Microbiological stabilization of tiger nuts' milk beverage by using ultra-high pressure homogenization

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Abstract

Tiger nuts' milk beverages are highly perishable products. Consequently, the interest of food industry for their commercialization makes necessary the application of preservation treatments to prolong their shelf-life. In the current study, the effect of ultra-high pressure homogenization (UHPH) on the microbiological and sensory quality of tiger nuts' milk beverage was evaluated. Characteristics of UHPH-treated products (at 200 and 300 MPa, with inlet temperature of 40 ºC) were compared with those of raw (RP) and conventionally homogenized-pasteurized (H-P) beverages, after treatment and during cold storage at 4 ºC. Microbiological quality of beverages was studied by enumerating total counts, psychrotrophic bacteria, lactobacilli, enterobacteria, molds and yeasts, and mesophylic spores. Physicochemical characteristics (pH and color) and sensory evaluation of beverages were also evaluated. Microbiological shelf-life of the tiger nuts' milk beverages was extended from 3 to 25, 30 and 57 days by applying H-P and UHPH treatments at 200 and 300 MPa, respectively. Although all treatments increased the brightness of tiger nuts' milk beverages, treated samples turned darker during storage, and their pH value decreased. Hence, UHPH treatments showed to be an alternative to the conventional H-P for obtaining tiger nuts' milk beverages with an improved microbiological shelf-life and good sensorial characteristics.

Keywords: Ultra-high pressure homogenization, tiger nuts' beverage, microbial stabilization

1. Introduction

Tiger nuts' milk beverages are one of the most appreciated vegetable beverages, which are obtained from the aqueous extract of tiger nuts tubers (*Cyperus esculentus* L.) (Coûkuner et al., 2002). These beverages are rich in carbohydrates (>50%), unsaturated fatty acids (75% in oleic and ~10% in linoleic acids, of total fat) and dietary fiber (~1%), and also contain a moderate percentage of nutritional minerals (phosphor, calcium, magnesium and iron) and vitamins (C and E) (Alegría-Torán and Farré-Rovira, 2003; Borges et al., 2008; Sánchez-Zapata et al., 2012). Different studies pointed out their suitability for lactose-intolerant and celiac patients, and also for preventing digestion disorders (Adejuyitan, 2011; Alegría-Torán et al., 2003). Due to the high microbiological loads of harvested tubers and the resistance of these spoilage microorganisms to disinfecting treatments, total aerobic counts in these beverages are usually in the range of 5-6 log cfu/mL (Gallart, 1999), thereby necessitating their immediate consumption (Corrales et al., 2012; Selma et al., 2002). In view of the global tendency towards an increase in the consumption of vegetable beverages, it is of particular interest to food producers to prolong the commercial shelf-life of these perishable products to enable worldwide distribution. Conventional heat preservation treatments such as pasteurization and sterilization are being the most commonly used in food industry (Selma et al., 2003). However, these treatments result in an undesirable loss of the most appreciated sensory characteristics (e.g. pale color or tiger nuts' flavor and taste). Owing to this, food industry looks for alternative technologies that improve the microbiological quality of these beverages while preserving their sensory characteristics (Corrales et al., 2012; Cortés et al., 2005; Selma et al., 2003). At present, few studies have demonstrated the potential of non-thermal technologies to reduce the spoilage-related microorganisms of tiger nuts' milk beverages. Selma et al. (2003)

evaluated the suitability of high-intensity pulsed electric fields for reducing the potential growth of *Enterobacter aerogenes*, but no more than 1.1 log reductions were reported to be achieved by any of the treatments applied. In line with this, Corrales et al. (2012) validated the feasibility of short wave ultraviolet radiation (UV-C) to inactivate psychrotrophic and mesophilic bacteria and yeasts and molds. These authors demonstrated that UV-C treatments at a fluence rate of 2.35 mW/cm^2 during 10 min achieved 3-3.5 log cycles reduction of these spoilage-related microorganisms, and the shelf-life of beverages was extended from 2 to 4 days (at 2 ºC).

Ultra-high pressure homogenization (UHPH) is a novel technology that allows the microbial inactivation and improves the colloidal stability of fluid foodstuffs maintaining, in most cases, both nutritional and sensory characteristics of untreated products (Dumay et al., 2012; Zamora & Guamis, 2015). This technology is based on the same principle as the conventional homogenization, but it is capable of working at pressures up to 350 MPa. The physical phenomena that fluid suffers when passing through the high-pressure valve gap and at the outlet (e.g. cavitation, pressure drop, shear stress, etc.) in combination with the sudden temperature jump promotes significant changes in the product characteristics, such as the disruption of vegetative microorganisms (Dumay et al., 2012; Zamora & Guamis, 2015) and in some cases, the reduction of spores counts (Georget et al., 2014; Zamora & Guamis, 2015). Some published contributions demonstrate the suitability of this technology for the improvement of microbiological quality in soymilk and almond-milk beverages, in comparison to the conventional heat treatments (Cruz et al, 2007; Ferragut et al., 2014; Poliseli-Scopel et al., 2012, 2014; Valencia-Flores et al., 2013). According to the literature, some of the most important processing parameters that influence in the effectiveness of this treatment are the operating pressure, the inlet temperature (T_i) and

the number of passes (Diels and Michiels, 2006). In line with this, studies performed with soymilk and almond-milk beverages reported that UHPH treatments at 200 and 300 MPa with the combination of T_i of 55-75 ºC were more effective than conventional pasteurization against almost all spoilage microorganism inactivation (Poliseli-Scopel et al., 2013; Valencia-Flores et al., 2013). Poliseli-Scopel et al. (2014) also showed that UHPH treatments at 300 MPa and T_i ≥75 ºC allowed the commercial sterility of soymilk. In this study, these sensory response of panelists after the evaluation of UHPH-pasteurized (200 MPa at T_i of 55-75 ºC) and UHPH-sterilized (300 MPa at T_i of 80 ºC) soymilks showed a positive trend.

Nevertheless, the potential effect of this stabilizing technology not only depends on the process parameters but also on the characteristics of the food matrix. In this way, the aim of the present work was to evaluate the potential of UHPH as a technology for improving microbiological quality and sensory characteristics of raw tiger nuts' milk beverages during cold storage (4 ºC), as alternative to the conventional heat treatment of homogenization-pasteurization.

2. Materials and Methods

2.1. Tiger nuts' milk beverage making

Tiger-nuts beverages were produced and processed at the Pilot Plant of Universitat Autònoma de Barcelona (UAB, Bellaterra, Spain), as described by Codina-Torrella et al. (2016). The general composition of tubers (as % dry matter), which were under the geographical origin *Chufa de Valencia,* corresponded to 8.66 ±0.04 moisture, 35.21 ±3.07 fat, 8.45 ±0.20 protein and 45.05 ±3.13 nitrogen free material (NFM), as previously described by Codina-Torrella et al. (2015). Raw product (RP) consisted in the liquid extract with 8% (w/w) of added sucrose. The composition of RP (%)

corresponded to: 12.99 ±0.18 total solids, 10.30 ±0.60 nitrogen free materials, 2.01 ±0.02 fat, 0.54 ±0.02 proteins and 0.13 ±0.01 ashes. Representative samples of the RP were bottled in sterilized glass bottles and stored at refrigeration (4 ºC) until analysis. Just before the application of the hygienizing 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 when heating. The holding time of the enzyme in RP before applying the technological treatments corresponded to 10 min. Qualitative determination of starch (Total Starch Assay Procedure kit, Amyloglucosidase/α-amylase method, K-TSTA 404-2009, Megazyme International Ireland Ltd., Wicklow, Ireland) in all samples demonstrated that after the treatment this component was totally hydrolyzed in all beverages.

2.2. Beverage treatments: UHPH, homogenization-pasteurization

Two different UHPH treatments were performed by using an ultra-high pressure homogenizer at a flow rate of 120 L/h (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 T_i of 40 ºC. The high-pressure homogenizer system consisted of two intensifiers driven by a 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 (Poliseli-Scopel et al., 2012). Temperatures during treatment, i.e., T_i, those before and after UHPH valve (T_1 and T_2, respectively) and at the outlet (T_o), were monitored.

A conventional treatment of Homogenization-Pasteurization (H-P) was also applied to RP sample using an indirect system composed by a double stage homogenizer

positioned upstream (Model X68, Soavi B. & Figli, S.P.A., Parma, Italy) and a multitube tubular heat exchanger at a flow rate of 1000 L/h (laminar flow) (6500/010, GEA Finnah GmbH, Ahaus, Germany). Beverages were homogenized at 18 + 4 MPa at 65 ºC and subsequently pasteurized at 80 ºC for a holding time of 15 s.

All samples (RP, H-P, 200 MPa and 300 MPa) were collected in sterilized glass bottles of 1 L of capacity with twist-off caps (Apiglass Envases y Material Apícola, S.L) inside a laminar flow cabinet (Mini-V/PCR cabinet, Telstar Technologies, S.L., Terrassa, Spain) and were stored at refrigeration temperature (4 ºC) until analyzed.

2.3. Microbiological analysis

Decimal dilutions in peptone water solution were used for microbiological enumeration. Aerobic mesophilic (AM) counts were enumerated on plate count agar (PCA, Oxoid Ltd., Basingstoke, UK) incubated at 30 ºC for 48 h. Psychrotrophic bacteria (PS) were enumerated on PCA, incubated at 21 ºC for 72 h. Lactobacilli (LB) were enumerated on Rogosa agar (Oxoid), incubated at 30 ºC for 72 h. *Enterobacteriaceae* (EB) counts were enumerated on violet red bile glucose agar (Oxoid), incubated at 37 ºC for 24 h. Faecal coliforms (FC) were enumerated on Coli ID selective chromogenic medium (bioMérieux S.A., Madrid, Spain), incubated at 37 ºC for 24 h. The *E. coli* presence was also evaluated by color difference using this chromogenic medium. For the total mesophylic spores (MS) enumeration, samples were heated at 80 ºC for 10 min and quickly-cooled in ice, and pour plated on PCA, incubated at 30 ºC for 48 h. Molds and yeast (MY) were enumerated on Rose Bengal agar (Oxoid) with chloramphenicol supplement (Oxoid), incubated at 25 ºC for 5 days.

Microbiological quality of beverages was monitored during their storage at 4 ºC, until microbiological counts exceed to the satisfactory microbial limit for their acceptance

(≤ 6 log cfu/mL) (Corrales et al., 2012). Regarding to this, microbiological analyses were performed at days 1 and 3 (for all samples), 14, 21 and 25 (for H-P and UHPH samples), 28 and 30 (for UHPH samples), 35, 42, 49, 54 and 58 for 300 MPa UHPH-treated samples.

For PS bacteria, lag time and the maximum velocity of growth in the exponential phase were calculated by using DMFit software, which is available at Combase website (ComBase, http://www.combase.cc). DMFit is a fitting tool for bacterial growth that displays different parameters for the selected model. In this case, predictions were based on the primary model of Baranyi and Roberts (1994). The pH and initial levels of PS bacteria were chosen based on the corresponding results obtained in the current study at day 1.

2.4. pH determination and color analysis

The determination of pH was performed in triplicates at 20 ºC by potentiometric measurement (Crison micropH 2001, Alella, Spain). Color analyses of beverages were conducted in triplicate with a Hunter Lab colorimeter (MiniScan XETM, Hunter Associates Laboratory Inc., Reston, USA). Color coordinates were measured with an illuminant of D65 and a standard observer angle of 10º. Colorimeter was calibrated with standard black and white tiles. Data was acquired in the CIELab color space: L* (luminosity), a* (red-green) and b* (blue-yellow). The total color differences ΔE* (Eq. 1) were calculated taking into account RP sample as the reference. During storage, for each type of product, this difference was calculated considering their respective homologue at day 1. Whiteness index (WI) was also calculated using L*, a* and b* parameters (Eq. 2).

(1) $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$

(2) $WI = [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$

2.5. Sensory evaluation

Ten trained judges from the staff at Universitat Autònoma de Barcelona (UAB) were pre-selected based on their previous experience in the evaluation of vegetal beverages and their habitual consumption of tiger nuts' milk beverages. Panelists were trained as described by Poliseli-Scopel et al. (2012). Beverages (~30 mL) were presented at the usual temperature of consumption of these products (~10 ºC), in transparent cups with a 3-digit random code. Sensory evaluation was carried out at days 3, 14, 21, 28 and 56 of storage, depending on the microbiological shelf-life of beverages. Owing to the short microbiological shelf-life of the reference sample, RP was produced each day of the sensory test. The evaluation consisted on a descriptive test, in which five attributes were evaluated: color (darkness), aroma (intensity), mouthfeel (cooked and strange) and thickness. Responses were recorded on an intensity scale from − 4 to 4 points, where differences were qualified as very considerable (-4 and 4), considerable (-3 and 3), noticeable (-2 and 2), minimal (-1 and 1) or unnoticeable (0 points) in comparison with the RP sample. Algebraic sign, i.e. negative or positive, indicates lower or greater perception.

2.6. Statistical analysis

The overall experiment was performed in triplicate on three different days, thereby consisted on three independent batches of 200 L of tiger nuts' milk beverage. Microbiological analyses were carried out in duplicate. Physicochemical analyses were performed in triplicate. Data exposed in the text corresponds to the mean ± standard error. 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. For sensory data, descriptive statistics (mode and relative frequency of either negative or positive values) were calculated. Means were analyzed for significance as previously described.

2. Results and Discussion

2.1. Temperature changes during UHPH processing

In the current study, the temperature of UHPH-treated tiger nuts' milk beverages increased 24.2 ºC between the pressure range of 200-300 MPa, as it was previously described by other authors who worked with the same UHPH equipment (Pereda et al., 2007; Poliseli-Scopel et al., 2012; Suárez-Jacobo et al., 2012). The rise of temperature is attributed to the physical phenomena that fluids experimented during this process and downstream of the valve, as a consequence of the adiabatic heating (Floury et al., 2000; Hayes & Kelly, 2003). Thereby, at the outlet of the high-pressure valve the product reached 92.1 ±1.7 and 116.3 ±4.3 ºC for the 200 and 300 MPa treatments, respectively, although the residence time of the product at this temperature was estimated to be very short (<0.7 s) in comparison with the conventional heat treatments (Poliseli-Scopel et al., 2012). In this study, T_o corresponded to 15.3 ±1.1 and 17.1 ±1.6 ºC in 200 and 300 MPa treatments, respectively.

2.2. Effect of treatments on tiger nuts' milk beverage characteristics

2.2.1. Microbiological quality and pH

Microbiological quality of vegetable beverages determines their shelf-life and also impacts on the biochemical changes that occur during their storage. Composition and neutral pH of tiger nuts' milk beverages strongly promote a fast microbiological growth,

therefore it is necessary the application of preservation treatments to extend their quality over time. In the current study, the pH value of the RP corresponded to 7.02 ±0.19, which did not significantly vary ($P > 0.05$) after the application of H-P and UHPH treatments.

Samples were analyzed for the microbial groups that are most typically found in this product (Corrales et al., 2012; Selma et al., 2002). As can be observed in Table 1, microbiological counts found for RP samples were in agreement with the results obtained by other authors in this type of beverages (Gallart, 1999; Hernández and Vilar, 1967). Initial counts of AM and PS bacteria (5.51 and 5.37 log cfu/mL, respectively) evidenced the high bacteria counts of this type of beverage after production, which are normally comprised between 5-6 log cfu/mL (Corrales et al., 2012; Selma et al., 2002). MS counts corresponded to 2.53 log cfu/mL. These microorganisms probably belonged to genus *Bacillus* (*B. subtilis*, *B. brevis* and *B. cereus*), the most typical spore-forming bacteria isolated in these type of beverages (Nyarko et al., 2011). EB and FC counts were 2.91 and 2.31 log cfu/mL respectively, and no *E. coli* bacteria was detected in any sample. The presence of these undesirable microorganisms in the RP could be related to the cropland and the use of animal manure as fertilizer. MY and LB counts were 2.87 and 2.58 log cfu/mL, respectively. These spoilage microorganisms are responsible for the quick alteration of tiger nuts' milk beverages during cold storage through fermentation of sugars and the concomitant production of acid and gas (Hernández et al., 1967; Monerrris-Aparisi et al., 1998).

All treatments reduced significantly ($P < 0.05$) the microbial counts of the RP (Table 1). Homogenization-pasteurization provoked the same microbial reduction of AM and PS bacteria (~2 log) than those observed in UHPH-treated samples at 200 MPa. However, increasing the pressure to 300 MPa triggered a reduction of ~3 log units for both groups

of microorganisms. In UHPH processing, the rate of microorganism inactivation mainly depends on the working pressure and the inlet temperature of products, showing better results with the increase of both parameters (Diels and Michiels, 2006; Picart et al., 2006) as has been demonstrated in the particular case of soymilk (Cruz et al., 2007; Poliseli-Scopel et al., 2012, 2014). At the same T_i of 40 ºC, Cruz et al. (2007) reported a major reduction of the total bacteria counts by increasing the working pressure from 200 to 300 MPa (~2 and 4 log, respectively). In line with this, Poliseli-Scopel et al. (2012) demonstrated that UHPH treatments at 200 MPa increased their efficiency in total counts reduction if T_i increased from 55 to 75 ºC with mesophilic bacterial counts of 3.2 and 0.3 log units, respectively. Furthermore, at 300 MPa and a T_i of 80 ºC, UHPH treatment resulted in soymilk microbiologically stable at room temperature for 6 months (Poliseli-Scopel et al., 2014).

In the present study, none of the stabilizing treatments resulted in a reduction in MS counts (Table 1), showing the high resistance of these microorganisms to the studied treatments. Some research has been conducted in order to elucidate the effect of UHPH on spore inactivation, concluding that successful inactivation could be achieved by combining higher pressures and T_i (Amador-Espejo *et al*., 2014; Georget et al., 2014). According to literature, for UHPH treatments below 200 MPa, microorganism inactivation is mainly dependent on the mechanical effects that fluid suffers when passing through the homogenization valve. However, at higher working pressures, microbial reduction is achieved by the synergic impact of pressure and temperature (Pathanibul et al., 2009). The capability of UHPH to inactivate bacterial spore appeared to be highly dependable on inlet and, consequently, valve temperature (Georget et al., 2014). As said before, soymilk sterilization was achieved by UHPH at 300 MPa and a T_i of 80 ºC, which corresponded to a valve temperature of 144 ºC (Poliseli-Scopel et al.,

2014). With almond milk, no bacterial growth after incubation at 30 ºC for 20 days was detected in UHPH-treated samples at 300 MPa with a T_i of 65 or 75 ºC, which corresponded to valve temperatures of 127 and 129 ºC, respectively (Valencia-Flores et al., 2013).

Concerning the other groups of microorganisms evaluated in the present study, all counts (EB, FC, LB or MY) were below the detection limit (<0.5 cfu/mL) for all treated beverages. In addition, *E. coli* was never detected. Such results were expected since these microorganisms have been reported to be highly susceptible to UHPH-treatments when the homogenization pressure is ≥200 MPa (Cruz et al., 2007; Hayes & Kelly, 2003; Pereda et al., 2007).

2.2.2. Sensory characteristics

In the present study, relevant attributes were included in the sensory evaluation of tiger nuts' milk beverages in order to get a general perception of their sensory profiles. Panelists did not perceive significant differences ($P > 0.05$) in aroma and taste attributes among all samples, although tiger-nuts' milk beverages treated by conventional heat treatments are characterized by the presence of different off-flavors, which are linked to the secondary compounds generated in lipid oxidative processes, and protein and sugar reactions. In a previous study of the present authors, it was demonstrated that after the H-P treatment, tiger nuts' milk beverage showed a higher level of oxidation than RP and both UHPH-treated beverages (Codina-Torrella et al., 2016). Nevertheless, the amount of these generated compounds was probably under the detection threshold, which could be in line with the results obtained in the sensory evaluation. The panel neither detected differences ($P > 0.05$) in the thicknesses between samples, although instrumental

rheological evaluation evidenced that 300 MPa treated beverage differed from the others (Codina-Torrella et al., 2016).

By the contrary, significant differences ($P < 0.05$) in the color were detected among all samples by the judges. The mode of scores obtained for H-P and 200 MPa treated beverages corresponded to -1 (with relative frequencies of 63.3 and 73.3%, respectively). Instead, for 300 MPa sample, the obtained value was -2 (with a relative frequency of 52%), which indicated that in most cases, panelists considered this beverage as the whitest. These subjective results were in agreement with those obtained in the current study by the instrumental determination of color. As seen in Table 2, global color differences (ΔE*) demonstrated that UHPH beverages were the most different with the highest values of lightness (L*), although both UHPH treatments did not differ. The increase of L* could be associated with the increase of light diffraction caused by the greater number of oil droplets dispersed in the continuous phase after homogenization (Codina-Torrella et al., 2016). As seen in Table 2, both a* (red-green) and b* (yellow-blue) parameters decreased significantly ($P < 0.05$) after the application of both homogenization treatments. In H-P sample, lower values in b* parameter could be associated with the non-enzymatic browning reactions of free sugars (and the consequent formation of secondary compounds) which are activated by the temperature during this processing.

2.3. *Evolution of beverages' characteristics during storage*

2.3.1. *Microbiological shelf-life and pH evolution*

Figure 2 shows the evolution of microbiological populations in tiger nuts' milk beverages during the cold storage. As can be seen, AM and PS counts increased over time in all samples, with the highest rate in RP sample. For all cases, AM and PS counts

over time presented the same values suggesting that most of the microorganisms in tiger-nuts' milk beverages were mainly PS bacteria. At day 3, AM and PS counts of RP sample reached ~6 log cfu/mL, which corresponds to the limit of the satisfactory microbial acceptance in this type of product (Corrales et al., 2012). In fact, the microbiological shelf-life of raw tiger nuts' milk beverages is generally not higher than 4 days, provided that the storage temperature is comprised between 4 and 6 ºC (Corrales et al., 2012; Monerris et al., 1998; Moreno-Seguí et al., 2000). By the contrary, counts of MS, EB, FC, LB and MY of RP sample remained unchanged probably due to the loss of viability of these microorganisms as a result of the low storage temperature (4 ºC) (Moreno-Seguí et al., 2000).

In H-P and UHPH-treated beverages, PS bacteria also showed an exponential growth over time (Figure 2B). Data obtained with DMFit modeling program allowed the calculation of the length of the lag phase for PS bacteria and the maximum velocity in their exponential phase of growth. The microbial lag phase corresponds to the time necessary for bacterial cells to modify themselves in order to settle in the new environment and start duplicating (initiate the exponential growth) (Swinnen et al., 2004). Lag phase of PS corresponded to 18.56 ±0.92, 26.96 ±0.68 and 43.18 ±2.06 days in H-P and UHPH-treated beverages at 200 and 300 MPa, respectively. These results showed that UHPH triggered longer lag phases than H-P processing. Among others, the extent of this phase is determined by the physiological state of microbial cells, which depends on the effects of the preservation treatment. Physical effects that fluids experiment downstream of the UHPH valve, together with the increase of product's temperature, probably caused a higher damage to the microbial cells than the conventional H-P. As seen in Figure 2B, after this lag phase, PS grew exponentially. During the exponential growing phase, healthy and sub-lethally injured cells are divided

at a constant rate. In the present study, the maximum velocity of growth varied according to the treatment, corresponding to 0.46 ±0.06, 0.60 ±0.07 and 0.26 ±0.05 log cfu/mL/day for H-P and UHPH at 200 and 300 MPa, respectively. Concomitantly, microbiological shelf-life of 200 and 300 MPa UHPH-treated products was extended to 30 and 57 days whilst the shelf-life of H-P beverage corresponded to 25 days. These results highlight the improvement on the microbiological quality of UHPH-treated products in comparison with conventional treatments. Furthermore, the shelf-life of tiger nuts' milk beverage UHPH-treated at 300 MPa was almost twice as long as the shelf-life of beverages treated at 200 MPa.

Concerning EB, FC, LB and MY, no microbiological growth was observed (< 0.5 cfu/mL) during cold storage of H-P and UHPH beverages. MS counts, which are reported to be partially responsible of the physicochemical changes occurred in these beverages over time (Lafuente et al., 1985), slightly increased (~ 1 log units) in all treated tiger nuts' milk beverages (Figure 2C).

As can be observed in Figure 3, during cold storage of beverages, as expected, the pH value decreased inversely proportionally to microbiological growth, evidencing the loss of quality of these beverages.

2.3.2. *Evolution of sensory characteristics*

Figure 4 shows the results obtained in the sensory evaluation of beverages stored under refrigeration. As shown at day 3, panelists were not able to detect differences ($P > 0.05$) in the thickness, aroma and taste attributes between treated beverages and RP sample, suggesting that biochemical and physicochemical changes occurred in these products during their shelf-life had no impact on their sensory profile. By the contrary, mode values of color reflected that all treated beverages were often given lower scores than

the RP (Table 3), which demonstrated that during storage panellists always considered the treated samples whither than the control. These color differences were in accordance with the results obtained in the instrumental evaluation of color. Global color of stored beverages changed over time, as evidenced by ΔE* parameter (Figure 5C). These differences were mainly explained by a decrease in L* and WI (Figure 5A and 5B). At the end of their microbiological shelf-life, RP sample showed the lowest WI values, followed by H-P and both UHPH samples. These changes may be related to the formation of new aggregates of particles, modifying the light diffraction of the food matrix, in addition to the chemical reactions occurred during storage (Codina-Torrella et al., 2016).

2.4. Conclusions

This study demonstrated that UHPH treatments performed at 200 and 300 MPa achieved a better microbial inactivation of tiger nuts' milk beverages in comparison to the conventional homogenization-pasteurization, being the 300 MPa UHPH-treated beverage the most stable. Microbiological shelf-life of the raw product was improved from ~3 to ~25, ~30 and ~57 days with the application of conventional homogenization-pasteurization and UHPH processing at 200 and 300 MPa, respectively. Results obtained in the sensory evaluation pointed out that the color of beverages was the only attribute that differentiated UHPH from the others, with greater luminosity and whiteness. In conclusion, UHPH processing is presented as an alternative treatment to conventional pasteurization for obtaining tiger nuts' milk beverages with an improved microbiological shelf-life and a good sensorial profile.

2.5. Acknowledgments

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Figure 1. Sensory parameters of beverages at day 3. RP: raw product; H-P: homogenized-pasteurized (18 + 4 MPa, 80 ºC for 15 s); 200 MPa: ultra-high pressure homogenized at 200 MPa and an inlet temperature of 40 ºC; 300 MPa: ultra-high pressure homogenized at 300 MPa and an inlet temperature of 40 ºC.

Figure 2. Microbial population (log cfu / mL) of RP and treated beverages during cold storage. A) Aerobic mesophilic, B) Psychrotrophic bacteria and c) Mesophylic spores. RP (■): raw product; H-P (♦): homogenized-pasteurized (18 + 4 MPa, 80 ºC for 15 s); 200 MPa (▲): ultra-high pressure homogenized at 200 MPa and an inlet temperature of 40 ºC; 300 MPa (●): ultra-high pressure homogenized at 300 MPa and an inlet temperature of 40 ºC.

Figure 3. Evolution of the pH value of beverages during refrigerated storage at 4 ºC. RP: raw product; H-P: homogenized-pasteurized (18 + 4 MPa, 80 ºC for 15 s); 200 MPa: ultra-high pressure homogenized at 200 MPa and an inlet temperature of 40 ºC; 300 MPa: ultra-high pressure homogenized at 300 MPa and an inlet temperature of 40 ºC.

Figure 4. Evolution of sensory parameters of beverages during their cold storage at day 14 (A), 21 (B), 28 (C) and 56 (D). RP: raw product; H-P: homogenized-pasteurized (18 + 4 MPa, 80 ºC for 15 s); 200 MPa: ultra-high pressure homogenized at 200 MPa and an

inlet temperature of 40 ºC; 300 MPa: ultra-high pressure homogenized at 300 MPa and an inlet temperature of 40 ºC.

Figure 5. Evolution of lightness (L*) (A), whiteness index (WI) (B) and color differences (ΔE*) (C) parameters during the cold storage of beverages. RP (■): raw product; H-P (♦): homogenized-pasteurized (18 + 4 MPa, 80 ºC for 15 s); 200 MPa (▲): ultra-high pressure homogenized at 200 MPa and an inlet temperature of 40 ºC; 300 MPa (●): ultra-high pressure homogenized at 300 MPa and an inlet temperature of 40 ºC.

Figure 1

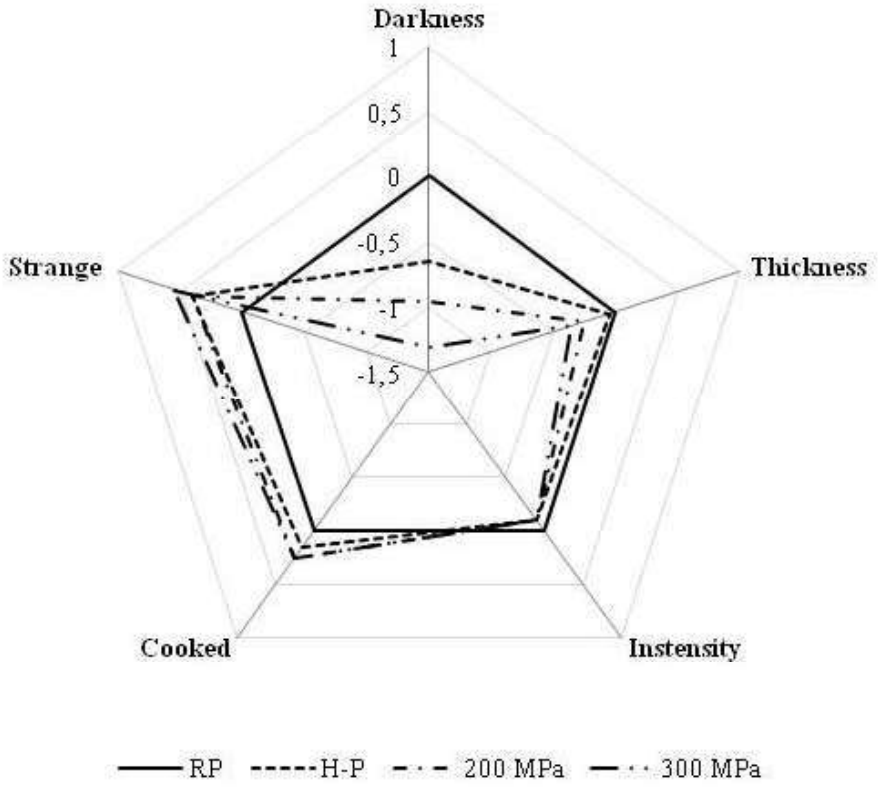

Darkness
1
0,5
0
-0,5
-1
-1,5
Thickness
Instensity
Cooked
Strange
RP
H-P
200 MPa
300 MPa

Figure 2

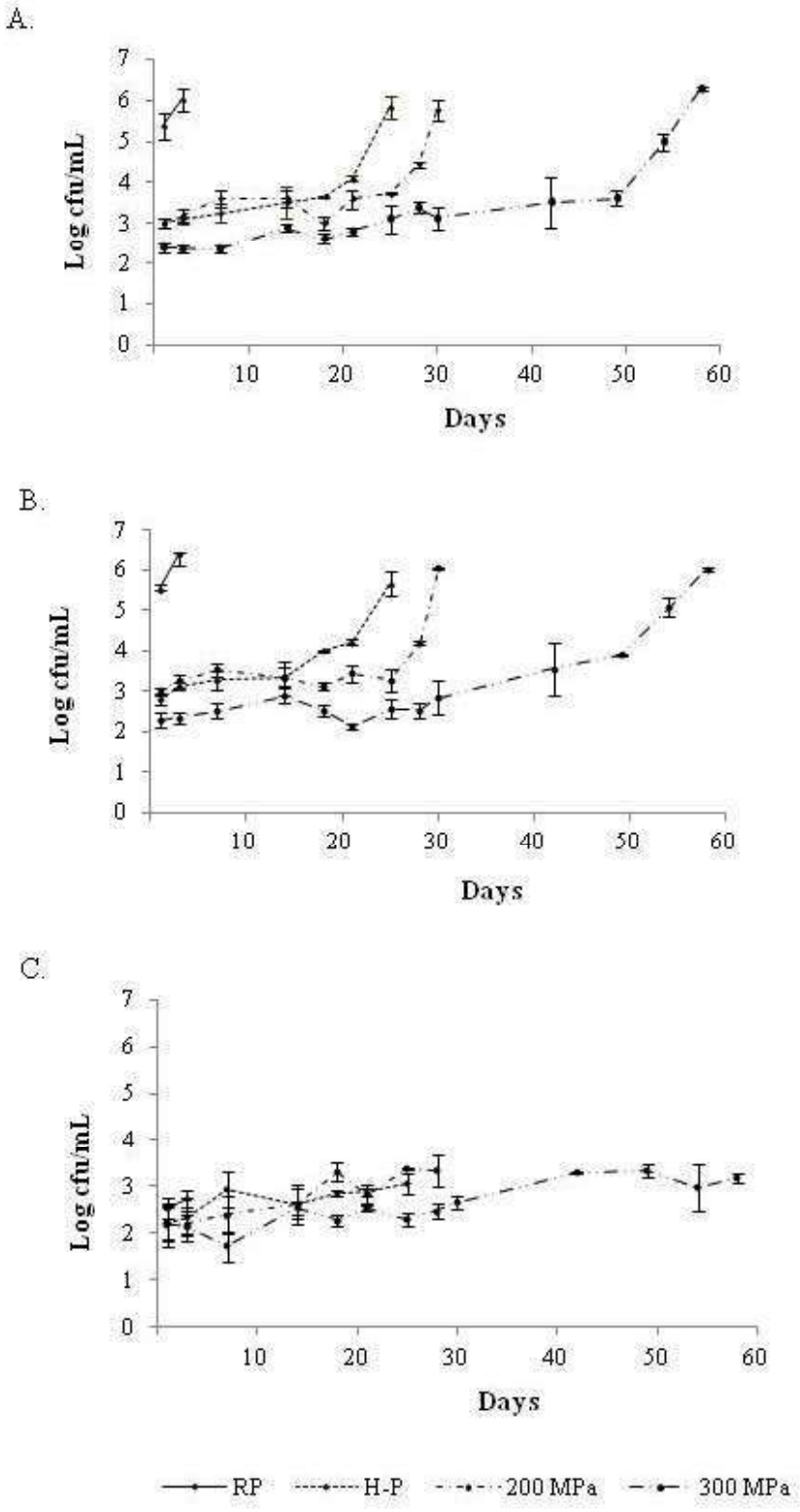

A.
Log cfu/mL
Days
B.
Log cfu/mL
Days
C.
Log cfu/mL
Days
RP
H-P
200 MPa
300 MPa

Figure 3

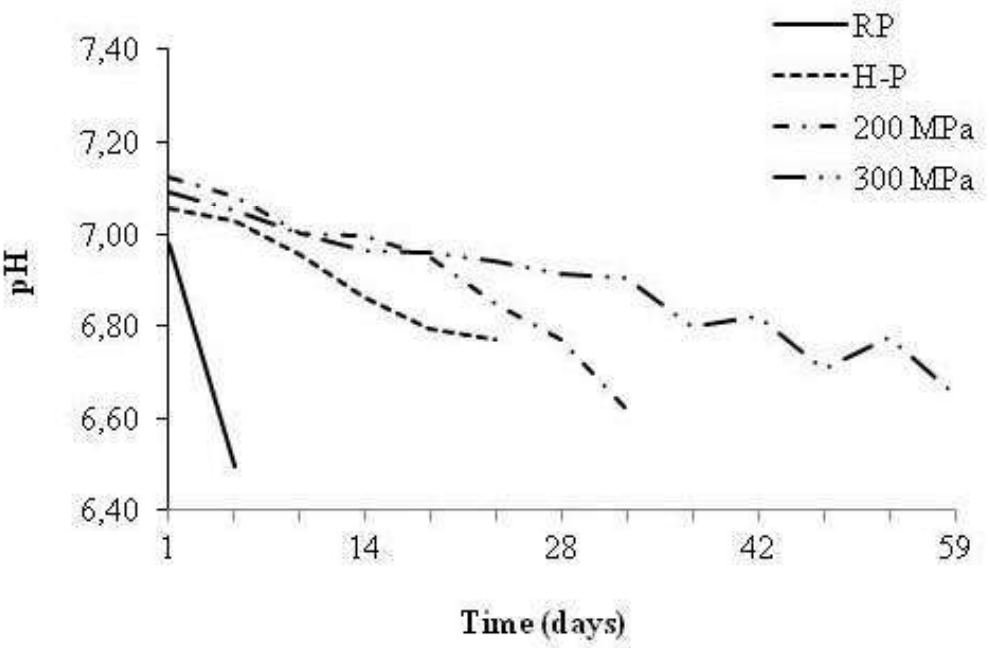

RP
H-P
200 MPa
300 MPa
7,40
7,20
7,00
6,80
6,60
6,40
pH
1
14
28
42
59
Time (days)

Figure 4

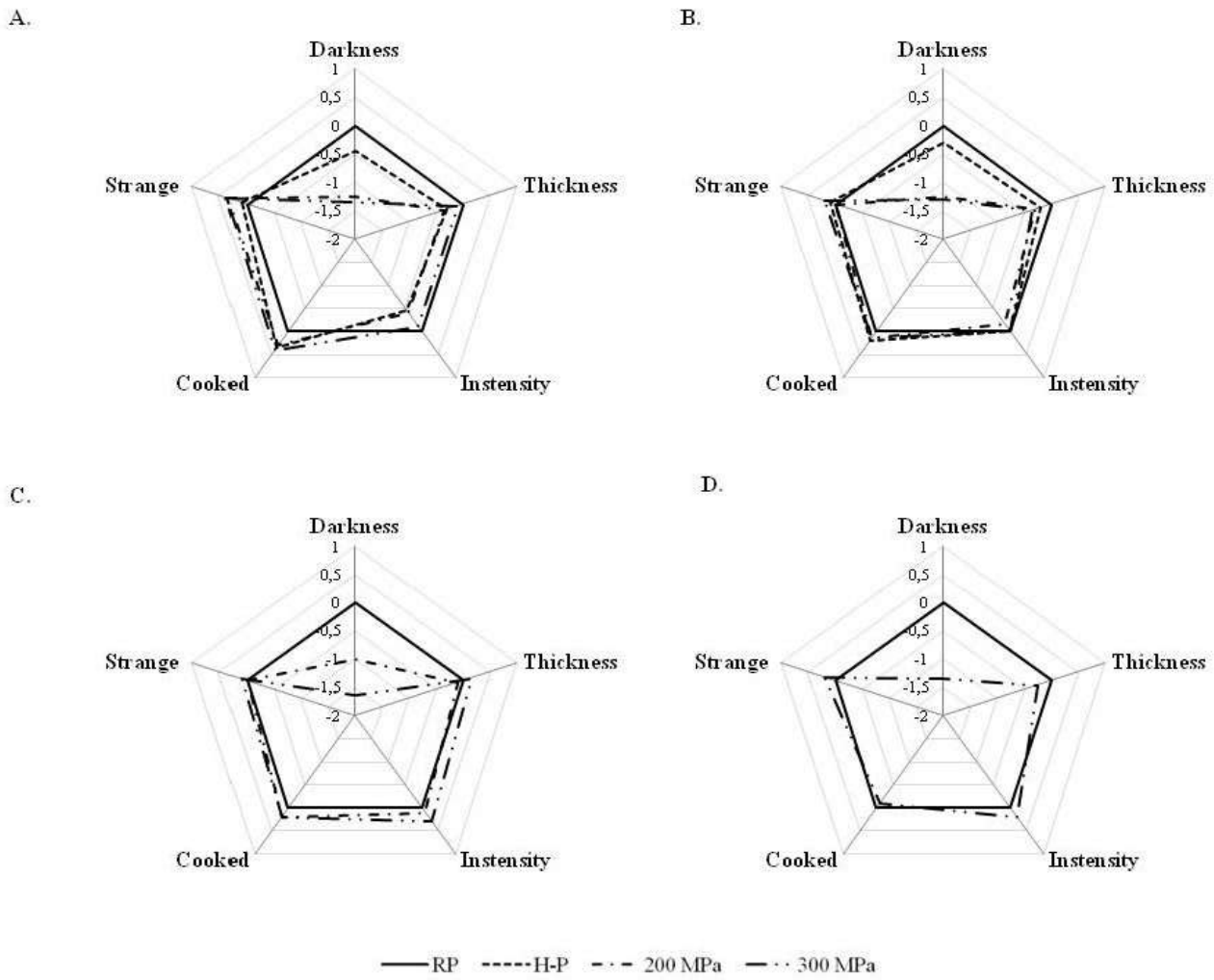
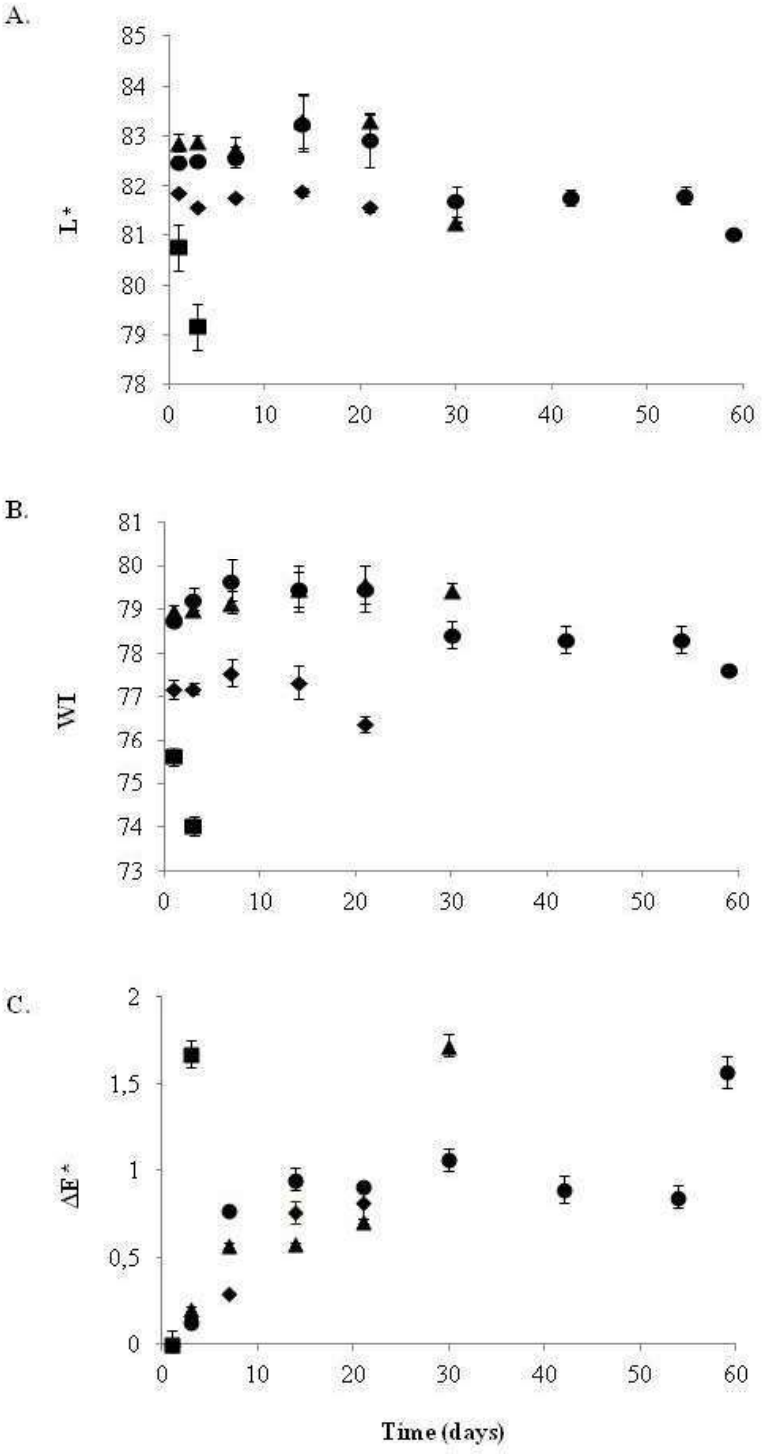

A.
B.
C.
D.
Darkness
Thickness
Instensity
Cooked
Strange
1
0,5
0
-0,5
-1
-1,5
-2
RP
H-P
200 MPa
300 MPa

Figure 5


A.
L*
85
84
83
82
81
80
79
78
0
10
20
30
40
50
60
B.
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81
80
79
78
77
76
75
74
73
0
10
20
30
40
50
60
C.
ΔE*
2
1,5
1
0,5
0
0
10
20
30
40
50
60
Time (days)

Table 1. Microbial populations (log cfu/mL) of raw and treated tiger nuts' milk beverages.

Microbial group	Treatment[1]			
	RP	**H-P**	**200 MPa**	**300 MPa**
Psychrotrophic	5,37^{a} ± 0,33	3,01^{b} ± 0,10	3,00^{b} ± 0,11	2,37^{c} ± 0,13
Aerobic mesophilic	5,51^{a} ± 0,23	2,78^{b} ± 0,25	3,03^{b} ± 0,07	2,29^{c} ± 0,17
Mesophylic spores	2,53^{a} ± 0,23	2,22^{a} ± 0,34	2,17^{a} ± 0,45	2,23^{a} ± 0,38
Enterobacteriaceae	3,61^{a} ± 0,13	ND^{b}	ND^{b}	ND^{b}
Escherichia coli	ND	ND	ND	ND
Lactobacilli	2,58^{a} ± 0,20	ND^{b}	ND^{b}	ND^{b}
Molds and yeasts	2,87^{a} ± 0,32	ND^{b}	ND^{b}	ND^{b}

$^{a\text{-}c}$ Mean value ± s.e.; ND: not detected, detection limit < 0.5 log ufc/mL; values without common superscripts per microorganism were significantly different ($P < 0.05$) by Tukey test.

[1] RP: raw product; H-P: homogenized-pasteurized (18 + 4 MPa, 80 ºC for 15 s); 200 MPa: ultra-high pressure homogenized at 200 MPa and an inlet temperature of 40 ºC; 300 MPa: ultra-high pressure homogenized 300 MPa and an inlet temperature of 40 ºC.

Table 2. Color parameters[1] of raw and treated tiger nuts' milk beverages.

Treatment [2]	L*	a*	b*	ΔE*	WI
RP	$80.76^{b} \pm 0.45$	$-0.48^{a} \pm 0.04$	$14.92^{a} \pm 0.17$	–	$75.62^{c} \pm 0.27$
H-P	$81.85^{a} \pm 0.27$	$-0.18^{b} \pm 0.03$	$13.80^{b} \pm 0.14$	$1.64^{b} \pm 0.12$	$77.19^{b} \pm 0.22$
200 MPa	$82.87^{a} \pm 0.18$	$-0.13^{b} \pm 0.01$	$12.19^{c} \pm 0.06$	$3.15^{a} \pm 0.01$	$78.97^{a} \pm 0.11$
300 MPa	$82.49^{a} \pm 0.11$	$-0.13^{b} \pm 0.02$	$12.04^{c} \pm 0.09$	$3.18^{a} \pm 0.08$	$78.74^{a} \pm 0.07$

[a-c] Mean value ± s.e.; Values without common superscripts per column were significantly different ($P < 0.05$) by Tukey test.

[1] L*: lightness; a*: green-red component; b*: blue-yellow component; ΔE*: total color differences calculated taking into account RP sample as reference; WI: whiteness index.

[2] RP: raw product; H-P: homogenized-pasteurized (18 + 4 MPa, 80 ºC for 15 s); 200 MPa: ultra-high pressure homogenized at 200 MPa and an inlet temperature of 40 ºC; 300 MPa: ultra-high pressure homogenized at 300 MPa and an inlet temperature of 40 ºC.

Table 3. Sensorial descriptive statistics (mode and relative frequency) of samples during the storage[1].

Treatment [2]	Storage day			
	14	**21**	**28**	**54**
H-P	− 1 (0.60)	− 1 (0.53)	-	-
200 MPa	− 1 (0.57)	− 2 (0.53)	− 1 (0.67)	-
300 MPa	− 1 (0.50)	− 2 (0.54)	− 2 (0.68)	− 2 (0.57)

[1] Mode (relative frequency). H-P, 200 MPa and 300 MPa samples were compared with RP; negative and positive values denote lower or greater perception, respectively.

[2] RP: raw product; H-P: homogenized-pasteurized (18 + 4 MPa, 80 ºC for 15 s); 200 MPa: ultra-high pressure homogenized at 200 MPa and an inlet temperature of 40 ºC; 300 MPa: ultra-high pressure homogenized at 300 MPa and an inlet temperature of 40 ºC.

Highlights

- UHPH processing was evaluated as a potential technology for tiger nuts' milk stabilization.
- UHPH-treated beverages showed better microbial shelf-life than the conventional homogenized-pasteurized product.
- Sensory evaluation suggested a consumers' acceptability towards the UHPH-treated products.