-Avila et al., 2016).

The emergent UHPH technology is able to produce fine and stable submicron emulsions (<1 μm), to modify the viscous properties of fluids due to the particle size reduction, to facilitate metabolite extraction, as well as to achieve inactivation of microorganisms, enzymes or even some viruses (Dumay et al., 2013; Fernandez-Avila et al., 2015; Hebishy et al., 2015).

Recently, the ability of oil-in-water emulsions (20% oil) treated by UHPH and CH and stabilized by soy protein isolates (4%) to deliver conjugated linoleic acid (CLA, 6%), has been studied by Fernandez-Avila and Trujillo (2015), showing that UHPH treatment is able to produce sterile emulsions with better physical and oxidative stabilities while maintaining similar percentages of bioavailability of CLA, in comparison to CH-treated emulsions. These results suggest the potential use of UHPH technology for delivering CLA in functional foods.

The aim of the study was to investigate the physical and the oxidative stability of an UHT milk-based product containing a soybean oil emulsion enriched with CLA in a free fatty acid form (FFA), stabilized by using UHPH (200 MPa) in comparison to the product containing the emulsion treated by CH (15 MPa).

2. Material and methods

2.1. Materials

A commercial SPI (PRO-FAM 974) was purchased from Lactotecnia (Barcelona, Spain). The composition of this commercial SPI according to manufacturer was: ≥90% protein, < 4% fat, < 6% moisture, and < 5% ash (dry basis, w/w). SPI PRO-FAM 974 has acid character and its isoelectric point is 4.6 (Kinsella 1979) due to the high content of glutamic acid (Glu, 19.2%) and aspartic acid (Asp, 11.5%). Solubility of PRO-FAM 974 at pH = 7 is 39.5% (Bissegger 2007). Soybean oil was purchased from Gustav Heess (Barcelona, Spain). Peroxide value of the soybean oil was below 10 meq O_2/kg and the acidity index was below 0.5 mg KOH/g. A free fatty acid (FFA) mixture high in isomers of CLA (Neobee®CLA80) was kindly donated from Stepan Specialty Products LLC (Maywood, USA), containing 80.4% of total CLA. The two main isomers present were c9, t11-CLA and t10.c12-CLA in a free form, in a 50:50 ratio. Other CLA isomers (*cis,cis* and *trans,trans*) were present in minor concentrations (<1.2%). Neobee®CLA80 was obtained from natural safflower oil by a gentle process without antioxidants added. Peroxide value of CLA was below 10 meq O_2/kg. All other chemicals used were of analytical or better grade.

2.2. Methods

2.2.1. *Preparation of oil-in-water CLA emulsions*

Oil-in-water emulsions were prepared with a fixed content of SPI (4%, w/v) and oil (20%, v/v), containing CLA (6%, v/v) and soybean oil (14%, v/v). Firstly, the stock protein dispersion (4%, w/v) was prepared by dispersing SPI in deionised water by using a high-speed dispersing unit at a rate of about 250 rpm for 1 h at 25 ºC. Protein dispersions were stored overnight at 4 ºC to allow complete hydration. Protein dispersions and oil were equilibrated at 20 ºC (inlet temperature) before mixing. Pre-emulsions (or coarse emulsions) were prepared by mixing the protein dispersions with the soybean oil using rotor stator emulsifying unit (model Diax 900; Heidolph, Kehlheim, Germany) at 15000 rpm for 2 min. The coarse emulsions were further homogenised by conventional homogenization (CH) or Ultra-High-Pressure Homogenization (UHPH) producing the corresponding emulsions (CH-E and UHPH-E). UHPH emulsions were treated at 200 MPa (single-stage) by an UHPH-Ypsicon equipment (Ypsicon Advanced Technologies, S.L., Barcelona, Spain) with a flow rate

of 150 L/h and provided with a high-pressure ceramic needle-seat valve. CH emulsions were homogenized at 25 MPa with a flow rate of 250-350 L/h, using Tetra Alex® Homogenizer (Model S05 A, Tetra Pak Processing Components AB, Lund, Sweden) with a cobalt carbide mushroom valve. Pre-emulsions were passed through both devices with an inlet temperature (T_{in}) of 25 ºC. The outlet temperature of UHPH emulsions was controlled by a heat exchanger located immediately after the high-pressure valve. During UHPH treatments, the T_{in}, the temperature after the high pressure valve (T1), and outlet temperature were monitored.

2.2.2. *Production of milk-based product*

To produce the milk-based products, skim milk was mixed with CH emulsion or UHPH emulsion, in a ratio of 1:10 (emulsion:skim milk), to obtain fat content of 2% in the products (CH-M or UHPH-M). Concentrations used in the final product would be in the range of what is necessary to deliver a sufficient amount of CLA to be nutritionally significant (0.6% CLA; 600 mg/100 mL). To both products indirect UHT treatment was applied in a tubular indirect Finamat heat exchanger (model 6500/010; GEA Finnah GmbH, Ahaus, Germany) at 140 °C for 4 s. Final milk-based products (CH-P and UHPH-P) were aseptically packed in 200 mL Tetra Brick Aseptic slim containers using aseptic packaging line (TBA9, Tetra Pak Processing Components AB). Products were held at 21 °C during 4 months. Physico-chemical composition of the fresh milk-based products was evaluated and their rheological properties, colour, pH, physical stability, proteolysis and lipid oxidation were evaluated over time.

2.2.3. Determination of oil droplet size distribution

The particle size distribution in the emulsions (CH-E and UHPH-E), the mixing of the emulsions and the skim milk before UHT treatment (CH-M and UHPH-M) and the final milk-based product samples (CH-P and UHPH-P) was determined immediately after each process, using a Beckman Coulter laser diffraction particle size analyzer (LS 13 320 series, Beckman Coulter, Fullerton, CA, USA). Emulsion samples were diluted in distilled water until an appropriate obscuration was obtained in the diffractometer cell. The optical parameters used were: a refractive index of 1.475 for the soybean oil and a refractive index of 1.332 for the water. The surface-weighted mean diameter ($d_{3.2}$, μm) and volume-weighted mean diameter ($d_{4.3}$, μm) were determined.

2.2.4. *Physico-chemical composition*

Fat content was measured by Gerber method (ISO 2446:2008/IDF 226:2008). Total protein content was determined by measuring total nitrogen using the Kjeldahl method (ISO 8968-3:2004/IDF 20-3:2004). Total solids content was enumerated based on a mass fraction of substances remaining after the heating process (ISO 6731:2010/IDF 21:2010). The pH of milk-based products was evaluated by electrode immersion with a micro pH-potenciometer (GLP 21model 2001; Crison Instruments SA, Alella, Spain).

2.2.5. Rheological measurements

Rheological measurements were performed using a controlled stress rheometer (Haake Rheo Stress 1, Thermo Electron Corporation, Karlsruhe, Germany) using a cone (1º, 60 mm diameter) and plate geometry probe at 21 ºC. Flow curves (shear stress compared to shear rate) were determined at increasing and decreasing shear rates between 0.001 s^{-1} and 100.0 s^{-1} in 1 min (up and down flow curves). Flow curves were fitted to the Newton model, $\tau = \eta\gamma$, where τ is the shear stress (Pa), η is the viscosity (Pa × s), and γ is the shear rate (s−1).

2.2.6. Colour

The colour values of the milk-based products were measured using a Hunter Lab colorimeter (MiniScan XE Hunter Associates Laboratory Inc., USA). Colour coordinates were measured with an illuminant of D65 and a standard observer of 10° and the colorimeter was calibrated against white and black tile standards. Data was acquired in the CIELab colour space, where L* value represents the lightness with values from 0 (black) to 100 (white), which indicates a perfect reflecting diffuser. The *a** and *b** axes have no specific numerical limits and represent chromatic components. The *a** values are a measure of redness (higher positive values indicate a redder colour, higher negative values indicate a greener colour); *b** values are a measure of yellowness (higher positive values indicate a more yellow colour, higher negative values indicate a more blue colour). Data were used then to calculate the total colour difference $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ formula equation. ΔL^*, Δa^* and Δb^* are the differences in the tristimulus coordinates between L*, a* and b* of the samples over time and the standard (day 0) of CH-P and UHPH-P, which gives an idea of the influence of the homogenization treatment contributing to the overall colour.

2.2.7. Physical stability

The optical characterization of milk-based products was analysed using a Turbiscan® MA 2000 (Formulaction, Toulouse, France), which is composed by a reading head (pulsed near-IR light source; λ = 850 nm, and two synchronous detectors of transmission and backscattering) which scans the sample glass tube. Milk-based product samples were placed in a cylindrical glass cell, under sterile conditions, on the day of production. Product destabilization (sedimentation and creaming) was analysed. When backscattered light is in the range of 0% to 100%, propagation of light goes respectively through a clear to an opaque dispersion. Thus, if backscattering is nearly 100%, it indicates sedimentation or creaming.

2.2.8. Proteolysis of the milk-based products

The pH 4.6-soluble fraction of milk-based products was obtained by isoelectric casein precipitation. Briefly, samples were centrifuged at 20.000 *g* for 10 min at 21 ºC to separate the fat content. The top cream layer was removed and the pH of skim milk-based products (1 mL) was adjusted to 4.6 by adding 15 µL of acetic acid of 33.3% (v/v), followed by addition of 15 µL of 3.33 M sodium acetate. The mix was centrifuged at 2800g for 5 min and 21 ºC, and the supernatant was collected. From this fraction the total free amino acids (FAA) was determine by the cadmium-ninhydrin method of Folkertsma and Fox (1992), measuring the total FAA concentrations (mg Leu/mL milk) at 507 nm and using a standard curve made from leucine (0.002-0.04 mg Leu/mL).

2.2.9. ***Analysis of primary and secondary oxidation***

The formation of lipid hydroperoxides was evaluated according to the method of (Hu, McClements, et al.,2004). In brief, aliquots (0.3 mL) of the emulsions, after storage of a specific incubation period, were mixed with 1.5 mL of isooctane/2-propanol (3:1, v/v) by vortexing. The organic solvent phase of the mixtures was collected by centrifugation at 1000 *g* for 2 min. The organic solvent phase (200 µL) was added to 2.8 mL of a methanol:1-butanol mixture (2:1, v/v), followed by addition of 15 µL of 3.94 M ammonium thiocyanate and 15 µL of ferrous iron solution (prepared by mixing 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$). The absorbance of the resultant solutions were measured at 510 nm 20 min after addition of the iron. Lipid hydroperoxide concentrations (mmol/L

of emulsion) were determined using a standard curve made from cumene hydroperoxide (0.5-5 mmol/L). Afterwards, data was expressed in mmol/L of final product.

To determine the formation of secondary oxidation products, TBARs technique was performed. It detects the formation of malondialdehyde, which reacts with thiobarbituric acid (TBA). Malondialdehyde or TBARs content of the emulsions upon storage at 37 °C for 14 days was determined using a process as described by Sørensen & Jørgensen (1996), with a few modifications. In brief, emulsions (3.0 mL) were mixed with 6.0 mL of tricholoracetic acid (TCA) reagent (7.5%, w/v) and were vortexed. The resultant mixtures were filtered with a 1 μm microporous membrane after 20 min of the TCA addition. The filtered (2.0 mL) were placed in test tubes containing TBA reagent (0.8%, w/v) and they were mixed by vortexing. The resultant mixtures were heated in a water bath (95 ºC) for 15 min, and then cooled immediately in an ice-bath to slow down the reaction. The absorbance of the final extracts was recorded at 532 and 450 nm (A_{450}). The TBARs concentration (mmol/L of emulsion) was determined according to a standard curve with hexanal.

2.2.10. *Statistical analyses*

Descriptive statistics, mean and standard deviation, were listed for each variable in this study. In order to evaluate the physico-chemical stability of the milk-based products containing CLA over time, one-way ANOVA was performed. The statistical analysis was performed using the SPSS® v17.0 package to a 95% level of significance and Tukey adjustment was performed ($P < 0.05$). Also, the Student's t-test for paired samples analysis was carried out to compare the changes between samples (CH and UHPH). Significance was defined as $P < 0.05$ for a two-sided test. Experiments were performed in duplicate as separate independent runs.

3. **Results and discussion**

3.1 Determination of oil droplet size distribution

Figure 1 shows the effect of UHPH (200 MPa) and CH (15 MPa) treatments on particle size distribution in emulsions (E), milk-based products containing those emulsions before UHT (M) and final UHT milk-based product (P).

The particle size distribution curve of CH-E showed a bimodal particle distribution between 0.07 and >100 μm, whereas UHPH-E showed a monomodal distribution and smaller particle size distribution, between 0.05 and <10 μm (Fig. 1A and B for emulsions). CH-E was characterized by a main peak of about 50 μm due to the formation of large particles or to coalesced fat globules, and UHPH-E was characterized by a main peak of about 0.7 μm and a second minor peak that could correspond to small particle aggregates.

Before UHT treatment, changes in particle size distribution could be observed when emulsions were mixed with skimmed milk. For UHPH-M the particle distribution changed from bimodal to monomodal, which size comprised between 0.03 and <1 μm (grey line) and characterised by a main peak of about 0.2 μm. In the case of the CH-M, a change in the typical bimodal distribution was visible, with a dramatic increase in the first peak intensity and a displacement of the second peak (Fig.1A and B for emulsions before UHT).

Finally, curves of size distribution of CH-P showed a polydisperse distribution profile with the presence of a second group of larger particles due to the possible disruption of particles owing to turbulences created during the UHT treatment. UHPH-P showed a displacement of the peak with a change of size distribution into the narrowest distribution (Fig. 1A and B).

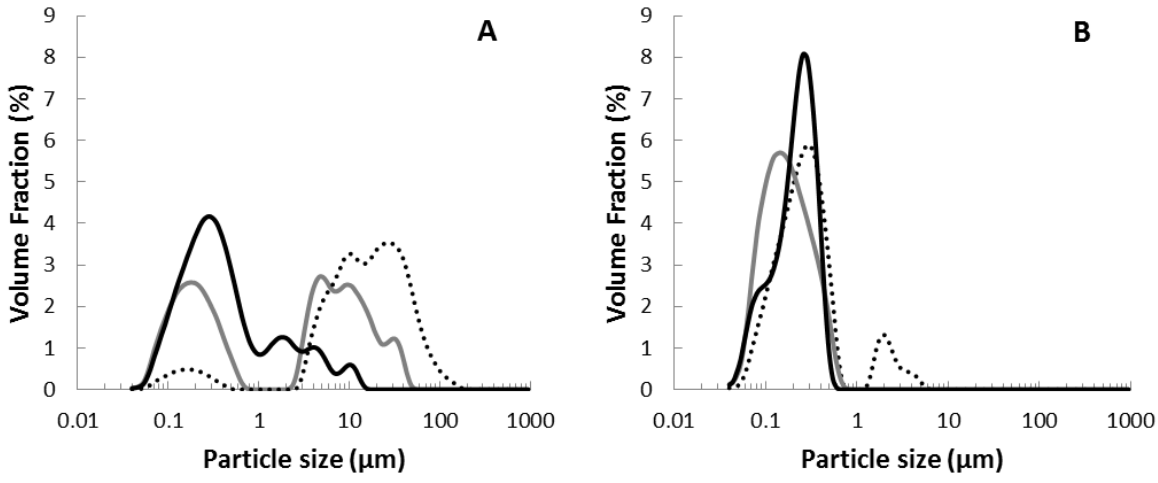

Fig 1. Size distribution profiles of emulsions (dotted line), the mixing of the emulsions and the skim milk before UHT treatment (grey line) and the final milk-based product (black line) samples treated by conventional (A) and by Ultra-high Pressure (B) homogenization.

The particle size values ($d_{3,2}$ and $d_{4,3}$) for E, M and P samples treated by CH and UHPH are listed in Table 1. The particle size ($d_{3,2}$ and $d_{4,3}$) were significantly ($P < 0.05$) reduced in CH-M and UHPH-M samples in comparison to their counterpart E samples, but not significant changes were found in CH-P and UHPH-P, in comparison to their respective M samples. As expected, the reduction in $d_{3,2}$ and $d_{4,3}$ values, achieved in UHPH samples was higher ($P < 0.05$) than that obtained by the CH treatment.

Table 1. Mean values ± SD of $d_{3,2}$ and $d_{4,3}$ indexes of emulsions (E), the mixing of the emulsions and the skim milk before UHT treatment (M) and the final UHT milk-based product samples (P) treated by conventional homogenization (CH) and Ultra-high Pressure Homogenization (UHPH).

Stage treatment	Droplet size distribution			
	$d_{3,2}$ (μm)		$d_{4,3}$ (μm)	
	CH	UHPH	CH	UHPH
E	$3.25^{a,x} \pm 1.86$	$0.21^{a,y} \pm 0.01$	$36.64^{a,x} \pm 14.73$	$1.72^{a,y} \pm 1.43$
M	$0.34^{b,x} \pm 0.02$	$0.15^{b,y} \pm 0.00$	$7.67^{b,x} \pm 0.65$	$0.23^{b,y} \pm 0.04$
P	$0.28^{b,x} \pm 0.01$	$0.16^{b,y} \pm 0.03$	$1.73^{c,x} \pm 0.65$	$0.24^{b,y} \pm 0.01$

[a, b] Means within a column for each treatment with a different superscript were significantly different ($P < 0.05$).
[x, y] Means within a row for each parameter with a different superscript were significantly different ($P < 0.05$)

The results obtained are in accordance with the results of Codina-Torrella (2016) who reported that UHPH treatment of milk results in a greater reduction of the size of milk fat globules than CH with increasing pressures up to 200 MPa, achieving a narrow particle size distribution in comparison to CH. This particle size reduction decrease the creaming rate and consequently improves shelf life of the product (Thiebaud et al., 2003). Furthermore, greater reduction of particle size achieved by UHPH at 200 MPa compared to CH has been observed in O/W emulsions containing vegetable oils and whey protein isolate as emulsifier (Hebishy et al., 2015), soy milk (Cruz et al., 2007; Poliseli-Scopel et al., 2012), almond milk (Valencia-Flores et al., 2013) and tiger nut milk (Codina-Torrella et al., 2016).

3.2 Physico-chemical composition

Chemical composition of UHT milk-based products containing CH and UHPH emulsions are showed in Table 2. No significant ($P > 0.05$) difference can be observed between both treatments, therefore homogenization process did not influence in protein, fat and total solid content of CH-P and UHPH-P.

Table 2. Mean values ± SD of protein, fat and total solid content in UHT milk-based products containing CLA-emulsions treated by conventional homogenization (CH-P) or by Ultra-high Pressure Homogenization (UHPH-P).

	Chemical composition		
	Protein (%)	Fat (%)	Total solid (%)
CH-P	3.31 ± 0.08	1.65 ± 0.00	9.88 ± 0.05
UHPH-P	3.32 ± 0.004	1.65 ± 0.00	10.08 ± 0.002

The pH of milk-based products was relatively unaffected by the homogenization treatments applied to the oil emulsions (Table 3), showing at day 1 acidic pH values closed to neutrality. However at 30, 60 and 120 days of storage, the pH values of CH-P were slightly but significantly lower ($P < 0.05$) than the pH values of UHPH-P. For both products the pH had a slight but significant ($P < 0.05$) decrease during the storage time at 21 ºC up to day 90, although a not significant increase in the pH values at 120 days of storage was evidenced (Table 3).

Table 3. Mean values ± SD of pH UHT milk-based products containing CLA-emulsions treated by conventional homogenization (CH-P) and Ultra-high Pressure Homogenization (UHPH-P).

	pH	
Time (days)	CH-P	UHPH-P
1	$6.33^{a} \pm 0.02$	$6.36^{a} \pm 0.04$
30	$6.28^{ab,y} \pm 0.01$	$6.32^{ab,x} \pm 0.01$
60	$6.29^{a,y} \pm 0.04$	$6.32^{ab,x} \pm 0.03$
90	$6.18^{c} \pm 0.04$	$6.24^{c} \pm 0.01$
120	$6.22^{bc,y} \pm 0.04$	$6.27^{bc,x} \pm 0.02$

^{a,b,c} Means within a column for each treatment with a different superscript were significantly different ($P < 0.05$).
$^{x, y}$ Means within a row for each parameter with a different superscript were significantly different ($P < 0.05$).

3.3 Rheological measurements

Viscosity affects the flow conditions in dairy processes and the mouthfeel sensory attributes. Furthermore, is an important physical property related to milk shelf life because it is associated with the rate of creaming (Amador-Espejo et al., 2013; Pereda et al., 2007). The fat globule size reduction of emulsions lowers the creaming rate and consequently improves shelf life (Thiebaud et al., 2003).

Table 4 shows the viscosity evolution (mPa × s) of UHT milk-based products containing CH and UHPH emulsions during storage at 21 ºC and measured by the Newton model ($n \approx 1$).Values measured in CH-P showed a slightly but significant ($P < 0.05$) decrease of viscosity during storage from day 60. Nevertheless, the UHPH-P viscosity values also indicated a slight decrease, but in this case not significant ($P > 0.05$). By comparing CH-P and UHPH-P no significant ($P > 0.05$) differences could be observed, although UHPH-P had always lower values than CH-P during storage.

Table 4. Evolution of viscosity (mPa × s) of UHT milk-based products containing CLA-emulsions treated by conventional homogenization (CH-P) and Ultra-high Pressure Homogenization (UHPH-P) during storage at 21 ºC.

Viscosity measurements		
Time (days)	CH-P	UHPH-P
1	$2.084^{a} \pm 0.039$	$2.020^{a} \pm 0.050$
30	$2.011^{ab} \pm 0.014$	$1.968^{a} \pm 0.088$
60	$1.965^{b} \pm 0.069$	$1.954^{a} \pm 0.004$
90	$1.960^{b} \pm 0.060$	$1.958^{a} \pm 0.058$
120	$1.972^{b} \pm 0.023$	$1.984^{a} \pm 0.114$

[a,b] Means within a column for each treatment with a different superscript were significantly different ($P < 0.05$).

As it has been already mentioned in other studies, viscosity can be influenced by particle size, casein micelle aggregates, and denaturation of whey proteins by heat (Pereda et al., 2007). Floury et al. (2000) working on O/W emulsions, in a range of pressures from 20 to 150 MPa, found that as pressure increased, the mean droplet diameters, the droplet size distributions, and viscosity of emulsions decreased. Moreover, the stable values of UHPH-P confirm the capacity of UHPH to produce stable emulsions.

3.4. Colour

The influence of treatments applied and storage time on the colour of CH-P and UHPH-P measured using a HunterLab colorimeter is shown in Table 5. For both products lightness (L*) and colour intensity (a* and b*) presented a significant ($P < 0.05$) increased tendency during storage time. L* values increased during the storage time, however the increase was only significant up to 60 days of storage. No significant differences were observed between treatments up to 60 days of storage; however, from 90 days of storage UHPH-P showed higher L* values (whiter colour) than CH-P.

Both products exhibited a slight but significant ($P < 0.05$) increase in a* values during storage up to day 30, which means that the product became less green during storage time. Significant differences ($P < 0.05$) in a* value between CH-P and UHPH-P were only detected at day 1, being CH-P greener than UHPH-P. In relation to b* values, CH-P and UHPH-P also show a significant increase ($P < 0.05$) from day 1 to day 30 but no differences were observed during the rest of the storage time. Significant differences ($P < 0.05$) in b* values between CH-P and UHPH-P were only detected from day 60, with CH-P being more yellowness than UHPH-P.

To compare general colour changes in the products a total colour difference (ΔE) from the tristimulus values was calculated measuring the colour coordinates of the milk-based products at day 1, and during storage time *t* related to the initial colour coordinates of the product at day 1 (Table 5). The ΔE between products at day 1 was 0.93 ± 1.50, while the ΔE of both products during storage was significantly ($P < 0.05$) different up to day 60, being the ΔE of UHPH-P during storage higher than CH-P. However, it would be necessary to assess by sensory evaluation whether these instrumental differences are detected or not by consumers and how they are considered in terms of quality.

Table 5. Colour parameters[1] of UHT milk-based products containing CLA-emulsions produced by conventional homogenization (CH-P) and Ultra-high Pressure Homogenization (UHPH-P).

Time (days)	Colour parameters							
	L*		a*		b*		$\Delta E^{day\ 1}$	
	CH-P	UHPH-P	CH-P	UHPH-P	CH-P	UHPH-P	CH-P	UHPH-P
1	$36.66^{b} \pm 1.06$	$35.99^{c} \pm 0.27$	$-1.37^{ab,y} \pm 0.14$	$-1.28^{a,x} \pm 0.15$	$3.53^{b} \pm 0.51$	$3.10^{b} \pm 0.46$	-	-
30	$37.06^{b} \pm 0.40$	$38.20^{b} \pm 0.61$	$-1.62^{b} \pm 0.11$	$-1.64^{b} \pm 0.05$	$4.55^{a} \pm 0.30$	$4.40^{a} \pm 0.10$	$0,97^{b,y} \pm 0,14$	$3,53^{b,x} \pm 1,61$
60	$38.74^{a} \pm 0.40$	$39.61^{a} \pm 1.12$	$-1.24^{a} \pm 0.16$	$-1.14^{a} \pm 0.13$	$4.93^{a,x} \pm 0.21$	$4.53^{a,y} \pm 0.16$	$3,90^{a,y} \pm 1,98$	$8,20^{a,x} \pm 3,62$
90	$37.56^{ab,y} \pm 0.72$	$38.37^{ab,x} \pm 0.27$	$-1.26^{a} \pm 0.16$	$-1.16^{a} \pm 0.09$	$4.85^{a,x} \pm 0.35$	$4.68^{a,y} \pm 0.27$	$2,27^{ab} \pm 1,31$	$4,32^{ab} \pm 0,28$
120	$38.04^{ab,y} \pm 0.34$	$38.34^{ab,x} \pm 0.33$	$-1.33^{ab} \pm 0.14$	$-1.18^{a} \pm 0.07$	$4.76^{a,x} \pm 0.20$	$4.36^{a,y} \pm 0.18$	$2,31^{ab,y} \pm 1,33$	$3,64^{b,x} \pm 0,61$

[1]Mean values ± SD of colour parameters. ΔE was calculated taking into account day 1 as reference sample.
[a, b] Means within a column for each treatment with a different superscript were significantly different ($P < 0.05$).
[x, y] Means within a row for each parameter with a different superscript were significantly different ($P < 0.05$).

3.5 Physical stability

Turbiscan allow us to scan the turbidity profile of an emulsion along the height of a glass tube filled with the emulsion, in order to follow the fate of the turbidity profile over time. These profiles constitute the macroscopic fingerprint of the emulsion samples at a given time (Herrera, 2011). The results expressed are based on the increase of the light signal refracted in the product (backscattering), which is directly proportional to the concentration of particles. This is translated into the possibility to detect the two major destabilisation phenomena affecting the homogeneity of dispersions: particle migration (creaming, sedimentation) and particle size variation or aggregation (coalescence, flocculation) (Mengual et al., 1999).

Figure 2 shows the turbidity profile for CH-P and UHPH-P. At the top and bottom of the vial stands creaming and sedimentation layer of the particles, respectively, are represented by an intense orange. As noted, creaming layer in CH-P appeared before 1 month and increased during the storage time. Onto the sedimentation zone, some clarification was observed in CH-P compared to UHPH-P (represented by the appearance of a diffuse white coloration), which is due to migration of the particles toward the surface container (Fig. 2A). This clarification phenomena may be related to physical instability of the product and produce a significant ($P < 0.05$) viscosity decrease of CH-P during storage time.

From Figure 2, it is clearly visible that CH-P was more unstable than UHPH-P due to larger extent in creaming during storage. A possible explanation could be the larger particle size compared to UHPH-P, as it has been already mentioned (see Section Determination of oil droplet size distribution).

Findings in the literature suggest that UHPH improves the creaming stability of emulsions by decreasing the droplet diameters (Floury et al., 2002; Lee et al., 2009). When the particle sizes are < 100 nm, creaming is greatly reduced and aggregation becomes a dominant mechanism for emulsion instability (McClements, 2005).

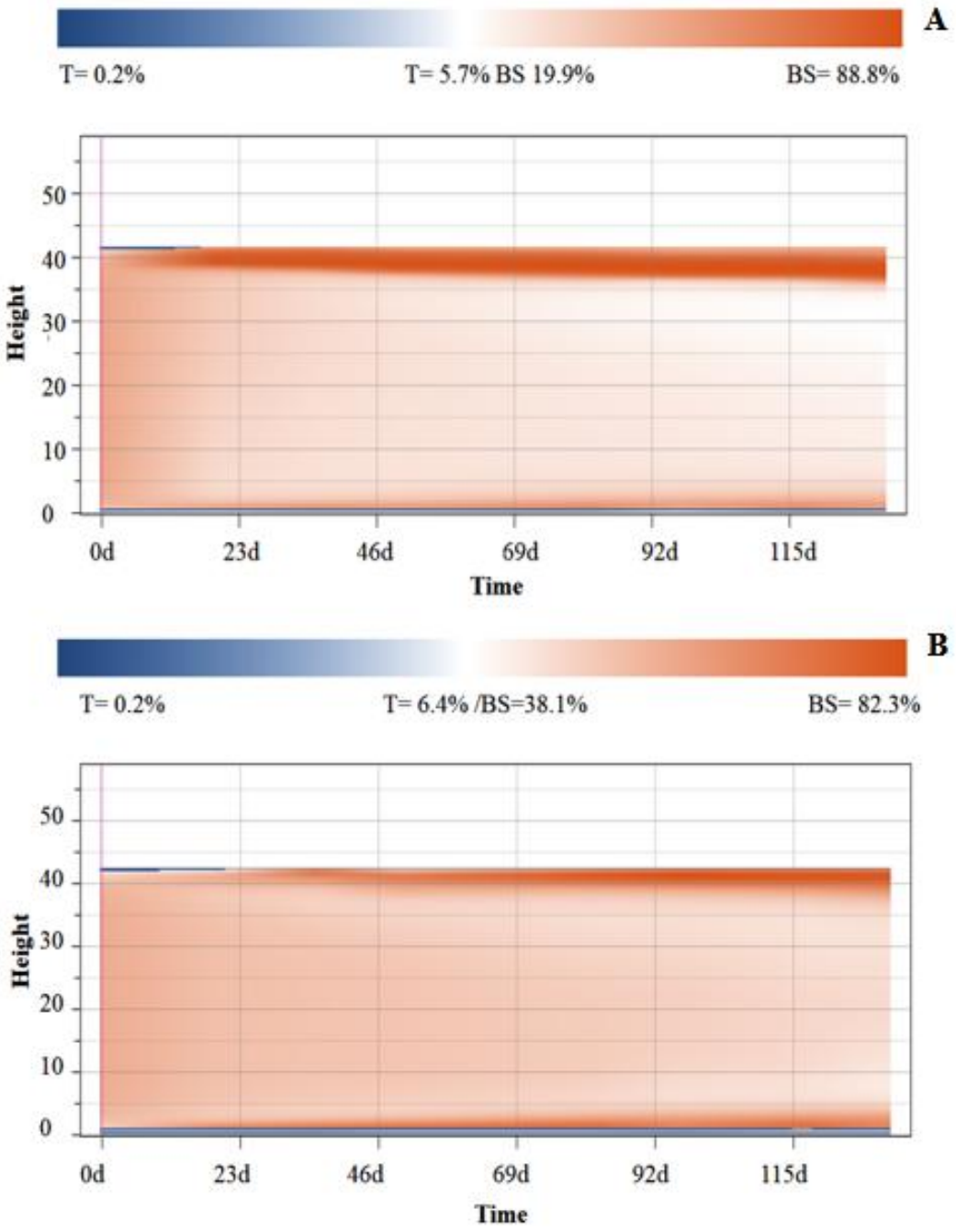

Figure 2. Evolution in backscattering (BS) and transmission (T) of UHT milk-based products containing CLA-emulsions treated by conventional (A) or Ultra-high Pressure (B) homogenization during storage time.

3.6. Proteolysis in milk-based products

Proteolysis of UHT milk be mainly caused by indigenous proteases and by those produced by psychotrophic bacteria during cold storage of raw milk (Datta & Deeth, 2003; Pereda et al., 2008). Plasmin (PL) and its inactive precursor plasminogen are the main proteolytic enzymes in milk, associated with the casein micelle and the milk fat globule membrane, that can survive severe heat treatments (Bastian & Brown, 1996; Enright et. al, 1999). PL, the principal indigenous milk proteinase, has optimum activity at 37 ºC and pH 7.4, hydrolyses mainly β- and α_{s2}-caseins, and more slowly α_{s1}-casein (Bastian & Brown, 1996), and its activity, in general, generates sensory problems in commercial sterile milks, because of the partial hydrolysis of these caseins causes development of bitter and astringent flavours, and often causes a loss of quality during storage (Datta & Deeth, 2003; Enright et al., 1999; Santos et al., 2003).

Among the treatments applied in this study the FAA concentration determined by the cadmium-ninhydrin method in milk-based products containing oil emulsions treated by CH and UHPH (data not shown) for both treatments was situated in the range of 0.05-0.07 mg Leu/ml and there were no significant ($P>0.05$) differences for products. These results suggesting a low or no plasmin activity and/or of other exogenous proteases, and a similar propensity to proteolysis in both UHT products.

3.7 Oxidation stability

Lipid oxidation is the main reason for the deterioration of fats and oils (Matthäus, 2010) that consists of the reaction of molecular oxygen with unsaturated fatty acids, resulting in unsaturated hydroperoxides (Gunstone & Martini, 2010). Lipid hydroperoxides, the primary oxidation products, are unstable molecules that can further decompose to a wide range of volatile and non-volatile secondary products, particularly aldehydes, responsible for undesirable odours and flavours (McClements & Decker, 2000).

Lipid hydroperoxides content can be determined by spectrophotometric method that measures the ability of lipid hydroperoxides to oxidize ferrous ions to ferric ions, which are complexed by thiocyanate. Ferric thiocyanate, a red-violet complex shows strong absorption at 500-510 nm (Dobarganes & Velasco, 2002). Lipid hydroperoxides content can signalise the initial stages of lipid oxidation in milk-based products (Ruiz et al., 2001).

Results obtained for primary oxidation (Table 6) showed an irregular behaviour with increasing and decreasing periods of lipid hydroperoxides in both milk-based products. As can be seen, a significant decrease ($P<0.05$) of hydroperoxides in CH-P and UHPH-P up to 60 days of storage was observed followed by a significant ($P<0.05$) increase to day 90 and finally by a significant ($P<0.05$) decrease. This irregular behaviour with increasing and decreasing periods of lipid hydroperoxides can be explained because of the instability of primary oxidation products. As peroxides are unstable primary oxidation products, they transform quickly into secondary oxidation products including aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds (McClements & Decker, 2000).

Table 6. Mean values ± SD of primary oxidation of UHT milk-based products contain CLA-emulsions treated by conventional homogenisation (CH-P) and ultra-high press homogenization (UHPH-P) expressed in mmol/L of final product.

	Primary oxidation	
Time (days)	CH-P	UHPH-P
1	$0.575^{a} \pm 0.010$	$0.592^{a} \pm 0.019$
30	$0.535^{b} \pm 0.017$	$0.525^{b} \pm 0.006$
60	$0.292^{e} \pm 0.021$	$0.280^{e} \pm 0.008$
90	$0.442^{c, x} \pm 0.015$	$0.415^{c, y} \pm 0.006$
120	$0.375^{d, x} \pm 0.006$	$0.320^{d, y} \pm 0.018$

[a-e] Means within a column for each treatment with a different superscript were significantly different ($P < 0.05$).
[x, y] Means within a row for each parameter with a different superscript were significantly different ($P < 0.05$).

By comparing both products, significant differences ($P < 0.05$) were detected from 90 days of storage, being the level of lipid hydroperoxides lower for UHPH-P than for CH-P. The examination of secondary oxidation in CH-P and UHPH-P measuring thiobarbituric acid-reactive substances (TBARS) showed no differences between treatments neither during storage time with a mean A_{450} of 0.04 ± 0.00 for all measurements.

Lipid oxidation is accelerated by reactions that take place at the surface of oil-in-water emulsion droplets. Based on this principle alone and as could be expected, the rate of lipid oxidation should increase in the UHPH-P rather than CH-P, as the droplet sized decreased in UHPH-P and hence the specific surface area increased. However, it is interesting to note that the UHPH-P presented better oxidation stability than CH-P, indicating that other factors than the particle size or specific surface area are important determinants for oxidative stability, i.e. the quantity of soy protein directly in contact with the lipid phase (adsorbed soy protein), the thickness and viscoelastic properties of interfaces formed by proteins around lipid droplets, and the partial denaturation of soy proteins due to the UHPH treatment, which would produce more exposition of the hydrophobic regions of proteins making the protein adsorbs even better to the oil-water interface (Fernandez-Avila et al., 2015 & 2016; Hebishy, 2013).

4 Conclusions

The UHT milk-based product containing the CLA-emulsion treated by UHPH presented similar rheological behaviour, but smaller particle size distribution and better physical and oxidative stability, compared to its homologous product containing CLA-emulsion treated by CH. Creaming rate of UHT milk-based product containing the UHPH CLA-emulsion was much slower and it contained lower hydroperoxide content than in the product with CH emulsion.

It can be concluded from this study, that the UHT milk-based products containing CLA emulsions treated by UHPH provide a more stable food matrix for CLA than the product with emulsion prepared by CH. However, it would be necessary to assess the sensory characteristics of the developed UHT milk-based products in order to assess the impact of the CLA fortification on the consumer´s acceptance.

5. References

Amador-Espejo, G. G., Suàrez-Berencia, A., Juan, B., Bárcenas, M. E., & Trujillo, A. J. 2014. Effect of moderate inlet temperatures in ultra-high-pressure homogenization treatments on physicochemical and sensory characteristics of milk. *Journal of Dairy Science,* 97 (2): 659–671.

Bastian, E. D., & Brown, R. J. 1996. Plasmin in milk and dairy products: An update. *International Dairy Journal*, 6: 435–457.

Bissegger, S. 2007. Production of bioactive soy peptides (Thesis) HES-SO Valais.

Campbell, W., Drake, M. A & Larick, D.K. 2003. The impact of fortification with conjugated linoleic acid (CLA) on the quality of fluid milk. *Journal of dairy science*, 86 (1): 43–51.

Choi, K.O, Ryu, J., Kwak, H & Ko, S. 2010. Spray-dried conjugated linoleic acid encapsulated with Maillard reaction products of whey proteins and maltodextrin. *Food Science and Biotechnology*, 19 (4): 957–965.

Codina-Torrella, I., Guamis, B., Ferragut, V. & Trujillo, A.J. 2016. Potential application of ultra-high pressure homogenization in the physico-chemical stabilization of tiger nuts' milk beverage. *Innovative Food Science & Emerging Technologies* (*in press*).

Costa, A. M. M., Nunes, J. C., Lima, B. N. B., Pedrosa, C., Calado, V. & Torres, A. G. 2015. Effective stabilization of CLA by microencapsulation in pea protein. *Food Chemistry*, 168: 157–166.

Cruz, N., Capellas, M., Hernández, M., Trujillo, A. J., Guamis, B., & Ferragut, V. 2007. Ultra high pressure homogenization of soymilk: Microbiological, physicochemical and microstructural characteristics. *Food Research International*, 40(6): 725–732.

Datta, N. & Deeth, H.C. 2003. Diagnosing the cause of proteolysis in UHT milk. *LWT - Food Science and Technology*, 36(2): 173–182.

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies) 2010. Scientific Opinion on the substantiation of health claims related to conjugated linoleic acid (CLA) isomers and contribution to the maintenance or achievement of a normal body weight, increase in lean body mass. *EFSA Journal*, 8(10), 26. 1794.

Enright, E., Bland, A. P., Needs, E. C., & Kelly, A. L. 1999. Proteolysis and

physicochemical changes in milk on storage as affected by UHT treatment, plasmin activity and KIO3 addition. *International Dairy Journal*, 9: 581–591.

Fernandez-Avila, C., Arranz, E., Guri, A., Trujillo, A. J. & Corredig, M. 2015. Vegetable protein isolate-stabilized emulsions for enhanced delivery of conjugated linoleic acid in Caco-2 cells. *Food Hydrocolloids*, 55: 144–154.

Fernandez-Avila, C., Trujillo, A.J. "*Ultra-High Pressure Homogenization enhances physical, microbiological and oxidative stability of emulsions containing conjugated linoleic acid"*, oral communication presented at 2nd UK Hydrocolloids Symposium, Birmingham (UK), 10th September (2015).

Fernandez-Avila, C., Trujillo, A.J. 2016. Ultra-High Pressure Homogenization improves oxidative stability and interfacial properties of soy protein isolate-stabilized emulsions. *Food Chemistryk*, 75: 357:366.

Ferreiro, T., Gayoso, L. & Rodríguez-Otero, J.L. 2015. Milk phospholipids: Organic milk and milk rich in conjugated linoleic acid compared with conventional milk. *Journal of dairy science*, 98(1): 9–14.

Floury J, Desrumaux A, Lardières J. 2000. Effect of high-pressure homogeniza- tion on droplet size distributions and rheological properties of model oil-in- water emulsions. Innov Food Sci Emerg Technol 1:127-134

Floury, J., Desrumaux, A., Axelos, M. & Legrand, J. 2002. Degradation of methylcellulose during ultra-high pressure homogenisation. *Food Hydrocolloids*, 16(1): 47–53.

Gao, Z.-M., Zhu, L.-P., Yang, X.-Q., He, X.-T., Wang, J.-M., Guo, J., et al. 2014. Soy lipophilic protein nanoparticles as a novel delivery vehicle for conjugated linoleic acid. *Food & Function*, 5(6): 1286–1293.

Griinari, J. M. & Bauman, D. E. 1999 Biosynthesis of conjugated linoleicacid and its incorporation into meat and milk in ruminants. In Advances in Conjugated Linoleic Acid Research; Yurawecz, M. P., Mossoba, M. M., Kramer, J. K. G., Pariza, M. W.,Nelson, G. J. *AOCS Press:Champaign, IL*,1: 180–200.

Ha, Y.L., Grimm, N.K., Pariza, M.W. 1987. Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. *Carcinogenesis* 8: 1881–1887.

He, H., Honga, Y., Gua, Liua, G., Chenga, L. & Lia, Z. 2016. Improved stability and controlled release of CLA with spray-dried microcapsules of OSA-modified starch and

xanthan gum. *Carbohydrate Polymers*, 147: 243–250.

Hebishy, E., Buffa, M., Guamis, B., Blasco-Moreno, A. & Trujillo, A.J. 2015. Physical and oxidative stability of whey protein oil-in-water emulsions produced by conventional and ultra high-pressure homogenization: Effects of pressure and protein concentration on emulsion characteristics. *Innovative Food Science and Emerging Technologies*, 32: 79–90.

Hebishy, E. H. 2013. Application of ultra high-pressure homogenization (UHPH) in the pro- duction of submicron/nano-oil-in-water emulsions using vegetable oils and milk proteins as emulsifiers. (Thesis) Autonomous University of Barcelona. Bellaterra.

Heo, W., Kim J.H., Pan H.P. & Kim. Y.J. 2016. Lecithin-Based Nano-emulsification Improves the Bioavailability of Conjugated Linoleic Acid. *Journal of Agricultural and Food Chemistry*, 64(6): 1355–1360.

Herrera, M.L., 2012. *Analytical Techniques for Studying the Physical Properties of Lipid Emulsions*, Springer, Buenos Aires. pp: 61.

Hu, M., Julian McClements, D. & Decker, E.A. 2004. Impact of chelators on the oxidative stability of whey protein isolate-stabilized oil-in-water emulsions containing ω-3 fatty acids. *Food Chemistry*, 88(1): 57–62.

IDF 21:2010. Milk, cream and evaporated milk – Determination of total solids content (Reference method). IDF Standard 21:2010/ISO 6731. International Dairy Federation, Brussels, Belgium.

IDF 226:2008. Milk – Determination of fat content. IDF Standard 226:2008/ISO 2446:2008. International Dairy Federation, Brussels, Belgium.

IDF. 2002. Milk and milk products – Determination of nitrogen content. IDF Standard 185:2002/ISO 14891. International Dairy Federation, Brussels, Belgium.

Jimenez, M., Garcia, H.S. & Beristain, C.I. 2008. Sensory evaluation of dairy products supplemented with microencapsulated conjugated linoleic acid (CLA). *LWT - Food Science and Technology*, 41(6): 1047–1052.

Khaskheli, A. A., Talpur, F. N., Demir, A. S., Cebeci, A., & Jawaid, S. 2013. A highly selective whole cell biocatalysis method for the production of two major bioactive conjugated linoleic acid isomers. *Biocatalysis and Agricultural Biotechnology, Biotechnology*, 2(4): 328–332.

Kim, J. H., Kim, Y., Kim, Y. J., & Park, Y. 2016. Conjugated Linoleic Acid: Potential Health Benefits as a Functional Food Ingredient. *Annual Review of Food Science and Technology*, *7*: 221–44.

Kinsella, J.E. 1979. Functional properties of soy proteins. *Journal of the American Oil Chemists' Society*, 56(3): 242–258.

Koba, K. & Yanagita, T. 2014. Health benefits of conjugated linoleic acid (CLA). *Obesity research & clinical practice*, 8(6): 525–32.

Lalush, I., Bar, H., Zakaria, I., Eichler, S., & Shimoni, E. 2004. Utilization of amylose- lipid complexes as molecular nanocapsules for conjugated linoleic Acid. *Bio-macromolecules*, 6(1): 121–130.

Lee, S.H., Lefèvre, T., Subirade, M., Paquin, P. 2009. Effects of ultra-high pressure homogenization on the properties and structure of interfacial protein layer in whey protein-stabilized emulsion. *Food Chemistry*, 113(1): 191–195.

McClements, D. & Decker, E. 2000. Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems. *Journal of Food Science*, 65(8): 1270–1282.

McClements, D. J. 2005. *Food emulsions: Principles, practices, and techniques*, 2nd Ed. CRC Pres, Florida. pp:632.

Pereda, J., Ferragut, V., Quevedo, J.M., Guamis, B., & Trujillo, A.J. 2007. Effects of ultra-high pressure homogenization on microbial and physicochemical shelf life ofmilk. *Journal of Dairy Science*, 90: 1081–1093.

Pereda, J., Jaramillo, D.P., Quevedo, J.M., Ferragut, V., Guamis, B., & Trujillo, A.J., 2008. Characterization of volatile compounds in ultra-high-pressure homogenized milk. *International Dairy Journal*, 18: 826–834.

Poliseli-Scopel, F.H., Hernández-Herrero, M., Guamis, B., & Ferragut, V. 2012. Comparison of ultra high pressure homogenization and conventional thermal treatments on the microbiological, physical and chemical quality of soymilk. LWT-*Food Science and Technology*, 46: 42–48.

Santos, M. V., Ma, Y., Caplan, Z. & Barbano D., M., 2003. Sensory threshold of off-flavors caused by proteolysis and lipolysis in milk. *Journal of dairy science*, 86(5): 1601–1607.

Sørensen, G., & Jørgensen, S. S. 1996. A critical examination of some experimental variables in the 2-thiobarbituric acid (TBA) test for lipid oxidation in meat products. *Zeitschrift Für Lebensmittel-Untersuchung Und-Forschung*, *202*(3): 205–210.

Valencia-Flores, D.C., Hernandez-Herrero, M., Guamis, B., Ferragut, V. 2013. Comparing the effects of ultra-high-pressure homogenization and conventional thermal treatments on the microbiological, physical, and chemical quality of Almond beverages. *Journal of Food Science* 78: 199-205.

Wallace, R.J., McKain, N., Shingfield, K. J. & Devillard, E. 2007. Isomers of conjugated linoleic acids are synthesized via different mechanisms in ruminal digesta and bacteria. *Journal of Lipid Research*, 48(10): 2247–2254.

Xiang, S. Yao, X., Zhang, W., Zhang, K., Fang, Y., Nishinai, K., Phillips, G.O. & Jiang, F. 2015. Gum Arabic-stabilized conjugated linoleic acid emulsions: Emulsion properties in relation to interfacial adsorption behaviors. *Food Hydrocolloids*, 48: 110–116.

Yang, L., Leung, L.K., Huang, Y. & Chen, Z.Y. 2000. Oxidative stability of conjugated linoleic acid isomers. *Journal of agricultural and food chemistry*, 48(8): 3072–6.

Yao, X., Xu, Q., Tian, D., Wang, N., Fang, Y., Deng, Z., et al. 2013. Physical and chemical stability of gum arabic-stabilized conjugated linoleic acid oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, 61(19): 4639–4645.

Yettella, R.R., Henbest, B. & Proctor, A. 2011. Effect of antioxidants on soy oil conjugated linoleic acid production and its oxidative stability. *Journal of Agricultural and Food Chemistry*, 59(13): 7377–7384.

Zhang, A. & Chen, Z.Y. 1997. Oxidative stability of conjugated linoleic acids relative to other polyunsaturated fatty acids. *Journal of the American Oil Chemists' Society*, 74(12): 1611–1613.