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4 **1 Microbiological stabilization of tiger nuts' milk beverage by using ultra-high**  
5 **2 pressure homogenization**  
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62 **Abstract**  
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64 Tiger nuts' milk beverages are highly perishable products. Consequently, the interest of  
65 food industry for their commercialization makes necessary the application of  
66 preservation treatments to prolong their shelf-life. In the current study, the effect of  
67 ultra-high pressure homogenization (UHPH) on the microbiological and sensory quality  
68 of tiger nuts' milk beverage was evaluated. Characteristics of UHPH-treated products  
69 (at 200 and 300 MPa, with inlet temperature of 40 °C) were compared with those of raw  
70 (RP) and conventionally homogenized-pasteurized (H-P) beverages, after treatment and  
71 during cold storage at 4 °C. Microbiological quality of beverages was studied by  
72 enumerating total counts, psychrotrophic bacteria, lactobacilli, enterobacteria, molds  
73 and yeasts, and mesophylic spores. Physicochemical characteristics (pH and color) and  
74 sensory evaluation of beverages were also evaluated. Microbiological shelf-life of the  
75 tiger nuts' milk beverages was extended from 3 to 25, 30 and 57 days by applying H-P  
76 and UHPH treatments at 200 and 300 MPa, respectively. Although all treatments  
77 increased the brightness of tiger nuts' milk beverages, treated samples turned darker  
78 during storage, and their pH value decreased. Hence, UHPH treatments showed to be an  
79 alternative to the conventional H-P for obtaining tiger nuts' milk beverages with an  
80 improved microbiological shelf-life and good sensorial characteristics.  
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104 **Keywords:** Ultra-high pressure homogenization, tiger nuts' beverage, microbial  
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121 **1. Introduction**  
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123 Tiger nuts' milk beverages are one of the most appreciated vegetable beverages, which  
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125 are obtained from the aqueous extract of tiger nuts tubers (*Cyperus esculentus* L.)  
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127 (Cokuner et al., 2002). These beverages are rich in carbohydrates (>50%), unsaturated  
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129 fatty acids (75% in oleic and ~10% in linoleic acids, of total fat) and dietary fiber  
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131 (~1%), and also contain a moderate percentage of nutritional minerals (phosphor,  
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133 calcium, magnesium and iron) and vitamins (C and E) (Alegría-Torán and Farré-Rovira,  
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135 2003; Borges et al., 2008; Sánchez-Zapata et al., 2012). Different studies pointed out  
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137 their suitability for lactose-intolerant and celiac patients, and also for preventing  
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139 digestion disorders (Adejuyitan, 2011; Alegría-Torán et al., 2003). Due to the high  
140  
141 microbiological loads of harvested tubers and the resistance of these spoilage  
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143 microorganisms to disinfecting treatments, total aerobic counts in these beverages are  
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145 usually in the range of 5-6 log cfu/mL (Gallart, 1999), thereby necessitating their  
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147 immediate consumption (Corrales et al., 2012; Selma et al., 2002). In view of the global  
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149 tendency towards an increase in the consumption of vegetable beverages, it is of  
150  
151 particular interest to food producers to prolong the commercial shelf-life of these  
152  
153 perishable products to enable worldwide distribution. Conventional heat preservation  
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155 treatments such as pasteurization and sterilization are being the most commonly used in  
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157 food industry (Selma et al., 2003). However, these treatments result in an undesirable  
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159 loss of the most appreciated sensory characteristics (e.g. pale color or tiger nuts' flavor  
160  
161 and taste). Owing to this, food industry looks for alternative technologies that improve  
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163 the microbiological quality of these beverages while preserving their sensory  
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165 characteristics (Corrales et al., 2012; Cortés et al., 2005; Selma et al., 2003). At present,  
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167 few studies have demonstrated the potential of non-thermal technologies to reduce the  
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169 spoilage-related microorganisms of tiger nuts' milk beverages. Selma et al. (2003)  
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180 76 evaluated the suitability of high-intensity pulsed electric fields for reducing the potential  
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182 77 growth of *Enterobacter aerogenes*, but no more than 1.1 log reductions were reported to  
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184 78 be achieved by any of the treatments applied. In line with this, Corrales et al. (2012)  
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186 79 validated the feasibility of short wave ultraviolet radiation (UV-C) to inactivate  
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188 80 psychrotrophic and mesophilic bacteria and yeasts and molds. These authors  
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190 81 demonstrated that UV-C treatments at a fluence rate of 2.35 mW/cm<sup>2</sup> during 10 min  
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192 82 achieved 3-3.5 log cycles reduction of these spoilage-related microorganisms, and the  
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194 83 shelf-life of beverages was extended from 2 to 4 days (at 2 °C).  
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197 84 Ultra-high pressure homogenization (UHPH) is a novel technology that allows the  
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199 85 microbial inactivation and improves the colloidal stability of fluid foodstuffs  
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201 86 maintaining, in most cases, both nutritional and sensory characteristics of untreated  
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203 87 products (Dumay et al., 2012; Zamora & Guamis, 2015). This technology is based on  
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205 88 the same principle as the conventional homogenization, but it is capable of working at  
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207 89 pressures up to 350 MPa. The physical phenomena that fluid suffers when passing  
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209 90 through the high-pressure valve gap and at the outlet (e.g. cavitation, pressure drop,  
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211 91 shear stress, etc.) in combination with the sudden temperature jump promotes  
212  
213 92 significant changes in the product characteristics, such as the disruption of vegetative  
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215 93 microorganisms (Dumay et al., 2012; Zamora & Guamis, 2015) and in some cases, the  
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217 94 reduction of spores counts (Georget et al., 2014; Zamora & Guamis, 2015). Some  
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219 95 published contributions demonstrate the suitability of this technology for the  
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221 96 improvement of microbiological quality in soymilk and almond-milk beverages, in  
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223 97 comparison to the conventional heat treatments (Cruz et al, 2007; Ferragut et al., 2014;  
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225 98 Poliseli-Scopel et al., 2012, 2014; Valencia-Flores et al., 2013). According to the  
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227 99 literature, some of the most important processing parameters that influence in the  
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229 100 effectiveness of this treatment are the operating pressure, the inlet temperature ( $T_i$ ) and  
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239 101 the number of passes (Diels and Michiels, 2006). In line with this, studies performed  
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241 102 with soymilk and almond-milk beverages reported that UHPH treatments at 200 and  
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243 103 300 MPa with the combination of  $T_i$  of 55-75 °C were more effective than conventional  
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245 104 pasteurization against almost all spoilage microorganism inactivation (Poliseli-Scopel et  
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247 105 al., 2013; Valencia-Flores et al., 2013). Poliseli-Scopel et al. (2014) also showed that  
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249 106 UHPH treatments at 300 MPa and  $T_i \geq 75$  °C allowed the commercial sterility of  
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251 107 soymilk. In this study, these sensory response of panelists after the evaluation of  
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253 108 UHPH-pasteurized (200 MPa at  $T_i$  of 55-75 °C) and UHPH-sterilized (300 MPa at  $T_i$  of  
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255 109 80 °C) soymilks showed a positive trend.  
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257 110 Nevertheless, the potential effect of this stabilizing technology not only depends on the  
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259 111 process parameters but also on the characteristics of the food matrix. In this way, the  
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261 112 aim of the present work was to evaluate the potential of UHPH as a technology for  
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263 113 improving microbiological quality and sensory characteristics of raw tiger nuts' milk  
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265 114 beverages during cold storage (4 °C), as alternative to the conventional heat treatment of  
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267 115 homogenization-pasteurization.  
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## 273 117 **2. Materials and Methods**

### 274 118 *2.1. Tiger nuts' milk beverage making*

275  
276 119 Tiger-nuts beverages were produced and processed at the Pilot Plant of Universitat  
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278 120 Autònoma de Barcelona (UAB, Bellaterra, Spain), as described by Codina-Torrella et  
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280 121 al. (2016). The general composition of tubers (as % dry matter), which were under the  
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282 122 geographical origin *Chufa de Valencia*, corresponded to 8.66 ±0.04 moisture, 35.21  
283  
284 123 ±3.07 fat, 8.45 ±0.20 protein and 45.05 ±3.13 nitrogen free material (NFM), as  
285  
286 124 previously described by Codina-Torrella et al. (2015). Raw product (RP) consisted in  
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288 125 the liquid extract with 8% (w/w) of added sucrose. The composition of RP (%)  
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298 126 corresponded to: 12.99  $\pm$ 0.18 total solids, 10.30  $\pm$ 0.60 nitrogen free materials, 2.01  
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300 127  $\pm$ 0.02 fat, 0.54  $\pm$ 0.02 proteins and 0.13  $\pm$ 0.01 ashes. Representative samples of the RP  
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302 128 were bottled in sterilized glass bottles and stored at refrigeration (4 °C) until analysis.  
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304 129 Just before the application of the hygienizing treatments of UHPH and conventional  
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306 130 pasteurization, 0.05% of  $\alpha$ -amylase enzyme (Bialfa, Biocon Española, S.A., Les  
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308 131 Franqueses del Vallès, Spain) was added to the RP, in order to hydrolyze the starch  
309  
310 132 granules and avoid their subsequent gelatinization when heating. The holding time of  
311  
312 133 the enzyme in RP before applying the technological treatments corresponded to 10 min.  
313  
314 134 Qualitative determination of starch (Total Starch Assay Procedure kit,  
315  
316 135 Amyloglucosidase/ $\alpha$ -amylase method, K-TSTA 404-2009, Megazyme International  
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318 136 Ireland Ltd., Wicklow, Ireland) in all samples demonstrated that after the treatment this  
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320 137 component was totally hydrolyzed in all beverages.  
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## 326 139 *2.2. Beverage treatments: UHPH, homogenization-pasteurization*

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328 140 Two different UHPH treatments were performed by using an ultra-high pressure  
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330 141 homogenizer at a flow rate of 120 L/h (Model: DRG No. FPG11300:400 Hygienic  
331  
332 142 Homogenizer, Stansted Fluid Power Ltd., Harlow, UK) at two different pressures, 200  
333  
334 143 and 300 MPa, and at the same  $T_i$  of 40 °C. The high-pressure homogenizer system  
335  
336 144 consisted of two intensifiers driven by a hydraulic pump, a high-pressure  
337  
338 145 homogenization valve and two spiral type heat exchangers located before the machine  
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340 146 entrance and after the high-pressure valve (Garvía, Barcelona, Spain), respectively  
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342 147 (Poliseli-Scopel et al., 2012). Temperatures during treatment, i.e.,  $T_i$ , those before and  
343  
344 148 after UHPH valve ( $T_1$  and  $T_2$ , respectively) and at the outlet ( $T_o$ ), were monitored.  
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346 149 A conventional treatment of Homogenization-Pasteurization (H-P) was also applied to  
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348 150 RP sample using an indirect system composed by a double stage homogenizer  
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357 151 positioned upstream (Model X68, Soavi B. & Figli, S.P.A., Parma, Italy) and a  
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359 152 multitube tubular heat exchanger at a flow rate of 1000 L/h (laminar flow) (6500/010,  
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361 153 GEA Finnah GmbH, Ahaus, Germany). Beverages were homogenized at 18 + 4 MPa at  
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363 154 65 °C and subsequently pasteurized at 80 °C for a holding time of 15 s.  
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366 155 All samples (RP, H-P, 200 MPa and 300 MPa) were collected in sterilized glass bottles  
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368 156 of 1 L of capacity with twist-off caps (Apiglass Envases y Material Apícola, S.L) inside  
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370 157 a laminar flow cabinet (Mini-V/PCR cabinet, Telstar Technologies, S.L., Terrassa,  
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372 158 Spain) and were stored at refrigeration temperature (4 °C) until analyzed.  
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### 378 160 *2.3. Microbiological analysis*

379 161 Decimal dilutions in peptone water solution were used for microbiological enumeration.  
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381 162 Aerobic mesophilic (AM) counts were enumerated on plate count agar (PCA, Oxoid  
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383 163 Ltd., Basingstoke, UK) incubated at 30 °C for 48 h. Psychrotrophic bacteria (PS) were  
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385 164 enumerated on PCA, incubated at 21 °C for 72 h. Lactobacilli (LB) were enumerated on  
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387 165 Rogosa agar (Oxoid), incubated at 30 °C for 72 h. *Enterobacteriaceae* (EB) counts were  
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389 166 enumerated on violet red bile glucose agar (Oxoid), incubated at 37 °C for 24 h. Faecal  
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391 167 coliforms (FC) were enumerated on Coli ID selective chromogenic medium  
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393 168 (bioMérieux S.A., Madrid, Spain), incubated at 37 °C for 24 h. The *E. coli* presence was  
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395 169 also evaluated by color difference using this chromogenic medium. For the total  
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397 170 mesophylic spores (MS) enumeration, samples were heated at 80 °C for 10 min and  
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399 171 quickly-cooled in ice, and pour plated on PCA, incubated at 30 °C for 48 h. Molds and  
400  
401 172 yeast (MY) were enumerated on Rose Bengal agar (Oxoid) with chloramphenicol  
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403 173 supplement (Oxoid), incubated at 25 °C for 5 days.  
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406 174 Microbiological quality of beverages was monitored during their storage at 4 °C, until  
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408 175 microbiological counts exceed to the satisfactory microbial limit for their acceptance  
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416 176 ( $\leq 6 \log \text{cfu/mL}$ ) (Corrales et al., 2012). Regarding to this, microbiological analyses  
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418 177 were performed at days 1 and 3 (for all samples), 14, 21 and 25 (for H-P and UHPH  
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420 178 samples), 28 and 30 (for UHPH samples), 35, 42, 49, 54 and 58 for 300 MPa UHPH-  
421  
422 179 treated samples.

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425 180 For PS bacteria, lag time and the maximum velocity of growth in the exponential phase  
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427 181 were calculated by using DMFit software, which is available at Combase website  
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429 182 (ComBase, <http://www.combase.cc>). DMFit is a fitting tool for bacterial growth that  
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431 183 displays different parameters for the selected model. In this case, predictions were based  
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433 184 on the primary model of Baranyi and Roberts (1994). The pH and initial levels of PS  
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435 185 bacteria were chosen based on the corresponding results obtained in the current study at  
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437 186 day 1.

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#### 441 188 *2.4. pH determination and color analysis*

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444 189 The determination of pH was performed in triplicates at 20 °C by potentiometric  
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446 190 measurement (Crison micropH 2001, Alella, Spain). Color analyses of beverages were  
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448 191 conducted in triplicate with a Hunter Lab colorimeter (MiniScan XETM, Hunter  
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450 192 Associates Laboratory Inc., Reston, USA). Color coordinates were measured with an  
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452 193 illuminant of D65 and a standard observer angle of 10°. Colorimeter was calibrated with  
453  
454 194 standard black and white tiles. Data was acquired in the CIELab color space: L\*  
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456 195 (luminosity), a\* (red-green) and b\* (blue-yellow). The total color differences  $\Delta E^*$  (Eq.  
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458 196 1) were calculated taking into account RP sample as the reference. During storage, for  
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460 197 each type of product, this difference was calculated considering their respective  
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462 198 homologue at day 1. Whiteness index (WI) was also calculated using L\*, a\* and b\*  
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464 199 parameters (Eq. 2).

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467 200 (1)  $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$   
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$$(2) \text{ WI} = [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$
  
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480 203 *2.5. Sensory evaluation*

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482 204 Ten trained judges from the staff at Universitat Autònoma de Barcelona (UAB) were  
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484 205 pre-selected based on their previous experience in the evaluation of vegetal beverages  
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486 206 and their habitual consumption of tiger nuts' milk beverages. Panelists were trained as  
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488 207 described by Poliselí-Scopel et al. (2012). Beverages (~30 mL) were presented at the  
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490 208 usual temperature of consumption of these products (~10 °C), in transparent cups with a  
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492 209 3-digit random code. Sensory evaluation was carried out at days 3, 14, 21, 28 and 56 of  
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494 210 storage, depending on the microbiological shelf-life of beverages. Owing to the short  
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496 211 microbiological shelf-life of the reference sample, RP was produced each day of the  
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498 212 sensory test. The evaluation consisted on a descriptive test, in which five attributes were  
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500 213 evaluated: color (darkness), aroma (intensity), mouthfeel (cooked and strange) and  
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502 214 thickness. Responses were recorded on an intensity scale from - 4 to 4 points, where  
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504 215 differences were qualified as very considerable (-4 and 4), considerable (-3 and 3),  
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506 216 noticeable (-2 and 2), minimal (-1 and 1) or unnoticeable (0 points) in comparison with  
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508 217 the RP sample. Algebraic sign, i.e. negative or positive, indicates lower or greater  
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510 218 perception.  
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516 220 *2.6. Statistical analysis*

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518 221 The overall experiment was performed in triplicate on three different days, thereby  
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520 222 consisted on three independent batches of 200 L of tiger nuts' milk beverage.  
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522 223 Microbiological analyses were carried out in duplicate. Physicochemical analyses were  
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524 224 performed in triplicate. Data exposed in the text corresponds to the mean ± standard  
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526 225 error. Statistical analyses were performed using the GLM procedure of Statgraphics  
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534 226 (Statgraphics Inc., Chicago, IL, USA). Tukey test was used for the data comparison and  
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536 227 significant differences were determined at the 5% level of probability. For sensory data,  
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538 228 descriptive statistics (mode and relative frequency of either negative or positive values)  
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540 229 were calculated. Means were analyzed for significance as previously described.  
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## 545 231 **2. Results and Discussion**

### 547 232 *2.1. Temperature changes during UHPH processing*

549 233 In the current study, the temperature of UHPH-treated tiger nuts' milk beverages  
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551 234 increased 24.2 °C between the pressure range of 200-300 MPa, as it was previously  
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553 235 described by other authors who worked with the same UHPH equipment (Pereda et al.,  
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555 236 2007; Polisel-Scopel et al., 2012; Suárez-Jacobo et al., 2012). The rise of temperature  
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557 237 is attributed to the physical phenomena that fluids experimented during this process and  
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559 238 downstream of the valve, as a consequence of the adiabatic heating (Floury et al., 2000;  
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561 239 Hayes & Kelly, 2003). Thereby, at the outlet of the high-pressure valve the product  
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563 240 reached 92.1 ±1.7 and 116.3 ±4.3 °C for the 200 and 300 MPa treatments, respectively,  
564  
565 241 although the residence time of the product at this temperature was estimated to be very  
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567 242 short (<0.7 s) in comparison with the conventional heat treatments (Polisel-Scopel et  
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569 243 al., 2012). In this study, T<sub>0</sub> corresponded to 15.3 ±1.1 and 17.1 ±1.6 °C in 200 and 300  
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571 244 MPa treatments, respectively.  
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### 577 246 *2.2. Effect of treatments on tiger nuts' milk beverage characteristics*

#### 579 247 *2.2.1. Microbiological quality and pH*

581 248 Microbiological quality of vegetable beverages determines their shelf-life and also  
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583 249 impacts on the biochemical changes that occur during their storage. Composition and  
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585 250 neutral pH of tiger nuts' milk beverages strongly promote a fast microbiological growth,  
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593 251 therefore it is necessary the application of preservation treatments to extend their quality  
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595 252 over time. In the current study, the pH value of the RP corresponded to  $7.02 \pm 0.19$ ,  
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597 253 which did not significantly vary ( $P > 0.05$ ) after the application of H-P and UHPH  
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599 254 treatments.  
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601 255 Samples were analyzed for the microbial groups that are most typically found in this  
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603 256 product (Corrales et al., 2012; Selma et al., 2002). As can be observed in Table 1,  
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605 257 microbiological counts found for RP samples were in agreement with the results  
606  
607 258 obtained by other authors in this type of beverages (Gallart, 1999; Hernández and Vilar,  
608  
609 259 1967). Initial counts of AM and PS bacteria (5.51 and 5.37 log cfu/mL, respectively)  
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611 260 evidenced the high bacteria counts of this type of beverage after production, which are  
612  
613 261 normally comprised between 5-6 log cfu/mL (Corrales et al., 2012; Selma et al., 2002).  
614  
615 262 MS counts corresponded to 2.53 log cfu/mL. These microorganisms probably belonged  
616  
617 263 to genus *Bacillus* (*B. subtilis*, *B. brevis* and *B. cereus*), the most typical spore-forming  
618  
619 264 bacteria isolated in these type of beverages (Nyarko et al., 2011). EB and FC counts  
620  
621 265 were 2.91 and 2.31 log cfu/mL respectively, and no *E. coli* bacteria was detected in any  
622  
623 266 sample. The presence of these undesirable microorganisms in the RP could be related to  
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625 267 the cropland and the use of animal manure as fertilizer. MY and LB counts were 2.87  
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627 268 and 2.58 log cfu/mL, respectively. These spoilage microorganisms are responsible for  
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629 269 the quick alteration of tiger nuts' milk beverages during cold storage through  
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631 270 fermentation of sugars and the concomitant production of acid and gas (Hernández et  
632  
633 271 al., 1967; Monerrris-Aparisi et al., 1998).  
634  
635 272 All treatments reduced significantly ( $P < 0.05$ ) the microbial counts of the RP (Table 1).  
636  
637 273 Homogenization-pasteurization provoked the same microbial reduction of AM and PS  
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639 274 bacteria (~2 log) than those observed in UHPH-treated samples at 200 MPa. However,  
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641 275 increasing the pressure to 300 MPa triggered a reduction of ~3 log units for both groups  
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652 276 of microorganisms. In UHPH processing, the rate of microorganism inactivation mainly  
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654 277 depends on the working pressure and the inlet temperature of products, showing better  
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656 278 results with the increase of both parameters (Diels and Michiels, 2006; Picart et al.,  
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658 279 2006) as has been demonstrated in the particular case of soymilk (Cruz et al., 2007;  
660  
661 280 Poliseli-Scopel et al., 2012, 2014). At the same  $T_i$  of 40 °C, Cruz et al. (2007) reported a  
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663 281 major reduction of the total bacteria counts by increasing the working pressure from 200  
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665 282 to 300 MPa (~2 and 4 log, respectively). In line with this, Poliseli-Scopel et al. (2012)  
666  
667 283 demonstrated that UHPH treatments at 200 MPa increased their efficiency in total  
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669 284 counts reduction if  $T_i$  increased from 55 to 75 °C with mesophilic bacterial counts of 3.2  
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671 285 and 0.3 log units, respectively. Furthermore, at 300 MPa and a  $T_i$  of 80 °C, UHPH  
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673 286 treatment resulted in soymilk microbiologically stable at room temperature for 6 months  
674  
675 287 (Poliseli-Scopel et al., 2014).

678 288 In the present study, none of the stabilizing treatments resulted in a reduction in MS  
679  
680 289 counts (Table 1), showing the high resistance of these microorganisms to the studied  
681  
682 290 treatments. Some research has been conducted in order to elucidate the effect of UHPH  
683  
684 291 on spore inactivation, concluding that successful inactivation could be achieved by  
685  
686 292 combining higher pressures and  $T_i$  (Amador-Espejo *et al.*, 2014; Georget et al., 2014).  
687  
688 293 According to literature, for UHPH treatments below 200 MPa, microorganism  
689  
690 294 inactivation is mainly dependent on the mechanical effects that fluid suffers when  
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692 295 passing through the homogenization valve. However, at higher working pressures,  
693  
694 296 microbial reduction is achieved by the synergic impact of pressure and temperature  
695  
696 297 (Pathanibul et al., 2009). The capability of UHPH to inactivate bacterial spore appeared  
697  
698 298 to be highly dependable on inlet and, consequently, valve temperature (Georget et al.,  
699  
700 299 2014). As said before, soymilk sterilization was achieved by UHPH at 300 MPa and a  $T_i$   
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702 300 of 80 °C, which corresponded to a valve temperature of 144 °C (Poliseli-Scopel et al.,  
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711 301 2014). With almond milk, no bacterial growth after incubation at 30 °C for 20 days was  
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713 302 detected in UHPH-treated samples at 300 MPa with a  $T_i$  of 65 or 75 °C, which  
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715 303 corresponded to valve temperatures of 127 and 129 °C, respectively (Valencia-Flores et  
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717  
718 304 al., 2013).  
719  
720 305 Concerning the other groups of microorganisms evaluated in the present study, all  
721  
722 306 counts (EB, FC, LB or MY) were below the detection limit (<0.5 cfu/mL) for all treated  
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724 307 beverages. In addition, *E. coli* was never detected. Such results were expected since  
725  
726 308 these microorganisms have been reported to be highly susceptible to UHPH-treatments  
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728 309 when the homogenization pressure is  $\geq 200$  MPa (Cruz et al., 2007; Hayes & Kelly,  
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730 310 2003; Pereda et al., 2007).  
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### 734 312 *2.2.2. Sensory characteristics*

735  
736 313 In the present study, relevant attributes were included in the sensory evaluation of tiger  
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738 314 nuts' milk beverages in order to get a general perception of their sensory profiles.  
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740 315 Panelists did not perceive significant differences ( $P > 0.05$ ) in aroma and taste attributes  
741  
742 316 among all samples, although tiger-nuts' milk beverages treated by conventional heat  
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744 317 treatments are characterized by the presence of different off-flavors, which are linked to  
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746 318 the secondary compounds generated in lipid oxidative processes, and protein and sugar  
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748 319 reactions. In a previous study of the present authors, it was demonstrated that after the  
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750 320 H-P treatment, tiger nuts' milk beverage showed a higher level of oxidation than RP and  
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752 321 both UHPH-treated beverages (Codina-Torrella et al., 2016). Nevertheless, the amount  
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754 322 of these generated compounds was probably under the detection threshold, which could  
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756 323 be in line with the results obtained in the sensory evaluation. The panel neither detected  
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758 324 differences ( $P > 0.05$ ) in the thicknesses between samples, although instrumental  
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770 325 rheological evaluation evidenced that 300 MPa treated beverage differed from the  
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772 326 others (Codina-Torrella et al., 2016).

774 327 By the contrary, significant differences ( $P < 0.05$ ) in the color were detected among all  
775  
776 328 samples by the judges. The mode of scores obtained for H-P and 200 MPa treated  
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778 329 beverages corresponded to -1 (with relative frequencies of 63.3 and 73.3%,  
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781 330 respectively). Instead, for 300 MPa sample, the obtained value was -2 (with a relative  
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783 331 frequency of 52%), which indicated that in most cases, panelists considered this  
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785 332 beverage as the whitest. These subjective results were in agreement with those obtained  
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787 333 in the current study by the instrumental determination of color. As seen in Table 2,  
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789 334 global color differences ( $\Delta E^*$ ) demonstrated that UHPH beverages were the most  
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791 335 different with the highest values of lightness ( $L^*$ ), although both UHPH treatments did  
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793 336 not differ. The increase of  $L^*$  could be associated with the increase of light diffraction  
794  
795 337 caused by the greater number of oil droplets dispersed in the continuous phase after  
796  
797 338 homogenization (Codina-Torrella et al., 2016). As seen in Table 2, both  $a^*$  (red-green)  
798  
799 339 and  $b^*$  (yellow-blue) parameters decreased significantly ( $P < 0.05$ ) after the application  
800  
801 340 of both homogenization treatments. In H-P sample, lower values in  $b^*$  parameter could  
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803 341 be associated with the non-enzymatic browning reactions of free sugars (and the  
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805 342 consequent formation of secondary compounds) which are activated by the temperature  
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807 343 during this processing.  
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### 814 345 *2.3. Evolution of beverages' characteristics during storage*

#### 816 346 *2.3.1. Microbiological shelf-life and pH evolution*

818 347 Figure 2 shows the evolution of microbiological populations in tiger nuts' milk  
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820 348 beverages during the cold storage. As can be seen, AM and PS counts increased over  
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822 349 time in all samples, with the highest rate in RP sample. For all cases, AM and PS counts  
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829 350 over time presented the same values suggesting that most of the microorganisms in  
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831 351 tiger-nuts' milk beverages were mainly PS bacteria. At day 3, AM and PS counts of RP  
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833 352 sample reached  $\sim 6$  log cfu/mL, which corresponds to the limit of the satisfactory  
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835 353 microbial acceptance in this type of product (Corrales et al., 2012). In fact, the  
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837 354 microbiological shelf-life of raw tiger nuts' milk beverages is generally not higher than  
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839 355 4 days, provided that the storage temperature is comprised between 4 and 6 °C (Corrales  
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841 356 et al., 2012; Moneris et al., 1998; Moreno-Seguí et al., 2000). By the contrary, counts  
842  
843 357 of MS, EB, FC, LB and MY of RP sample remained unchanged probably due to the loss  
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845 358 of viability of these microorganisms as a result of the low storage temperature (4 °C)  
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847 359 (Moreno-Seguí et al., 2000).

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851 360 In H-P and UHPH-treated beverages, PS bacteria also showed an exponential growth  
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853 361 over time (Figure 2B). Data obtained with DMFit modeling program allowed the  
854  
855 362 calculation of the length of the lag phase for PS bacteria and the maximum velocity in  
856  
857 363 their exponential phase of growth. The microbial lag phase corresponds to the time  
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859 364 necessary for bacterial cells to modify themselves in order to settle in the new  
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861 365 environment and start duplicating (initiate the exponential growth) (Swinnen et al.,  
862  
863 366 2004). Lag phase of PS corresponded to  $18.56 \pm 0.92$ ,  $26.96 \pm 0.68$  and  $43.18 \pm 2.06$  days  
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865 367 in H-P and UHPH-treated beverages at 200 and 300 MPa, respectively. These results  
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867 368 showed that UHPH triggered longer lag phases than H-P processing. Among others, the  
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869 369 extent of this phase is determined by the physiological state of microbial cells, which  
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871 370 depends on the effects of the preservation treatment. Physical effects that fluids  
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873 371 experiment downstream of the UHPH valve, together with the increase of product's  
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875 372 temperature, probably caused a higher damage to the microbial cells than the  
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877 373 conventional H-P. As seen in Figure 2B, after this lag phase, PS grew exponentially.  
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879 374 During the exponential growing phase, healthy and sub-lethally injured cells are divided  
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888 375 at a constant rate. In the present study, the maximum velocity of growth varied  
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890 376 according to the treatment, corresponding to  $0.46 \pm 0.06$ ,  $0.60 \pm 0.07$  and  $0.26 \pm 0.05$  log  
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892 377 cfu/mL/day for H-P and UHPH at 200 and 300 MPa, respectively. Concomitantly,  
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895 378 microbiological shelf-life of 200 and 300 MPa UHPH-treated products was extended to  
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897 379 30 and 57 days whilst the shelf-life of H-P beverage corresponded to 25 days. These  
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899 380 results highlight the improvement on the microbiological quality of UHPH-treated  
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901 381 products in comparison with conventional treatments. Furthermore, the shelf-life of  
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903 382 tiger nuts' milk beverage UHPH-treated at 300 MPa was almost twice as long as the  
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905 383 shelf-life of beverages treated at 200 MPa.

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907 384 Concerning EB, FC, LB and MY, no microbiological growth was observed  
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909 385 ( $< 0.5$  cfu/mL) during cold storage of H-P and UHPH beverages. MS counts, which are  
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911 386 reported to be partially responsible of the physicochemical changes occurred in these  
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913 387 beverages over time (Lafuente et al., 1985), slightly increased ( $\sim 1$  log units) in all  
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915 388 treated tiger nuts' milk beverages (Figure 2C).

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917 389 As can be observed in Figure 3, during cold storage of beverages, as expected, the pH  
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919 390 value decreased inversely proportionally to microbiological growth, evidencing the loss  
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921 391 of quality of these beverages.

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### 925 926 393 **2.3.2. Evolution of sensory characteristics**

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929 394 Figure 4 shows the results obtained in the sensory evaluation of beverages stored under  
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931 395 refrigeration. As shown at day 3, panelists were not able to detect differences ( $P > 0.05$ )  
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933 396 in the thickness, aroma and taste attributes between treated beverages and RP sample,  
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935 397 suggesting that biochemical and physicochemical changes occurred in these products  
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937 398 during their shelf-life had no impact on their sensory profile. By the contrary, mode  
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939 399 values of color reflected that all treated beverages were often given lower scores than  
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947 400 the RP (Table 3), which demonstrated that during storage panellists always considered  
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949 401 the treated samples whither than the control. These color differences were in accordance  
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951 402 with the results obtained in the instrumental evaluation of color. Global color of stored  
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953 403 beverages changed over time, as evidenced by  $\Delta E^*$  parameter (Figure 5C). These  
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955 404 differences were mainly explained by a decrease in  $L^*$  and WI (Figure 5A and 5B). At  
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957 405 the end of their microbiological shelf-life, RP sample showed the lowest WI values,  
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959 406 followed by H-P and both UHPH samples. These changes may be related to the  
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961 407 formation of new aggregates of particles, modifying the light diffraction of the food  
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963 408 matrix, in addition to the chemical reactions occurred during storage (Codina-Torrella et  
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965 409 al., 2016).  
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## 972 411 **2.4. Conclusions**

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975 412 This study demonstrated that UHPH treatments performed at 200 and 300 MPa  
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977 413 achieved a better microbial inactivation of tiger nuts' milk beverages in comparison to  
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979 414 the conventional homogenization-pasteurization, being the 300 MPa UHPH-treated  
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981 415 beverage the most stable. Microbiological shelf-life of the raw product was improved  
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983 416 from ~3 to ~25, ~30 and ~57 days with the application of conventional homogenization-  
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985 417 pasteurization and UHPH processing at 200 and 300 MPa, respectively. Results  
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987 418 obtained in the sensory evaluation pointed out that the color of beverages was the only  
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989 419 attribute that differentiated UHPH from the others, with greater luminosity and  
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991 420 whiteness. In conclusion, UHPH processing is presented as an alternative treatment to  
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993 421 conventional pasteurization for obtaining tiger nuts' milk beverages with an improved  
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995 422 microbiological shelf-life and a good sensorial profile.  
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1014  
1015 428 team for their technical support in the production and processing of tiger nut milk  
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1017 429 beverages.  
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1023 431 **2.6. References**  
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**Figure 1.** Sensory parameters of beverages at day 3. RP: raw product; H-P: homogenized-pasteurized (18 + 4 MPa, 80 °C for 15 s); 200 MPa: ultra-high pressure homogenized at 200 MPa and an inlet temperature of 40 °C; 300 MPa: ultra-high pressure homogenized at 300 MPa and an inlet temperature of 40 °C.

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**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 °C for 15 s); 200 MPa (▲): ultra-high pressure homogenized at 200 MPa and an inlet temperature of 40 °C; 300 MPa (●): ultra-high pressure homogenized at 300 MPa and an inlet temperature of 40 °C.

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

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**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 °C for 15 s); 200 MPa: ultra-high pressure homogenized at 200 MPa and an

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591 inlet temperature of 40 °C; 300 MPa: ultra-high pressure homogenized at 300 MPa and  
592 an inlet temperature of 40 °C.

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594 **Figure 5.** Evolution of lightness ( $L^*$ ) (A), whiteness index (WI) (B) and color  
595 differences ( $\Delta E^*$ ) (C) parameters during the cold storage of beverages. RP (■): raw  
596 product; H-P (◆): homogenized-pasteurized (18 + 4 MPa, 80 °C for 15 s); 200 MPa (▲):  
597 ultra-high pressure homogenized at 200 MPa and an inlet temperature of 40 °C; 300  
598 MPa (●): ultra-high pressure homogenized at 300 MPa and an inlet temperature of 40  
599 °C.

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**Figure 1**

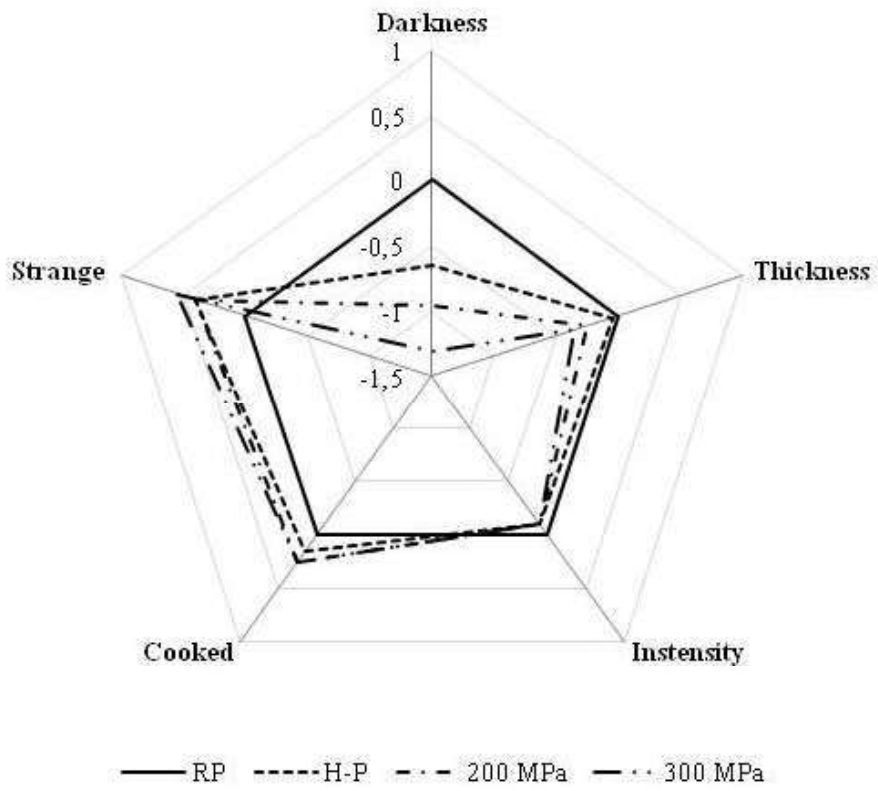
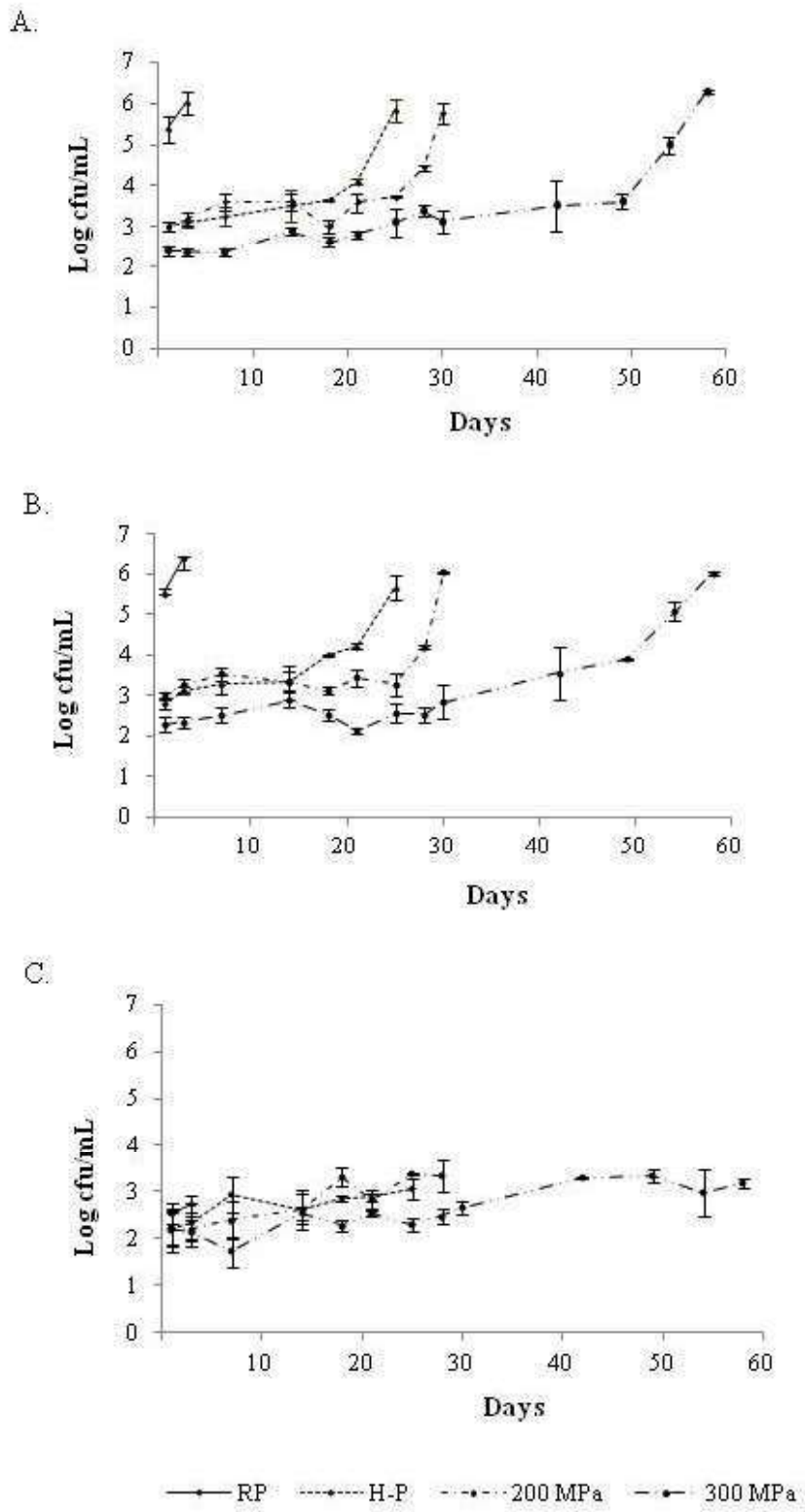
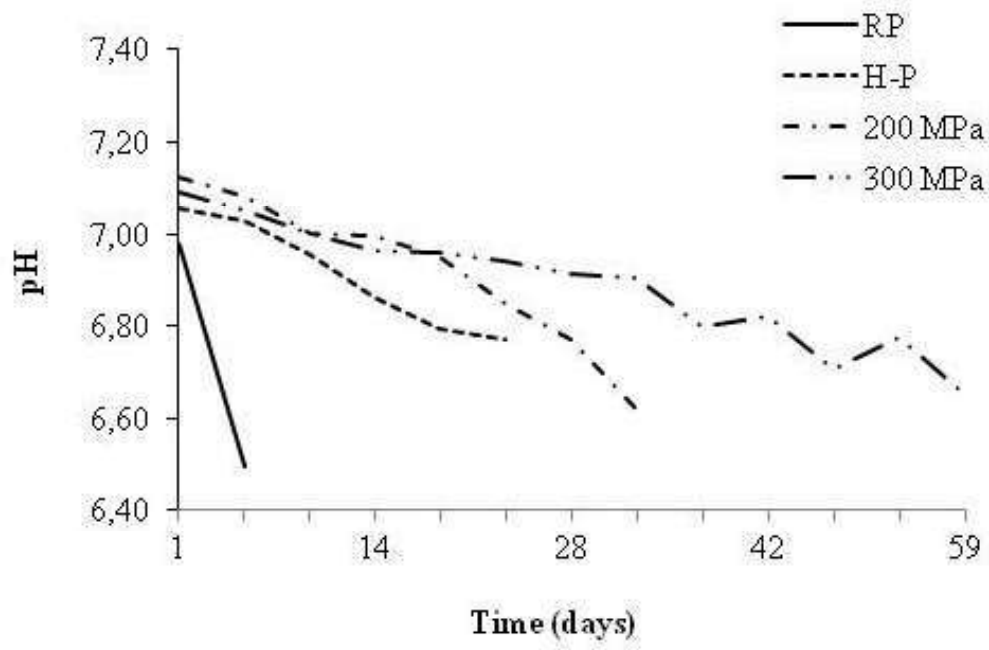


Figure 2



**Figure 3**



**Figure 4**

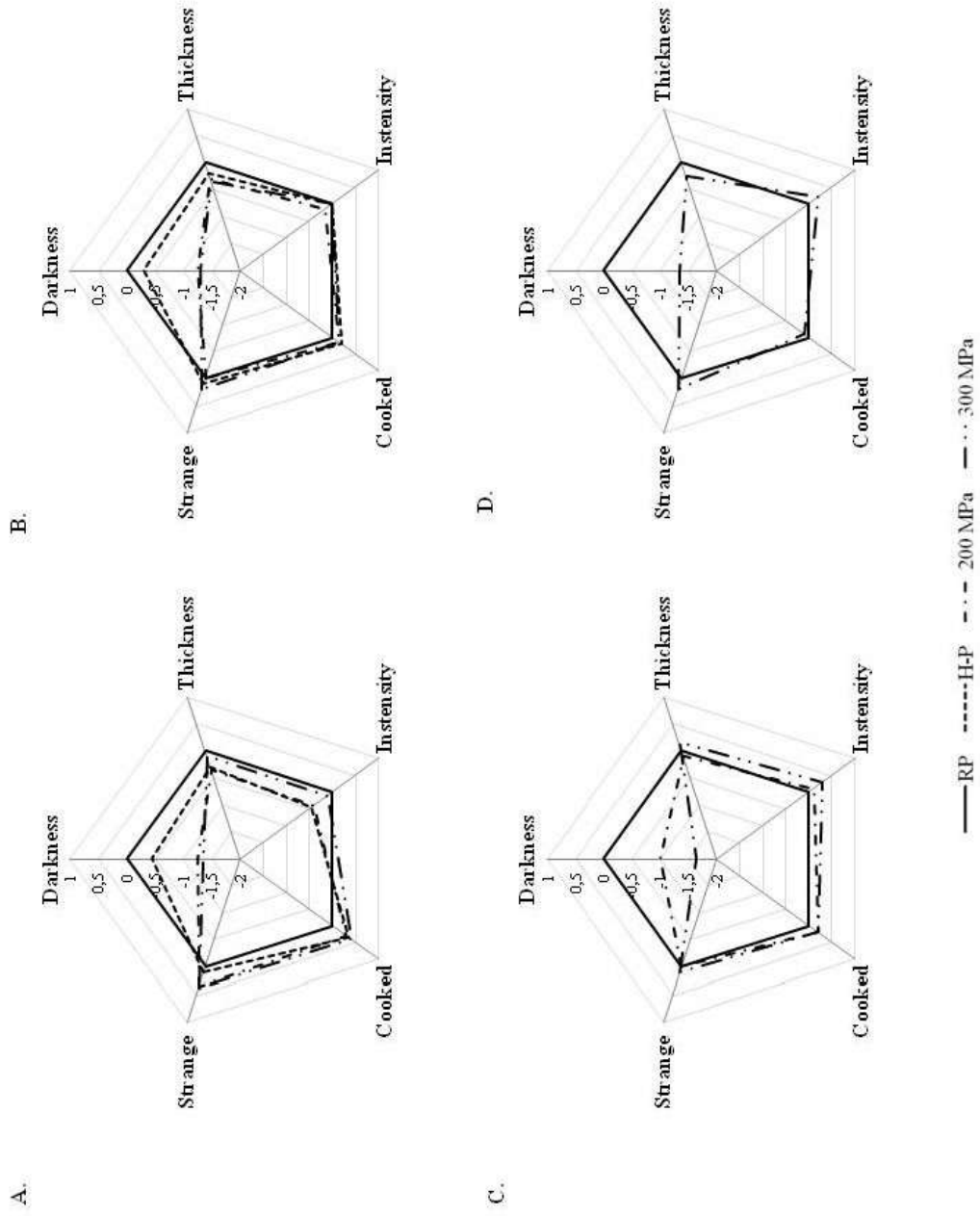
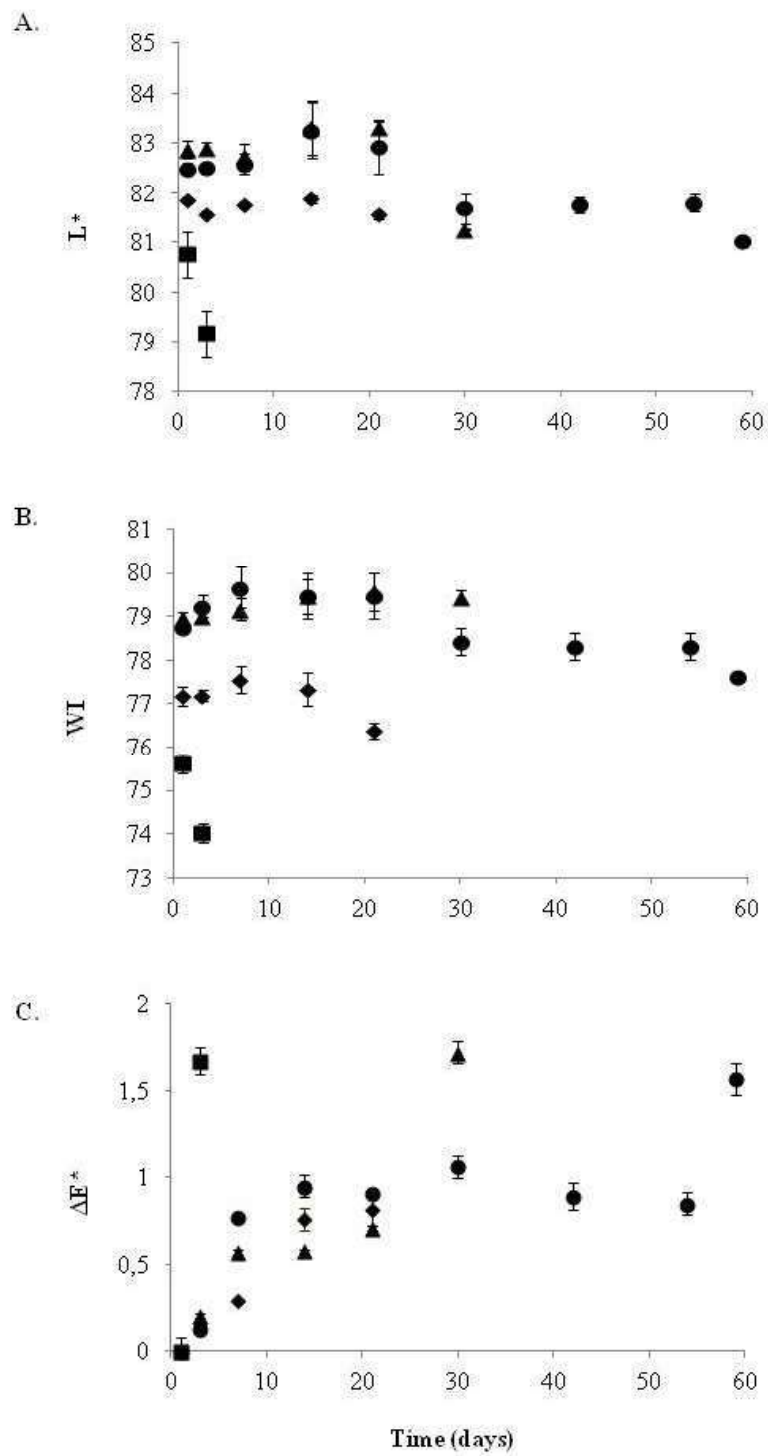


Figure 5



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

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

<sup>a-c</sup> 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.

<sup>1</sup> RP: raw product; H-P: homogenized-pasteurized (18 + 4 MPa, 80 °C for 15 s); 200 MPa: ultra-high pressure homogenized at 200 MPa and an inlet temperature of 40 °C; 300 MPa: ultra-high pressure homogenized 300 MPa and an inlet temperature of 40 °C.

**Table 2.** Color parameters<sup>1</sup> of raw and treated tiger nuts' milk beverages.

Treatment <sup>2</sup>	L*	a*	b*	$\Delta E^*$	WI
<b>RP</b>	80.76 <sup>b</sup> ± 0.45	- 0.48 <sup>a</sup> ± 0.04	14.92 <sup>a</sup> ± 0.17	—	75.62 <sup>c</sup> ± 0.27
<b>H-P</b>	81.85 <sup>a</sup> ± 0.27	- 0.18 <sup>b</sup> ± 0.03	13.80 <sup>b</sup> ± 0.14	1.64 <sup>b</sup> ± 0.12	77.19 <sup>b</sup> ± 0.22
<b>200 MPa</b>	82.87 <sup>a</sup> ± 0.18	- 0.13 <sup>b</sup> ± 0.01	12.19 <sup>c</sup> ± 0.06	3.15 <sup>a</sup> ± 0.01	78.97 <sup>a</sup> ± 0.11
<b>300 MPa</b>	82.49 <sup>a</sup> ± 0.11	- 0.13 <sup>b</sup> ± 0.02	12.04 <sup>c</sup> ± 0.09	3.18 <sup>a</sup> ± 0.08	78.74 <sup>a</sup> ± 0.07

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

<sup>1</sup> L\*: lightness; a\*: green-red component; b\*: blue-yellow component;  $\Delta E^*$ : total color differences calculated taking into account RP sample as reference; WI: whiteness index.

<sup>2</sup> RP: raw product; H-P: homogenized-pasteurized (18 + 4 MPa, 80 °C for 15 s); 200 MPa: ultra-high pressure homogenized at 200 MPa and an inlet temperature of 40 °C; 300 MPa: ultra-high pressure homogenized at 300 MPa and an inlet temperature of 40 °C.

**Table 3.** Sensorial descriptive statistics (mode and relative frequency) of samples during the storage<sup>1</sup>.

<b>Treatment</b> <sup>2</sup>	<b>Storage day</b>			
	<b>14</b>	<b>21</b>	<b>28</b>	<b>54</b>
<b>H-P</b>	- 1 (0.60)	- 1 (0.53)	-	-
<b>200 MPa</b>	- 1 (0.57)	- 2 (0.53)	- 1 (0.67)	-
<b>300 MPa</b>	- 1 (0.50)	- 2 (0.54)	- 2 (0.68)	- 2 (0.57)

<sup>1</sup> 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.

<sup>2</sup> RP: raw product; H-P: homogenized-pasteurized (18 + 4 MPa, 80 °C for 15 s); 200 MPa: ultra-high pressure homogenized at 200 MPa and an inlet temperature of 40 °C; 300 MPa: ultra-high pressure homogenized at 300 MPa and an inlet temperature of 40 °C.

## *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.