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Microbiological stabilization of tiger nuts' milk beverage using ultra-high pressure homogenization. A preliminary study on microbial shelf-life extension

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ABSTRACT

Tiger nuts' milk beverages are highly perishable products. For this reason, 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 qualities of tiger nuts' milk beverage was evaluated. Characteristics of UHPH-treated products (at 200 and 300 MPa, with inlet temperature of 40 °C) were compared with those of raw (RP) and conventionally homogenized-pasteurized (H-P) beverages, after treatment and during cold storage at 4 °C. Microbiological quality of beverages was studied by enumerating total counts, psychrotrophic bacteria, lactobacilli, enterobacteria, molds and yeasts, and mesophilic spores. Evolution of color and sensory characteristics of beverages were also determined. 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. Color of beverages was the only attribute that differentiated UHPH samples from the others, with greater luminosity and whiteness. 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.

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.) (Coşkuner 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 and Farré-Rovira, 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). At present, few studies have demonstrated the potential of non-thermal technologies to reduce the spoilage-related

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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 °C).

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 and 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) and in some cases, the reduction of spores counts (Georget et al., 2014; Zamora and 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; 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 °C 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 $T_i \geq 75$ °C allowed the commercial sterility of soymilk. In this study, the sensory response of panelists after the evaluation of UHPH-pasteurized (200 MPa at T_i of 55–75 °C) and UHPH-sterilized (300 MPa at T_i of 80 °C) 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 °C), 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. (2017). 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 °C) 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 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 °C. 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. 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. Residence time of the product at T_2 temperature was estimated to be < 0.7 s (Poliseli-Scopel et al., 2012).

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