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Potential application of ultra-high pressure homogenization in the physico-chemical stabilization of tiger nuts' milk beverage

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Abstract

The present study aimed to evaluate the effect of UHPH treatments (at 200 and 300 MPa and 40 °C of inlet temperature) on the physico-chemical characteristics of tiger nuts' milk beverages, in comparison with the raw beverage and the product treated by a conventional homogenization-pasteurization (18 + 4 MPa, 80 °C, 15 s) without stabilizers (H-P1) or with them (H-P2). The long term evaluation of samples showed that UHPH-treated beverages presented the highest colloidal stability, principally against creaming, due to the reduction in particle size and the new particle interactions, even if comparing with H-P2 beverage. Peroxidase activity showed the highest reduction after applying 300 MPa treatment, and regarding fat oxidation reactions, 200 MPa-treated beverage appeared to be the most stable. In this sense, the UHPH is presented as an emerging technology for obtaining tiger nuts' milk beverages free of additives with improved physico-chemical characteristics.

Keywords: Tiger nuts' milk beverage; ultra-high pressure homogenization (UHPH); colloidal stability; physico-chemical characteristics; additive-free.

1. Introduction

Tiger nuts' milk beverages are complex dispersions that are obtained from the aqueous extract of tiger nuts tubers (*Cyperus esculentus* L.) (Coşkuner, Ercan, Karababa & Nazlıcan, 2002). These beverages were traditionally produced in different Mediterranean countries, but nowadays, their consumption has been spread to all over the world (Tigernuts Traders, 2014). The nutritional profile of this product is characterized by the high content in carbohydrates (~ 12-17%), the amount of solids in suspension from the grinding of tubers, the moderate fat content (~ 2%) and the low percentage of protein (< 1%). Their potential in the food market is limited by their microbial shelf-life but also by the physico-chemical reactions that occur during the storage, mainly when temperatures are > 8 °C. Enzymatic activity, autoxidation of lipids and colloidal instabilities (by aggregation of particles, sedimentation of heavier bodies and creaming, among others) are some of the quality parameters that are involved in their loss of quality (Corrales, de Souza, Stahl & Fernández, 2012; Selma, Valero, Fernández & Salmerón, 2002). In order to extend the commercial shelf-life of these beverages, and facilitate their distribution, these beverages are subjected to conventional heat treatments. Regrettably, these processes compromise the quality characteristics of tiger nuts' milk beverages (loss of their sensory and nutritional properties, composition changes, etc.), which directly affects the consumer's acceptability (Corrales et al., 2012; Selma et al., 2002). Furthermore, the addition of stabilizers becomes indispensable to guarantee the physico-chemical stability of these beverages during their shelf-life.

To our knowledge, ultra-high pressure homogenization (UHPH) has demonstrated to be a promising technology for obtaining stabilized liquid foods (Cruz, Capellas, Hernández, Trujillo, Guamis & Ferragut, 2007; Georget, Miller, Aganovic, Callanan, Heinz & Mathys, 2014; Pereda, Ferragut, Quevedo, Guamis & Trujillo, 2007; Poliseli-

76 Scopel, Hernández-Herrero, Guamis & Ferragut, 2012; Toro-Funes, Bosch-Fusté,
77 Veciana-Nogués & Vidal-Carou, 2014).; Valencia-Flores, Hernández-Herrero, Guamis
78 & Ferragut, 2013). This technology is based on the same principle as the conventional
79 homogenization, but it is capable of working at pressures up to 400 MPa. The physical
80 phenomena that fluid suffers when it passes through the high-pressure valve gap and at
81 the outlet, in combination with the sudden (< 0.7 s) jump of temperature, involve
82 microbiological, physico-chemical, sensorial and nutritional changes on product
83 characteristics (Dumay, Chevalier-Lucia, Picart-Palmade, Benzaria, Gràcia-Julià &
84 Blayo, 2012). Since this moment, this technology has been widely studied in milk and
85 dairy products (Amador-Espejo, Hernández-Herrero, Juan & Trujillo, 2014; Pereda et
86 al., 2007; Sandra & Dalgleish, 2005; Serra, Trujillo, Quevedo, Guamis & Ferragut,
87 2007; Zamora, Ferragut, Jaramillo, Guamis & Trujillo, 2007), but there is still few
88 references in the bibliography about its application in vegetable beverages (Briviba,
89 Gräf, Walz, Guamis & Butz, 2016; Ferragut et al., 2014). Different authors have
90 demonstrated that UHPH can be considered as an alternative treatment to the
91 conventional heat pasteurization and/or sterilization in the obtaining hygienic soymilk
92 and almond milk beverages, by inactivation of microorganisms. These studies have also
93 pointed that UHPH improves the colloidal stabilization of these beverages, through the
94 particle size reduction and the induced changes on the functional properties of their
95 proteins as emulsifiers (Cruz et al., 2007; Poliseli-Scopel et al., 2012; Valencia-Flores et
96 al., 2013). Regarding the chemical stability of UHPH-treated beverages, Poliseli-
97 Scopel, Hernández-Herrero, Guamis & Ferragut (2014) showed that UHPH treatment
98 (300 MPa, 80 °C inlet temperature) caused stable levels of primary oxidation in
99 soymilk, in comparison to conventional heat treatment of ultra high temperature (UHT)
100 (142 °C for 6 s), which attributed to the protective effect of proteins by covering oil

droplet surface, and also to the new interactions between proteins and fat droplets during the storage. In this study, these authors also reported an important decrease of advanced levels of oxidation in UHPH-treated soymilk over time, in comparison to the heat-sterilized beverage. Besides of the physico-chemical and microbiological improvements observed in UHPH-treated vegetable beverages, these treatment may lead also to changes in the nutritional value. In line with this, Briviva et al. (2016) demonstrated that UHPH treatment at 350 MPa and 85 °C inlet temperature preserved B₁ and B₂ vitamins of almond milk and also decreased the antigenicity of the untreated beverage. Toro-Funes et al. (2014) also showed that UHPH treatments at the higher combination of pressure and temperature (300 MPa, 75 °C inlet temperature) lead to obtain an almond milk with increased amounts of bioactive compounds (total phytosterols and isoflavones), in comparison to raw base. Nevertheless, results obtained in these studies demonstrated that the effect of this technology is highly dependable on the characteristics of the food matrix. For this reason, it is interesting for food industry to investigate the application of UHPH in other vegetable beverages, in order to extend its application within this group of products.

Considering that the application of UHPH in the physico-chemical stabilization of tiger nuts' milk beverages have not been studied yet, the purpose of this work was to evaluate the effect of this technology for improving the physico-chemical stability of these vegetable beverages during their shelf-life, in comparison to the conventional heat treatment of homogenization-pasteurization.

2. Materials and Methods

2.1. Tiger nuts' milk beverage making

Tiger nuts' milk beverages were produced and processed at the Pilot Plant of Universitat Autònoma de Barcelona (UAB, Bellaterra, Spain). Tubers were washed, hydrated (12 h at room temperature) and disinfected according to Gallart (1999). Then, their skin was mechanically removed (Mejisa Mectufry Mecanica Jijonenca S.A., Jijona, Spain), and peeled tubers were ground in a hammer mill (Mejisa Mectufry Mecanica Jijonenca S.A.), with decalcified water at the proportion of water:tubers 1:8 (w:w). The ground product was separated by pressing and filtering (100 μ m, Mejisa Mectufry Mecanica Jijonenca S.A.) and finally, 8% (w/w) of sucrose was mixed with the liquid extract. This mixture was considered the raw product (RP), and its composition corresponded to: 12.99 ± 0.18 total solids, 10.30 ± 0.60 nitrogen free materials, 2.01 ± 0.02 fat, 0.54 ± 0.02 protein and 0.13 ± 0.01 ash. The pH value of the RP corresponded to 7.02 ± 0.19 . Representative samples of the RP were bottled and stored at refrigeration (4 °C) until their analysis.

Before the application of the sanitation treatments of UHPH and conventional pasteurization, 0.05% of α -amylase enzyme (Bialfa, Biocon Española, S.A., Les Franqueses del Vallès, Spain) was added to the RP, in order to hydrolyze the starch granules and avoid their subsequent gelatinization during the treatments.

2.2. Beverage treatments: UHPH, homogenization-pasteurization (H-P)

Two different UHPH treatments were performed by using an ultra-high pressure homogenizer (Model: DRG No. FPG11300:400 Hygienic Homogenizer, Stansted Fluid Power Ltd., Harlow, UK) at two different pressures, 200 and 300 MPa, and at the same inlet temperature (T_i) of 40 °C. The high-pressure homogenizer system consisted of two intensifiers driven by an hydraulic pump, a high-pressure homogenization valve and two spiral type heat exchangers located before the machine entrance and after the high-

pressure valve (Garvía, Barcelona, Spain), respectively. T_i , the temperatures before and after the UHPH valve (T_1 and T_2 , respectively) and the outlet temperature (T_o) of all beverages were monitored during the treatments. In the current study, T_2 corresponded to 92.1 ± 1.7 and 116.3 ± 4.3 °C, for the 200 and 300 MPa treatments, respectively. T_o corresponded to 15.3 ± 1.1 and 17.1 ± 1.6 °C in 200 and 300 MPa treatments, respectively.

A conventional treatment of H-P were also applied to the RP (H-P1), using a double stage homogenizer at $18 + 4$ MPa (LAB type: 22.51, Rannie, Copenhagen, Denmark) and a tubular heat exchanger (6500/010, GEA Finnah GmbH, Ahaus, Germany) at 80 °C for 15 s. Previously to the H-P treatment, a second batch of the RP was mixed with 0.2 % (w/v) of a mixture of emulsifiers (citric acid esters of mono- and diglycerides of fatty acids and sucrose esters of fatty acids) commercialized as Base HC9 (Cargill, S. L., Barcelona, Spain) (H-P2).

All samples (RP, H-P1, H-P2, 200 MPa and 300 MPa) were collected in sterile bottles inside a laminar flow cabin (Mini-V cabin, Telstar Technologies, S.L., Terrassa, Spain) and were stored at refrigeration temperature (4 °C) until their analyses. The overall experiment was performed in triplicate.

2.3. Physico-chemical analysis

Physico-chemical analyses of all beverages were performed during their microbiological shelf-life. Microbiological shelf-life of the RP was improved from 3 to 25, 30 and 57 days with the application of conventional H-P and UHPH processes at 200 and 300 MPa, respectively (Codina-Torrella, Quevedo, Guamis & Trujillo, 2014), according to the satisfactory microbial limit for their acceptance (≤ 6 log cfu/mL).

2.3.1. Particle size determination

The particle size determination was determined using a Beckman Coulter Laser Diffraction particle size analyzer (LS 13 320 series, Beckman Coulter, Fullerton, CA, USA), as described by Cruz et al. (2007). The particle size distribution was characterized by the Sauter diameter (surface-weighted mean diameter, $d_{3,2}$) and the volume-weighted mean diameter ($d_{4,3}$) values. Determinations were performed at day 1 and 21 (for all beverages), 30 (for 200 and 300 MPa-treated beverages), and 54 (for 300 MPa-treated beverage).

2.3.2. Rheological behavior

Rheological determination were performed in a rheometer (Haake RheoStress 1: Thermo Electron Corp., Karlsruhe, Germany), at 20 °C, using a cone (DC60/2° Ti L) and plate geometry probe. Flow curves were obtained at shear rates between 0.1 to 100 s⁻¹, for 60 s. The experimental flow curves were fitted to the Oswald de Waele rheological model ($\tau = K\dot{\gamma}^n$), where the consistency index (K) and flow behavior index (n) were calculated.

Determinations were performed at day 1 and 21 (for all beverages), 30 (for 200 and 300 MPa-treated beverages), and 54 (for 300 MPa-treated beverage).

2.3.3. Transmission electronic microscopy

Microstructure of samples was observed by transmission electron microscopy, according to Cruz et al. (2007). Samples were evaluated with a Jeol 1400 transmission electron microscope (Jeol Ltd, Tokyo, Japan) equipped with a Gatan Ultrascan ES1000 CCD camera. All microscopic images were taken at day 1.

2.3.4. Colloidal stability: Turbiscan[®]

The colloidal stability of tiger nuts' milk beverages was evaluated through the optical analyser Turbiscan MA 2000 (Formulaction, Toulouse, France). Turbiscan[®] works by measuring the backscattered and transmitted light into the sample, whose values are linked to particles average diameter and their volume fraction. This way, this equipment is useful to characterize the state of colloidal systems, as well as the changes of these dispersions over time by colloidal instabilities (creaming, sedimentation, flocculation, coalescence). Samples were transferred into borosilicate glass tubes of 27.5 × 70.0 mm up to 40 mm of height. Three tubes of each product (RP, H-P1, H-P2, 200MPa and 300 MPa) were prepared and stored at constant temperature at 4 °C, and physical stability was assessed during their microbiological shelf-life. Results were obtained using the specific software TurbiSoft 2.0 (Formulaction Toulouse, France). The height of the top and the bottom layers, which corresponded to the oil droplets creamed and the solid particles settled, respectively, were calculated during the storage of samples. In addition, the Turbiscan Stabiliby Index (TSI) was also calculated, which provides information about the global stability of the sample. TSI coefficient is obtained from the following formula:

$$TSI = \sqrt{\frac{\sum_{i=1}^n (x_i - x_{BS})^2}{n - 1}}$$

where n corresponds to the number of scans, x_i corresponds to the average backscattering for each minute of measurement and x_{BS} is the average value of x_i . This index values change in the range from 0 to 100, and lower values of TSI corresponded to a more stable system.

Scans were performed at day 0 (just after their production) and 3 (for all beverages), 5, 7, 14, 21 and 24 (for H-P and UHPH-treated beverages), 30 (for 200 and 300 MPa-treated beverages), and 42, 49 and 57 (for 300 MPa-treated beverage).

2.3.5. Peroxidase activity

Peroxidase activity was determined according to the method described by Shindler, Childs & Bardsley (1976), with some modifications. 50 μ L of the RP and 100 μ L of H-P and UHPH samples were mixed independently with 5 mL of 1 mM ABTS solution (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid, Sigma Aldrich Co., Spain) in phosphate buffer (0.1 M at pH 6) and 5 mM H₂O₂. Enzymatic activity was determined spectrophotometrically at 413 nm (CECIL 9000, CECIL Instruments, Cambridge, UK), at 20 °C. Change in absorbance during the first 5 min of the reaction was recorded, using the straight line section of the activity curve to express the enzyme activity. Enzymatic activity was defined as an increase in one absorbance unit per minute under the conditions of the assay. Determinations were performed at day 1 of storage.

2.3.6. Lipid oxidation

Primary oxidation was evaluated through hydroperoxides determination, by using the method described by Ostdal, Andersen & Nielsen (2000), with some modifications. Samples (4 mL) were mixed with methanol (4 mL) and chloroform (8 mL), and the mixture was stirred 30 min approximately. After centrifugation (12000 $\times$ g, 10 min, 20 °C), 1 mL of the chloroform phase was transferred to a test tube and subsequently mixed with 1 mL of Fe (II)/thiocyanate in methanol/chloroform. The mixture was allowed to react for 10 min at room temperature. Following, the absorbance of the mixture was read at 500 nm. The calibration curve was prepared according to the methodology

described by FIL-IDF (1991) and determinations were performed in triplicate during the shelf-life of beverages.

Hexanal, nonanal and total aldehydes were also determined as indicative products of secondary reactions of oxidation. These volatile compounds were analyzed and quantified by SPME-GC, as described by Klein, Gallardo-Chacón, Codina-Torrella, Trujillo & Juan, B (2014). These compounds were adsorbed in a SPME fiber (85 μ m DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) and desorbed by heat onto a column model J&W HP-INNOWAX (60 m $\times$ 0.251 mm $\times$ 0.25 μ m), with a bonded polyethylene glycol (PEG) high polarity stationary phase. Compounds were identified by comparison based on the Saturn spectra reported on Willey 7n.l. Determinations were performed at day 1 (for RP), 14 and 21 (for H-P1, H-P2, 200 and 300 MPa-treated beverages), 30 (for 200 and 300 MPa-treated beverages), and 54 (for 300 MPa-treated beverage) of storage.

2.4. Statistical analysis

Statistical analyses were performed using the GLM procedure of Statgraphics (Statgraphics Inc., Chicago, IL, USA). Tukey test was used for the data comparison, and significant differences were determined at the 5% level of probability. In this paper, data exposed in the tables and in the text corresponds to the mean $\pm$ standard error of every determination.

3. Results and Discussion

3.1. Physical stability

Vegetal beverages are unstable colloidal systems characterized by the presence of immiscible (or partially miscible) colloidal particles that are dispersed in the aqueous

phase. Among others, several aspects are involved in the colloidal stability of these products, such as their particle size distribution, the surface properties of dispersed particles and the difference of density between these particles and the aqueous phase.

3.1.1. Particle size

Figure 1 shows the particle size distribution of tiger nuts' milk beverages analyzed in the current study. In this type of product it is complex to distinguish what type of particles this determination is giving information about. Regarding the range of particle sizes, this analysis probably involved not only single fat droplets but also some aggregates of protein with fat and, in some cases, starch granules. All beverages followed a polydisperse distribution, which was characterized by the presence of a wider peak and a secondary group of bigger-sized particles. As shown in Figure 1, in all products the diameter of particles was $\leq 150 \mu\text{m}$, due to the filtration step of beverages during their production. In the RP, this main peak was the broadest and ranged between $0.6\text{-}20 \mu\text{m}$, accounting for 92.7% of the total volume of particles. Fat droplets and starch granules may be represented under this curve (Manek, Builders, Kolling, Emeje & Kunle, 2012). H-P and UHPH treatments reduced significantly ($P < 0.05$) the size of particles, which mainly corresponded to fat droplets and fat-protein aggregates, since starch granules had been previously hydrolyzed. Both UHPH treatments provoked a greater reduction in particles size than the conventional H-P processing. This reduction was also evidenced by the Sauter mean diameter, $d_{3,2}$ (Table 1), whose value decreased according to $\text{RP} > \text{H-P1} = \text{H-P2} > \text{UHPH-treated beverages}$. Hence, H-P1 and H-P2 beverages were characterized by a main peak in the range of $0.06\text{-}3.7$ and $0.1\text{-}3.7 \mu\text{m}$, respectively, which represented 80-95% of the total volume of particles, but conversely, both UHPH-treated beverages exhibited a narrower peak in the range of $0.05\text{-}0.9 \mu\text{m}$,

which accounted for ~ 74-88% of the total volume. As shown in Table 1, in this study, the increase of pressure (from 200 to 300 MPa) did not let to reduce ($P > 0.05$) the mean diameter of particles.

Particle size distribution (Fig. 1) also showed a secondary group of particles in the range of 2-150 μm . H-P2 and 300 MPa-treated beverages presented the highest percentages of bigger-sized particles and/or aggregates, which accounted 20 and 27% of total volume, respectively. Table 1 shows the values obtained in $d_{4,3}$ parameter, which is very sensitive to the presence of particle aggregates. These two beverages (H-P2 and 300 MPa-treated) presented by far the highest $d_{4,3}$ values, even if comparing with the RP. Cruz et al. (2007) treating soymilk by UHPH at 200 and 300 MPa ($T_i = 40\text{ }^\circ\text{C}$), also observed that $d_{4,3}$ value increased according to the pressure, which also attributed to the formation of aggregates.

As seen in Table 1, the average diameter of particles ($d_{3,2}$) did not change during storage. Concerning the evolution of $d_{3,4}$ parameter, it was only observed a significant ($P < 0.05$) increase in particles aggregates in both UHPH-treated beverages, which could be attributed to the flocculation of fat droplets over time. Besides, during the storage of beverages, the amount of particle aggregates in the 300 MPa-treated was always the highest.

3.1.2. Rheological behavior

Table 2 shows the rheological parameters of tiger nuts' milk beverages. All products exhibited a no-Newtonian flow behavior ($n < 1$), although n values were close to 1. The hydrolysis of starch granules previously to the application of heat treatments probably brought these beverages to a similar behavior to the Newtonian fluids. Previously, Schwartz, Vila, Gasque, Navarro & Lafuente (1984) mentioned that tiger nuts' milk

beverages heated at ≥ 75 °C exhibited a high pseudoplastic flow behavior, which was attributed to the gelatinization of starch. Owing to this, industrial processing of tiger nuts' milk beverages includes the hydrolysis of starch, in order to maintain the quality characteristics of these diluted emulsions after heating.

Viscosity values in the flow curves for all beverages slightly decrease as shear rate increase, indicating their pseudoplastic character, which was more accused in H-P2 and 300 MPa beverages. In these products, n showed lowest values, if comparing with the rest of treated samples. The consistency index (K) is related to the apparent viscosity in no-Newtonian fluids, which represents a relevant quality parameter in this type of beverages, in relation to the consistency perceived in mouth. K values of RP, H-P1 and 200 MPa treatments showed similar values ($P > 0.05$) in spite of homogenized samples showed a particle size decrease and an increase in the percentage of dispersed particles, which may involve viscosity changes. By the contrary, H-P2 and 300 MPa-treated beverages showed the highest values of K , which did not show differences between them ($P > 0.05$). The presence of large clusters of particles and the new linkages created between them (see microstructure, 3.1.3 section) made these systems more flow-resistant (Hayes & Kelly, 2003).

During the cold storage, no change were observed ($P > 0.05$) in the rheological parameters of beverages (data not shown), which indicated that not relevant further changes took place over time in relation to flow behavior characteristics.

3.1.3. Tiger-nuts' milk beverages microstructure

Figure 2 shows the electron micrographs of beverages at day 1 of storage. In all cases, low magnification images evidenced that fat droplets were distributed in the continuous phase with the propensity of forming clusters of particles. The RP was characterized by

the presence of some isolated big fat droplets dispersed in the aqueous phase (Figure 2, A2), which were surrounded by other smaller spherically-shaped droplets. By the contrary, and as it was expected, the size of fat droplets was reduced with both homogenization processes (H-P and UHPH), with the subsequent increase in their number. No great differences were observed between H-P1 and H-P2 or between 200 and 300 MPa-treated products (Figure 2, B to E), although when comparing these images, UHPH samples showed the higher number of dispersed droplets. More detailed images evidenced that fat droplets in H-P and UHPH-treated beverages were covered by a diffuse layer, probably formed by denatured protein and other materials. According to the literature, thermal treatment affects tiger nuts' protein stability, causing their partial denaturalization (Krog, 2004; Lee, Lefèvre, Subirade & Paquin, 2007). It has been demonstrated that the temperature of tiger nuts' protein denaturation corresponds ~70 °C (Kizzie-Hayford, Jaros, Schneider & Rohm, 2015), which was exceeded by large in the H-P treatments performed in the current study. Heat-denatured proteins tend to migrate to the oil-water interface and are absorbed onto fat surface creating a new viscous coverage. These absorbed proteins create protein-bridges with others fat droplets, forming new clusters of particles (protein and fat aggregates, PFA). In soymilk, Poliseli-Scopel et al. (2012) demonstrated that the conventional treatment of heat-pasteurization (95° C, 30 s) increased the hidrophobicity of soy proteins, and consequently, these authors observed that the colloidal stability of the emulsion was improved. If comparing the high magnification micrographs of H-P1 and H-P2 (Fig. 2, B to C) beverages, it was also appreciated that the shape of fat droplets in H-P2 appeared to be more distorted than in H-P1, which suggested the existence of differences between the materials adsorbed onto their corresponding surfaces. H-P2 fat droplets may also be covered by the low molecular weight emulsifier, which should be

adsorbed in preference onto fat surface and seemed to provoke stronger interactions than the droplet surface tension forces. Concerning UHPH-treated samples, previous studies reported that these treatments also induce protein denaturation, which is directly proportionally to the pressure increase (Floury, Desrumaux & Lardières, 2000). Cruz et al. (2007) demonstrated that at 200 MPa ($T_i = 40\text{ }^{\circ}\text{C}$) soy-globulins were partially denatured, while at 300 MPa ($T_i = 40\text{ }^{\circ}\text{C}$), thermograms obtained by differential scanning calorimetry pointed their total denaturation. A part from the pressure-induced dissociation of proteins, this phenomenon was also attributed to the sudden raise of temperature of the product after the high-pressure valve, which in the current study corresponded to 92.1 ± 1.7 and $116.3 \pm 4.3\text{ }^{\circ}\text{C}$ in 200 and 300 MPa-treated beverages, respectively. This phenomenon probably explained the newly recovery of fat droplets in both UHPH-treated beverages (Fig. 2, images D2-3 and E2-3).

In images corresponding to H-P and 300 MPa-treated samples (Figure 2, B, C and E), it was observed some protein aggregates (PA) in suspension, which perhaps corresponded to totally-denatured proteins due to the effect of the temperature. As shown, some of these PA are located onto the droplet surface, which also act as bridges among droplets enhancing their flocculation.

3.1.4. Colloidal stability

In this study, the evaluation of the impact of the treatments on the colloidal stability of beverages was evaluated in Turbiscan[®] equipment. Turbiscan[®] graphics registered the curves of backscattering along the height of flasks designed to this purpose. As reflected in Figure 3, the RP showed the most pronounced colloidal desestabilization, since at the end of its shelf-life, it was observed the faster and greater increase of the concentration of particles at the bottom ($\sim 40\text{ } \Delta\text{BS}$) and at the top of the analysis tube ($\sim 40\text{-}50\text{ } \Delta\text{BS}$)

ΔBS). These results corresponded to the sedimentation of the heaviest particles (insoluble fibers, starch granules, etc.) and the creaming of fat droplets and fat aggregates, respectively. In the RP, both destabilization phenomena started just after the beverage production. During the first day of storage, the velocity of particles migration showed the maximum values, which corresponded to 3.9 ± 0.3 and 2.6 ± 0.2 mm/day for creaming and sedimentation phenomena, respectively. According to Stokes' law, this parameter is directly proportional to particle size, so that the spontaneous coalescence of fat droplets after RP production probably favoured the kinetics of creaming. At the end of RP shelf-life (day 3), the height of creaming and sedimentation layers reached 3.6 ± 0.1 and 1.6 ± 0.3 mm, respectively.

Colloidal stability of RP was improved after the H-P and UHPH processes. The evolution of TSI indexes (Fig. 4) demonstrated that 300 MPa-treated sample was the most stable (lower values of this index), followed by the others according to $200 \text{ MPa} > \text{H-P2} > \text{H-P1} > \text{RP}$. As seen in Figure 3, these samples (H-P and UHPH) only showed the ΔBS on the top of the vial, which corresponded to the destabilization phenomenon of creaming. The maximum increase of BS signal in H-P1 and H-P2 beverages corresponded to ~45 and 40%, respectively. Therefore, at the end their shelf-life (25 days), the thickness of their corresponding creaming layers reached 4.0 ± 0.2 and 2.5 ± 0.3 mm, respectively. At first 7 days, creaming rate in H-P1 showed the maximum (0.33 ± 0.05 mm/day), while the rate in H-P2 exhibited an order of magnitude lower (0.04 ± 0.00 mm/day), which pointed that this phenomenon occurred faster in the beverage without emulsifier (H-P1). H-P2 beverage showed the maximum velocity of creaming (0.25 ± 0.02 mm/day) at day 18 of storage.

Concerning UHPH-treated beverages, both samples showed an increase of BS corresponding to 30% at the end of their shelf-life (30 and 57 days for 200 and 300

MPa-treated beverages, respectively). Maximum velocity of particles migration in both beverages was similar than the observed in H-P2 beverage, which corresponded to 0.25 ± 0.03 and 0.23 ± 0.04 mm/day for 200 and 300 MPa beverages, respectively. The thickness of the creaming layer in UHPH-treated samples also showed similar values than the observed in H-P2 beverage at day 25, which corresponded to 2.1 ± 0.3 (day 30) and 2.7 ± 0.3 mm (day 57) for the 200 and 300 MPa samples, respectively. These results demonstrated the improvement on the colloidal stability of the RP with UHPH processing and also underlined again the role of the emulsifier in the H-P2 beverage.

3.2. Oxidation stability

Oxidation processes in tiger nuts' milk beverages could be related to lipid autooxidation by free radical chain reactions and also to the changes in the catalytic activity of their intrinsic enzymes, such as the peroxidase (Corrales et al., 2012). Hence, both aspects were taken into account in the current study.

3.2.1. Peroxidase activity

Peroxidase is one of the most stable enzymes of food, which catalyzes the peroxidative degradation of unsaturated fatty acids yielding volatile and flavorful carbonyl compounds that may contributed to oxidized flavor (Stanley, Bourne & Stone, 1995; Reid & Paice, 1998). The activity of peroxidase decreased in treated tiger nuts' milk beverages, in comparison to the values observed in the RP (0.0387 ± 0.0018 Δ AU/min). H-P treatments caused the same reduction than UHPH treatment at 200 MPa ($P > 0.05$), corresponding to ~80 % of initial activity. By the contrast, no residual activity was detected in the 300 MPa-treated beverage, probably due to the higher temperature achieved for the product after the high-pressure valve ($T_1 \sim 116$ °C). This enzyme had

been reported to be high-thermal resistant, and its inactivation depends on the time-temperature combination applied (Cano, Lobo & De Ancos, 1998; Suha, Babiker & Babiker, 2012). In milk, Pereda et al. (2007) reported a peroxidase residual activity < 1 % working at 200 and 300 MPa and $T_i = 40\text{ }^{\circ}\text{C}$ (where T_1 corresponded to 84 and 103 $^{\circ}\text{C}$, respectively), while in milk treated at 200 MPa but at lower inlet temperature ($T_i = 30\text{ }^{\circ}\text{C}$), the residual peroxidase activity was higher, corresponding to 35 % ($T_1 = 79\text{ }^{\circ}\text{C}$).

3.2.2. *Fat oxidation stability*

In vegetal beverages, lipid oxidation reactions are one of the main causes of their lost of quality. This process is related with the existence of undesirable changes in the product that limits its sensorial acceptability and undermines its nutritional quality, as well (Badui, 2006).

Peroxide index is one of the widely used techniques for the detection of primary products of the fat oxidation reactions, in which the maximum concentration of hydroperoxides is detected. In the current study, none of the analyzed samples gave a positive result in the hydroperoxide determination. The high reactivity of these compounds with other molecules in the presence of oxygen, light, enzymes or temperature could explain the obtained results. In fact, similar results were previously reported by Cortés, Esteve, Frígola & Torregrosa (2005) in tiger nuts' milks beverages treated by pulsed electric fields (100 - 475 μs , 20 - 35 kV/cm) during the cold storage of beverages at 2-4 $^{\circ}\text{C}$.

Hydroperoxides are readily decomposed into a wide range of volatile compounds, such as hydrocarbons, carbonyls, aldehydes, ketones and esters, causing rancidity and undesirable odors (Frankel, 1984). Hexanal is often used as an indicator of secondary reactions of fat oxidation. In certain vegetable products, this aldehyde can be originate

471 through two oxidation pathways (autooxidation and enzymatic oxidation reactions), so
472 some authors suggest also using nonanal as a complementary indicator of the
473 autooxidation of fat (Plutowska & Wardencki, 2007; Vichi, Pizzale, Conte, Buxaderas &
474 López-Tamames, 2003). Therefore, in this study, these two compounds, in addition with
475 the total aldehydes content, were taken into consideration to estimate the secondary
476 reactions of fat oxidation. As shown in Figure 5, at day 1 of storage, 200 MPa-treated
477 beverage showed the lowest content of total aldehydes, while 300 MPa-treated beverage
478 did not show significant differences ($P > 0.05$) in comparison to the RP levels. Nonanal
479 was the most important aldehyde in the 200 MPa-treated and RP samples, while in 300
480 MPa-treated sample, the dominant aldehyde was hexanal. By the contrary, both H-P
481 samples presented the highest content in total aldehydes, according to $H-P2 > H-P1$, in
482 which nonanal was also the dominant compound. As seen, 200 MPa treatment improved
483 the oxidative stability of the RP, in comparison with the 300 MPa and H-P treatment.
484 Previous studies have demonstrated that in the homogenization processes, the disruption
485 of fat droplets produce a considerable increase of the total surface exposed to oxidative
486 reactions, so lipid oxidation reactions in these homogenized products might be expected
487 to be faster (Gohtani, Sirendi, Yamamoto, Kajikawa & Yamano, 1999). Nevertheless,
488 data obtained in the current study have shown the improvement of lipid oxidation
489 reactions in both UHPH beverages, in which there was the highest amount of newly fat
490 droplets. Fat oxidative reaction not only depends on the result of fat droplet size, but
491 rather as a consequence of the combination of different factors related with the emulsion
492 characteristics. Protein composition at interface, the interactions between denatured
493 proteins and the other components of the dispersion or the interfacial thickness also
494 resulted in a significant protective effect against fat droplet's oxidation (Hebishy, Buffa,
495 Guamis, Blasco-Moreno & Trujillo, 2015). Differences observed between UHPH

samples were probably attributed to the temperature reached by the product after the high-pressure valve. In 200 MPa-treated beverage, T_1 corresponded to ~ 96 °C, while this value increased to ~ 116 °C in 300 MPa treatment, which may involve the loss of functionality of proteins as antioxidative surfactants (Hebisy et al., 2015). Concerning H-P beverages, highest lipid oxidation reactivity of these samples was probably related to the thermal effect of this treatment. Differences observed between them could be explained by the emulsifier-type of droplets coating. Low molecular weight emulsifiers, which were adsorbed in preference in H-P2 fat droplets surface, are reported to be not as effective against lipid oxidation reactions as macromolecular type surfactants, such as denatured proteins that mainly cover fat droplets in H-P1 beverage.

During the cold storage of beverages, lipid oxidation reactions were evaluated according to the evolution of the total aldehydes content, which mainly corresponded to nonanal. As shown in Figure 6, total aldehydes content exhibited a different behavior in H-P and UHPH-treated beverages. During the first 14 days, total aldehydes decrease ($P < 0.05$) in both H-P beverages, probably to their subsequent transformation in other components (such as ketones, esters, etc.) resulting from advanced oxidation degree, which therefore may also indicate that these beverages were the most oxidized. By the contrary, in both UHPH-treated beverages the total content of aldehydes increased ($P < 0.05$) according to the storage time, until day 14 of storage, and then experienced a tendency to remain stable ($P > 0.05$). As shown in Figure 6, total content of aldehydes in 200 MPa-treated beverage at the end of its shelf-life (day 30) corresponded to half total content of aldehydes of H-P1 at day 1, whereas in UHPH-treated beverage at 300 MPa, no significant differences ($P > 0.05$) were observed between the total content of aldehydes at day 54 and the corresponding of H-P2 at day 1. These results pointed again the improvement in the oxidative stability of the RP with the UHPH treatments.

521

522 **4. Conclusions**

523 The present study has demonstrated that UHPH treatment is able to produce higher
524 colloidal stable tiger nuts milk's beverages, in comparison to the conventional heat
525 homogenization-pasteurization treatment. The long term evaluation of samples showed
526 that UHPH-treated beverages presented the highest colloidal stability, principally
527 against creaming, due to the reduction in particle size and the new network created
528 between these newly particles. 300 MPa-treated beverage showed the best colloidal
529 stability, even if comparing with the conventional H-P product with stabilizing
530 additives. Both UHPH treatments also improved the enzymatic stability of tiger nuts
531 milk's beverages, by reducing the peroxidase activity. Furthermore, UHPH treatment at
532 200 MPa also enhanced the lipid oxidative stability of this vegetable beverage. These
533 results are promising for the UHPH application in vegetable beverages, concerning the
534 improvement of the physico-chemical stability of these products.

535

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541 milk beverages.

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543 **References**

- 544 Amador-Espejo, G.G., Hernández-Herrero, M.M., Juan, B. & Trujillo, A.J. (2014).
545 Inactivation of *Bacillus* spores inoculated in milk by Ultra High Pressure
546 Homogenization. *Food Microbiology*, 44, 204-210.
- 547 Badui, S. (2006). *Química de los Alimentos*. (4^a ed.). Mexico, Pearson Educación.
- 548 Briviba, K., Gräf, V., Walz, E., Guamis, B. & Butz, P. (2016). Ultra high pressure
549 homogenization of almond milk: Physico-chemical and physiological effects. *Food*
550 *Chemistry*, 192, 82-89.
- 551 Cano, M.P., Lobo, M.G. & De Ancos, B. (1998). Peroxidase and Polyphenol Oxidase in
552 Long-Term Frozen Stored Papaya Slices. Differences among Hermaphrodite and
553 Female Papaya Fruits. *Journal of the Science and Food Agriculture*, 76, 135-141.
- 554 Codina-Torrella, I., Quevedo, J.M., Guamis, B. & Trujillo, A.J. (2014). Comparison of
555 UHPH (*Ultra-high Pressure Homogenization*) and thermal pasteurization effects on
556 physicochemical, microbiological and sensory characteristics of tiger nut beverage.
557 International Conference on High Pressure Science and Technology, Joint AIRAPT-
558 25th & EHPRG-53rd. August/2014, Madrid (Spain).
- 559 Corrales, M., de Souza, P.M., Stahl, M.R., & Fernández, A. (2012). Effects of the
560 decontamination of a fresh tiger nuts' milk beverage (horchata) with short wave
561 ultraviolet treatments (UV-C) on quality attributes. *Innovative Food Science &*
562 *Emerging Technologies*, 13, 163-168.
- 563 Cortés, C., Esteve, M.J., Frígola, A., & Torregrosa, F. (2005). Quality characteristics of
564 horchata (a Spanish vegetable beverage) treated with pulsed electric fields during
565 shelf-life. *Food Chemistry*, 91, 319-325.
- 566 Coşkuner, Y., Ercan, R., Karababa, E. & Nazlıcan, A.N., (2002). Physical and chemical
567 properties of chufa (*Cyperus esculentus* L) tubers grown in the Çukurova region of
568 Turkey. *Journal of the Science of Food and Agriculture*, 82, 625-631.

- Cruz, N., Capellas, M., Hernández, M., Trujillo, A.J., Guamis, B. & Ferragut, V. (2007). Ultra high pressure homogenization of soymilk: microbiological, physicochemical and microestructural characteristics. *Food Research International*, 40, 725-732.
- Dumay, E., Chevalier-Lucia, D., Picart-Palmade, L., Benzaria, A., Gràcia-Julià, A. & Blayo, C. (2012). Technological aspects and potential applications of (ultra) high-pressure homogenisation. *Trends in Food Science & Technology*, 31,13-26.
- Ferragut, V., Hernández-Herrero, M., Veciana-Nogués, M.T., Borrás-Suarez, M., González-Linares, J., Vidal-Carou, M.C. & Guamis, B. (2014). Ultra-high-pressure homogenization (UHPH) system for producing high-quality vegetable-based beverages:physicochemical, microbiological, nutritional and toxicological characteristics. *Journal of the Science of Food Agriculture*, 95, 953-961.
- FIL - IDF. (1991). Peroxide index determination. Standard 74A. Brussels: International Dairy Federation, Brussels, Belgium.
- Floury, J., Desrumaux, A. & Lardières, J. (2000). Effect of high-pressure homogenization on droplet size distributions and rheological properties of model oil-in-water emulsions. *Innovative Food Science & Emerging Technologies*, 1, 127-134.
- Frankel, E.N. (1984). Lipid oxidation: Mechanisms, products and biological significance. *Journal of the American Oil Chemists' Society*, 61, 1908-1917.
- Gallart, J. (1999). Guía para la aplicación del sistema de análisis de riesgos y control de puntos críticos en industrias elaboradoras de horchata de chufa natural. Valencia, Generalitat Valenciana, Conselleria de Sanitat, Valencia, Spain.
- Georget, E., Miller, B., Aganovic, K., Callanan, M., Heinz, V. & Mathys, A. (2014). Bacterial spore inactivation by ultra-high pressure homogenization. *Innovative Food Science & Emerging Technologies*, 26, 116-123.

- 594 Gohtani, S., Sirendi, M., Yamamoto, N., Kajikawa, K. & Yamano, Y. (1999). Effect of
595 droplet size on oxidation of docosahexanoic acid in emulsion system. *Journal of*
596 *Dispersion Science and Technology*, 20, 1319-1325.
- 597 Hayes, M.G. & Kelly, A.L. (2003). High pressure homogenization of raw whole bovine
598 milk (a) Effects on fat globule size and other properties. *Journal of Dairy Research*,
599 70, 297-305.
- 600 Hebishy, E., Buffa, M., Guamis, B., Blasco-Moreno, A. & Trujillo, A.J. (2015).
601 Physical and oxidative stability of whey protein oil-in-water emulsions produced by
602 conventional and ultra high-pressure homogenization: Effects of pressure and protein
603 concentration on emulsion characteristics. *Food Science and Emerging*
604 *Technologies*, 32, 79-90.
- 605 Kizzie-Hayford, N., Jaros, D., Schneider, Y. & Rohm, H. (2015). Characteristics of
606 tiger nut milk: effects of milling. *International Journal of food science &*
607 *Technology*, 50, 381-388.
- 608 Klein, B., Gallardo-Chacón, J.J., Codina-Torrella, I., Trujillo, A.J. & Juan, B. (2014).
609 Evaluation of volatile compounds of “Tiger nut beverage” (Orxata de xufla)
610 headspace by optimized solid-phase micro-extraction. *Open Access Library Journal*,
611 1: e1015. <http://dx.doi.org/10.4236/oalib.1101015>.
- 612 Krog, N. (2004). Emulsifiers and emulsions in dairy foods. *Encyclopedia of dairy*
613 *sciences*. In Fuquay, J., Fox, P. & Roginsky, H. (eds). Amsterdam (Netherlands),
614 Academic Press.
- 615 Lee, S.H., Lefèvre, T., Subirade, M. & Paquin, P. (2007). Changes and roles of
616 secondary structures of whey protein for the formation of protein membrane at soy
617 oil/water interface under high-pressure homogenization. *Journal of Agricultural and*
618 *Food Chemistry*, 55, 10924-10931.

- 619 Manek, R.V., Builders, P.F., Kolling, W.M., Emeje, M. & Kunle, O.O. (2012).
620 Physicochemical and binder properties of starch obtained from *Cyperus esculentus*.
621 *AAPS PharmSciTech*, 13, 1-10.
- 622 Ostdal, H., Andersen, H.J. & Nielsen, J.H. (2000). Antioxidative activity of urate in
623 bovine milk. *Journal of Agricultural and Food Chemistry*, 48, 5588-5592.
- 624 Pereda, J., Ferragut, V., Quevedo, J.M., Guamis, B. & Trujillo, A.J. (2007). Effects of
625 ultra-high pressure homogenization on microbial and physicochemical shelf-life of
626 milk. *Journal of Dairy Science*, 90, 1081-1093.
- 627 Plutowska, B. & Wardencki, W. (2007). Aromagrams-Aromatic profiles in the
628 appreciation of food quality. *Food Chemistry*, 101, 845-872.
- 629 Polisel-Scopel, F.H., Hernández-Herrero, M., Guamis, B. & Ferragut, V. (2012).
630 Comparison of ultra high pressure homogenization and conventional thermal
631 treatments on the microbiological, physical and chemical quality of soymilk. *Food*
632 *Science and Technology*, 46, 42-48.
- 633 Polisel-Scopel, F.H., Hernández-Herrero, M., Guamis, B. & Ferragut, V. (2014).
634 Sterilization and aseptic packaging of soymilk treated by ultra high pressure
635 homogenization. *Innovative Food Science & Emerging Technologies*, 22, 81-88.
- 636 Reid, I.D. & Paice, M.G. (1998). Effects of Manganese Peroxidase on Residual Lignin
637 of Softwood Kraft Pulp. *Applied and Environmental Microbiology*, 64, 2273-2274.
- 638 Sandra, S. & Dalgleish, D.G. (2005). Effects of ultra-high-pressure homogenization and
639 heating on structural properties of casein micelles in reconstituted skim milk powder.
640 *International Dairy Journal*, 15, 1095-1104.
- 641 Schwartz, M., Vila R., Gasque, F., Navarro, J.L. & Lafuente, B. (1982). Heat
642 stabilization of horchata beverage obtained from chufa tubers (*Cyperus esculentus*,
643 *L.*). *Revista de Agroquímica y Tecnología de Alimentos*, 22, 531-538.

- Selma, M.V., Valero, M., Fernández, P.S. & Salmerón, C. (2002). Application of HACCP in microbiological quality assurance of horchata de chufa. *Alimentación, Equipos y Tecnología*, 21, 83-89.
- Serra, M., Trujillo, A.J., Quevedo, J.M., Guamis, B. & Ferragut, V. (2007). Acid coagulation properties and suitability for yogurt production of cows' milk treated by high-pressure homogenisation. *International Dairy Journal*, 17, 782-790.
- Shindler, J.S., Childs, R.E. & Bardsley, G. (1976). Peroxidase from human cervical mucus. *European Journal of Biochemistry*, 65, 325-331.
- Suha, O.A., Babiker, E.M. & Babiker, E.E. (2012). Thermostability at different pH levels of peroxidase extracted from four vegetables. *International Food Research Journal*, 20, 715-719.
- Stanley, D.W., Bourne, M.C. & Stone, A.P. (1995). Low temperature blanching effects on chemistry, firmness and structure of canned green beans and carrots. *Journal of Food Science*, 60, 327-333.
- Tigernuts Traders (2014). Accessed on 27/09, 2014. <http://www.tigernuts.com>.
- Toro-Funes, N., Bosch-Fusté, J., Veciana-Nogués, M.T. & Vidal-Carou, M.C. (2014). Effect of ultra high pressure homogenization treatment on the bioactive compounds of soya milk. *Food Chemistry*, 152, 597-602.
- Valencia-Flores, D.C., Hernández-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.
- Vichi, S., Pizzale, L., Conte, L.S., Buxaderas, S. & López-Tamames, E. (2003). Solid-phase microextraction in the analysis of virgin olive oil volatile fraction:

668 modifications induced by oxidation and suitable markers of oxidative status. *Journal*
669 *of Agricultural and Food Chemistry*, 51, 6564-6571.

670 Zamora, A., Ferragut, V., Jaramillo, P.D., Guamis, B. & Trujillo, A.J. (2007). Effects of
671 ultra-high pressure homogenization on the cheese-making properties of milk. *Journal*
672 *of Dairy Science*, 90, 13-23.

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Figure captions

Figure 1. Particle size distribution curves of tiger nuts milk's beverages estimated by static light scattering. RP (■): raw product; H-P1 (■): homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s); H-P2 (■): homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s) with emulsifier; 200 MPa (■): ultra-high pressure homogenized (200 MPa, $T_i = 40$ °C); 300 MPa (■): ultra-high pressure homogenized (300 MPa, $T_i = 40$ °C).

Figure 2. Transmission electron microscopy images of tiger nuts' milks beverages. RP (A): raw product; H-P1 (B): homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s); H-P2 (C): homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s) with emulsifier; 200 MPa (D): ultra-high pressure homogenized (200 MPa, $T_i = 40$ °C); 300 MPa (E): ultra-high pressure homogenized (300 MPa, $T_i = 40$ °C), at (1) $\times 3000$, (2) $\times 25000$, (3) $\times 60000$ and (4) $\times 150000$ magnifications.

Figure 3. Changes in Backscattering profiles of tiger nuts' milk beverages during their cold storage. RP (A); H-P1 (B): homogenized - pasteurized (18 + 4 MPa, 80 °C, 15 sec.); H-P2 (C): homogenized - pasteurized (18 + 4 MPa, 80 °C, 15 s) with emulsifier; 200 MPa (D): ultra-high pressure homogenized (200 MPa, $T_i = 40$ °C); 300 MPa (E): ultra-high pressure homogenized (300 MPa, $T_i = 40$ °C).

Figure 4. Turbiscan Stability Index (TSI) of tiger nuts' milk beverages. RP: raw product; H-P1: homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s); H-P2: homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s) with emulsifier; 200 MPa: ultra-

high pressure homogenized (200 MPa, $T_i = 40\text{ }^{\circ}\text{C}$); 300 MPa: ultra-high pressure homogenized (300 MPa, $T_i = 40\text{ }^{\circ}\text{C}$).

Figure 5. Total aldehydes, nonanal and hexanal content of tiger nuts' milk beverages at day 1 of storage. RP: raw product; H-P1: homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s); H-P2: homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s) with emulsifier; 200 MPa: ultra-high pressure homogenized (200 MPa, $T_i = 40\text{ }^{\circ}\text{C}$); 300 MPa: ultra-high pressure homogenized (300 MPa, $T_i = 40\text{ }^{\circ}\text{C}$). ^{a-c} Different letters in each compound indicate significant differences ($P < 0.05$).

Figure 6. Evolution of total aldehydes content of tiger nuts' milk beverages at day 1, 14, 21, 30 and 54 of their cold storage. H-P1: homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s); H-P2: homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s) with emulsifier; 200 MPa: ultra-high pressure homogenized (200 MPa, $T_i = 40\text{ }^{\circ}\text{C}$); 300 MPa: ultra-high pressure homogenized (300 MPa, $T_i = 40\text{ }^{\circ}\text{C}$). ^{a-d} Different letters indicate significant differences ($P < 0.05$).

Table 1

Particle size parameters ($d_{3,2}$ and $d_{4,3}$) of tiger nuts’ milk beverages.

Parameters (μm) ¹	Day	Treatment ²				
		RP	H-P1	H-P2	200	300
$d_{3,2}$	1	1.43 ^a ±	0.55 ^{bx} ±	0.48 ^{bcx} ±	0.35 ^{cx} ±	0.36 ^{cx} ±
		0.12	0.01	0.01	0.01	0.01
	21	-	0.57 ^{ax} ±	0.43 ^{bx} ±	0.31 ^{cx} ±	0.34 ^{cx} ±
			0.03	0.03	0.02	0.03
	30	-	-	-	0.32 ^{ax} ±	0.35 ^{ax} ±
					0.03	0.01
	54	-	-	-	-	0.36 ^x ±
						0.01
	1	5.13 ^{cd} ±	4.01 ^{dx} ±	7.54 ^{bx} ±	3.87 ^{dz} ±	8.34 ^{ay} ±
		0.37	0.47	0.41	0.29	0.21
$d_{4,3}$	21	-	3.85 ^{dx} ±	6.77 ^{cx} ±	7.90 ^{by} ±	9.16 ^{ay} ±
			0.15	0.08	0.16	0.26

30	-	-	-	10.54 ^{bx} ± 1.18	12.59 ^{ax} ± 0.25
54	-	-	-	-	12.76 ^x ± 0.90

¹ Data are means ± standard error of triplicate analyses from 3 different productions.

² RP: raw product; H-P1: homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s); H-P2: homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s) with emulsifier; 200 MPa: ultra-high pressure homogenized (200 MPa, T_i = 40 °C); 300 MPa: ultra-high pressure homogenized (300 MPa, T_i = 40 °C).

^{a-d} Different letters in the same raw indicate significant differences (*P* < 0.05).

^{x-z} For each parameter, different letters in the same column indicate significant differences (*P* < 0.05).

Table 2

Rheological characteristics of beverages after their production (day 1).

Treatment	Rheological parameters		
	K (mPa × s) ⁿ	n	r ²
RP	1.699 ^b ± 0.049	0.969 ^a ± 0.008	0.992 ± 0.002
H-P1	1.679 ^b ± 0.023	0.978 ^a ± 0.009	0.997 ± 0.000
H-P2	3.225 ^a ± 0.066	0.944 ^b ± 0.008	0.999 ± 0.001
200 MPa	1.759 ^b ± 0.040	0.968 ^a ± 0.006	0.998 ± 0.002
300 MPa	3.551 ^a ± 0.072	0.938 ^b ± 0.009	0.999 ± 0.001

¹ Data are means ± standard error of triplicate analyses from 3 different productions.

² RP: raw product; H-P1: homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s); H-P2: homogenized-pasteurized (18 + 4 MPa, 80 °C, 15 s) with emulsifier; 200 MPa: ultra-high pressure homogenized (200 MPa, T_i = 40 °C); 300 MPa: ultra-high pressure homogenized (300 MPa, T_i = 40 °C).

^{a-b} For each parameter, different letters in the same column indicate significant differences (*P* < 0.05).

Fig. 1

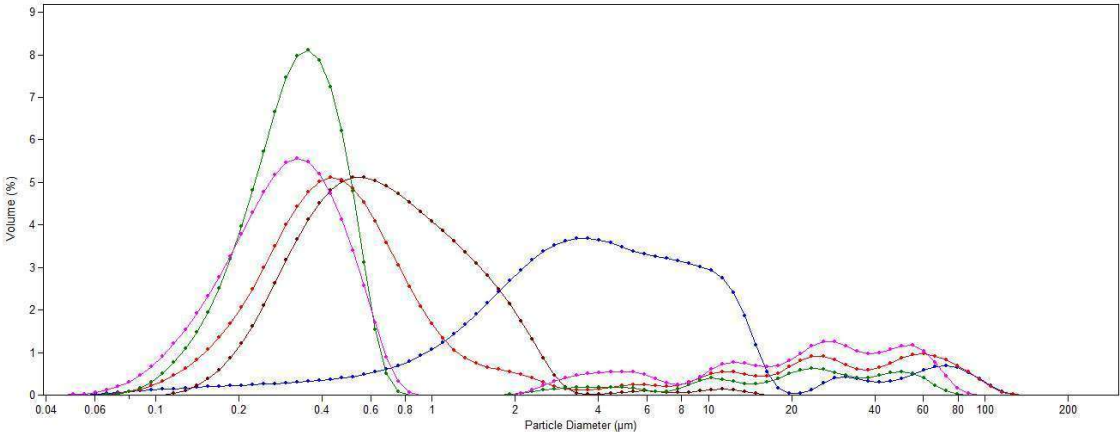


Fig. 2

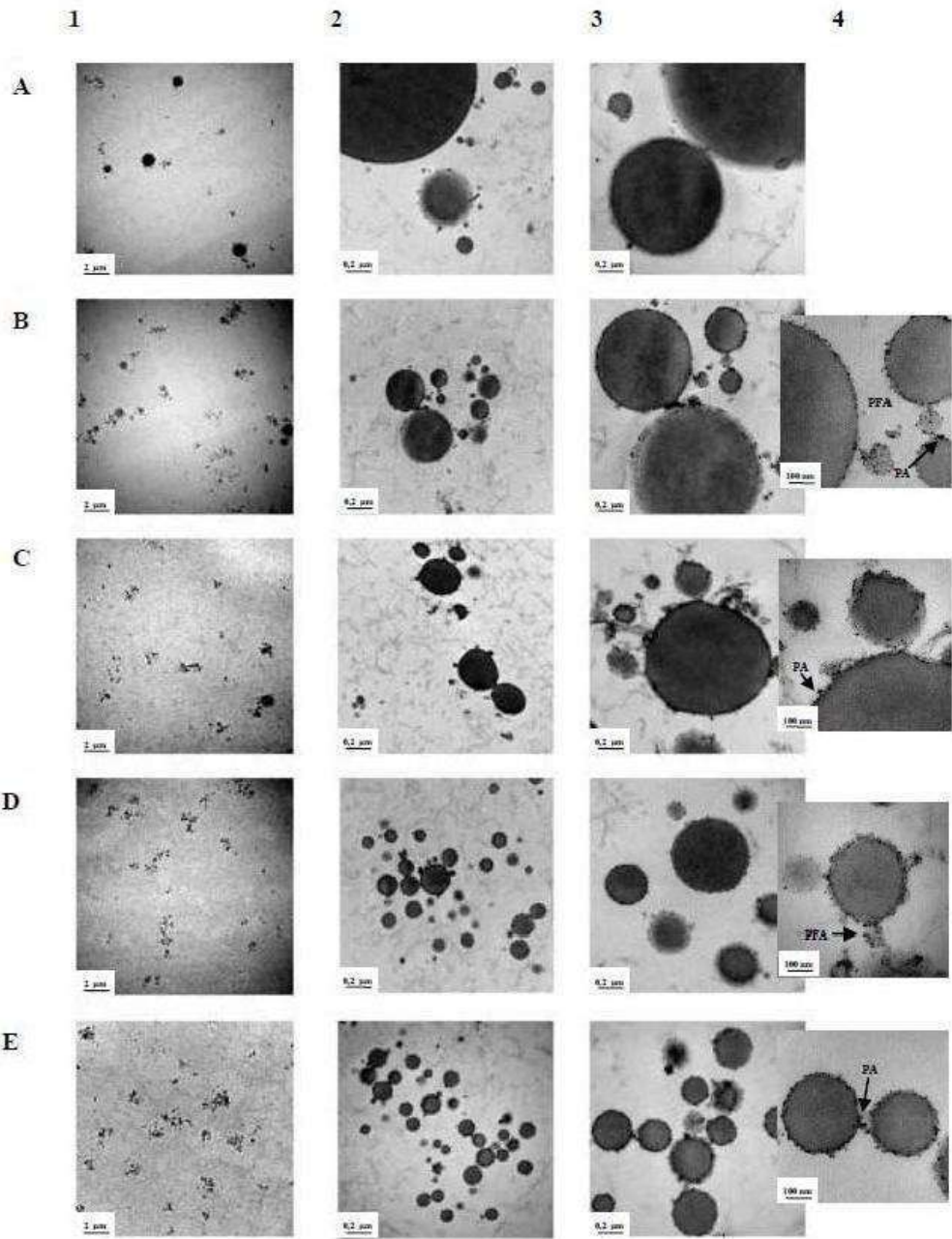


Fig. 3

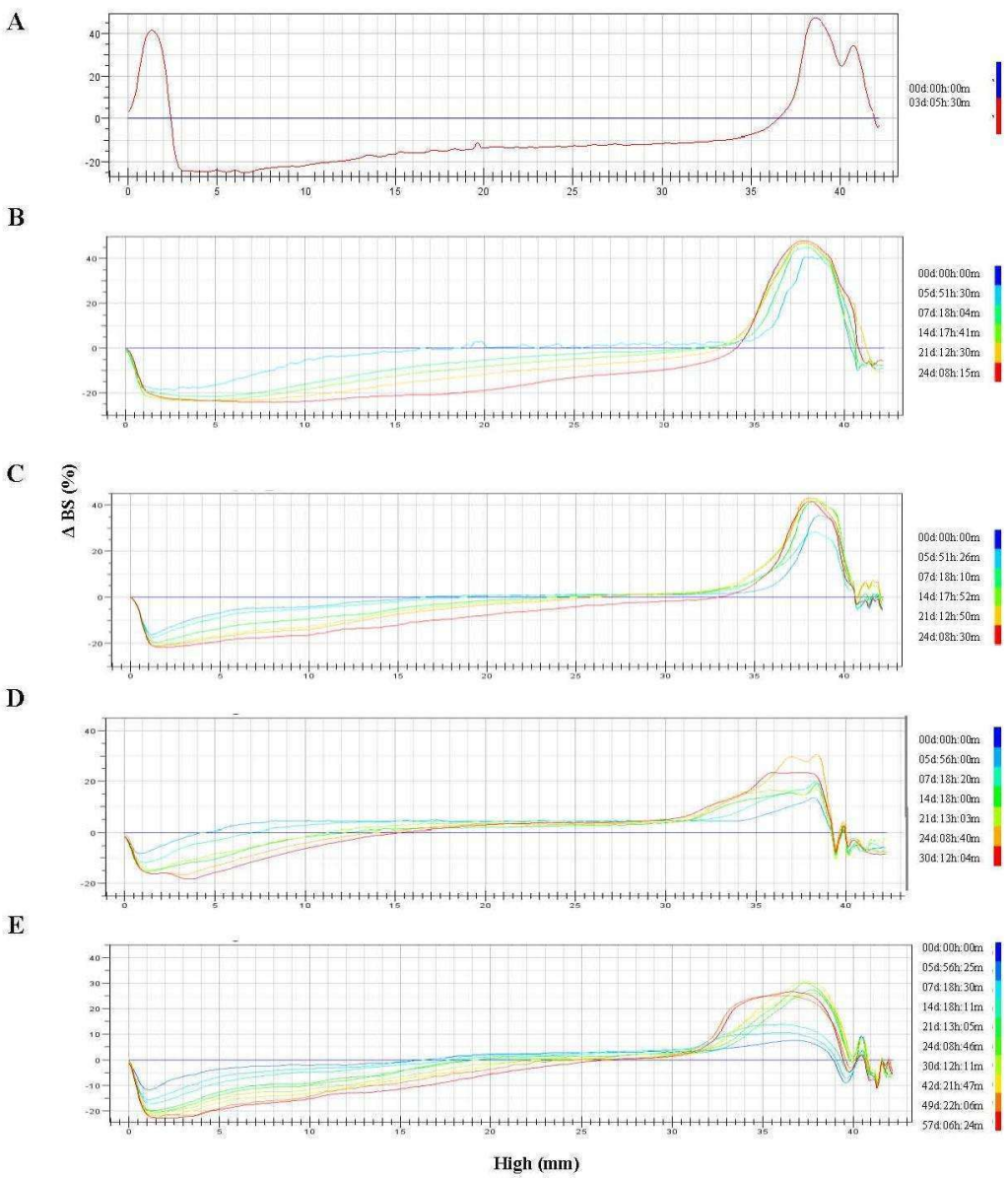


Fig. 4

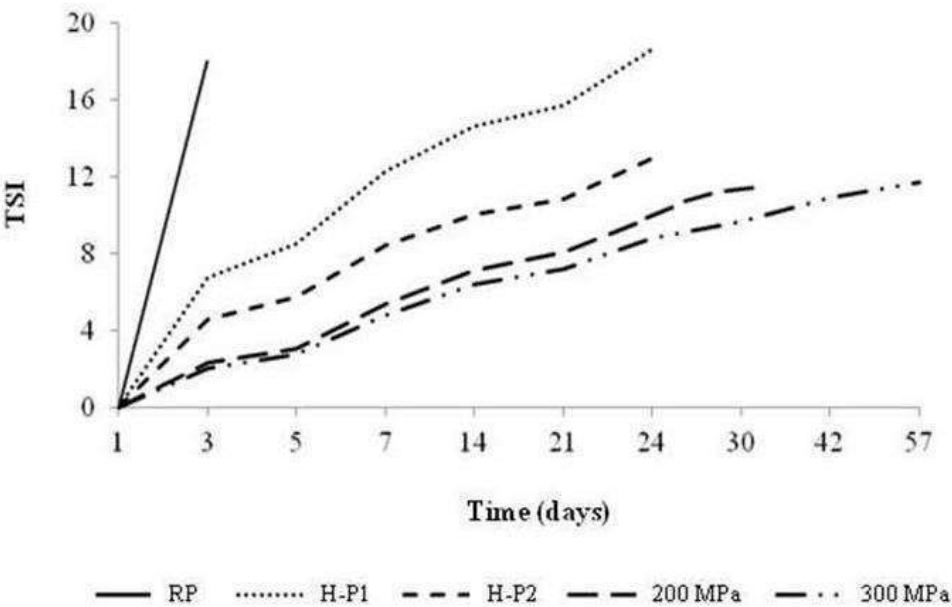


Fig. 5

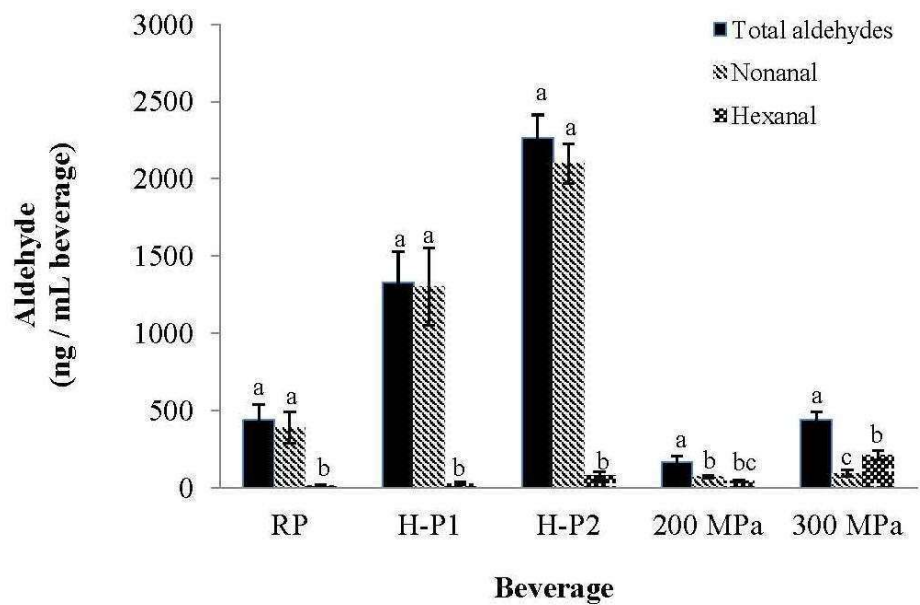


Fig. 6

