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Validation of High-Pressure Homogenization Process to Pasteurize Brazil Nut (*Bertholletia excelsa*) Beverages: Sensorial and Quality Characteristics during Cold Storage

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Abstract: The effect of high-pressure homogenization (HPH) on the inactivation of Escherichia coli and the stability of the quality properties of Brazil nut beverages were studied. E. coli was used as target microorganism to validate the HPH process (pressures from 50 to 180 MPa and inlet temperatures (Ti) from 25 to 75 °C). Cold storage (5 °C) for 21 days was conducted to establish the shelf-life of BN beverages, in terms of their microbiological, physical, physicochemical, and sensorial stability. HPH-treated samples were compared to pasteurized BN beverages (63 °C for 20 min). The combination of Ti and the pressure of the HPH process (50 to 150 MPa/75 °C and 180 MPa/25 °C) had a significant effect on *E. coli* inactivation (8.2 log CFU/mL). During storage at 5 °C, the growth of mesophilic aerobes in processed BN beverages was controlled by the HPH process. Oxidative stability (TBAR assay) and physicochemical properties (pH, acidity, and °Brix) were evaluated during cold storage, showing good stability. Additionally, HPH-treated beverages showed a reduction in their particle size and the formation of more stable protein aggregates, which favored the beverages' whiteness (color). The HPH process could be an alternative to pasteurization to obtain Brazil nut beverages with an acceptable microbiological shelf life (≥21 days at 5 °C) and high-quality characteristics without the use of any additives.

Keywords: Brazil nut; plant-based beverage; ultra-high-pressure homogenization; microbial inactivation; sensorial analysis; shelf-life period; *E. coli*

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1. Introduction

Brazil nut beverages are considered an alternative vegetable beverage with important nutritional and functional value, due to their composition of unsaturated fatty acids, selenium, magnesium, copper, and bioactive compounds (phenolic compounds, squalene, tocopherols, and phytosterols) [1]. Thermal treatments to pasteurize are the traditional preservation processes for vegetable beverages to ensure microbiological food safety, but they can degrade bioactive compounds (vitamins, volatile compounds, and phenolic compounds, among others) and deteriorate the nutritional and sensory quality. In this sense, in the last decade, the use of non-thermal technologies for microbial inactivation and minimizing the negative effect of heat on compositional, sensory, and functional characteristics have been intensively studied [2]. These technologies include high-pressure homogenization (HPH), high hydrostatic pressure (HHP), ohmic heating, pulsed electric fields (PEF), microwave heating, cold plasma, gamma irradiation, UV processing, and ultrasound.

HPH, also known as high-pressure dynamic homogenization, is considered one of the most promising alternatives to traditional thermal treatments for food preservation (microbial and enzymatic inactivation). The fluid is forced to pass through a gap, causing

mechanical stress (shear, hydrodynamic, and cavitation effects) and an increase in temperature, inducing cell disruption. Its effectiveness in deactivating spoilage microorganisms in models and real matrices is well documented, which is affected by several factors, such as the process; microbial, physiological, and matrix-related aspects; and the composition of the treated fluid [3].

At the level of vegetable beverages, certain microbiological criteria are required by sanitary authorities; in the case of Brazil nut beverages, a matrix similar to fruit juices and the Spanish beverage "horchata", it will require the absence of *Salmonella* and *Staphylococcus aureus* and levels of *E. coli* less than 1000 cfu/g [4,5]. In this sense, several studies demonstrate the effectiveness of microbial inactivation by HPH in vegetable beverages; in the case of "horchata", a greater microbial reduction and stability were obtained at 300 MPa with an inlet temperature (Ti) of 40 °C [6]; in almond milk, over 200 MPa at 75 °C (Ti) achieved the complete inactivation of total bacteria, total spores, and *B. cereus* [7–9]; and in soy milk, the application of 300 MPa had a better microbial reduction than 200 MPa, both at 40 °C (Ti) [10]. Despite previous evidence, there are limited reports of inactivation studies with *E. coli* (target microorganism), an indicator of the healthiness of the process and raw material in the production of vegetable beverages. *E. coli* O157:H7, a bacterial pathogen, was first confirmed as the epidemiological agent in juices following an apple cider outbreak in 1991. These and other foodborne illness incidents involving fruit juices led the U.S. Food and Drug Administration (FDA) to propose a Hazard Analysis and Critical Control Point (HACCP) regulation for juices, which includes the performance of a 5-log pathogen reduction [11]. The few studies on fruit juices provide favorable suggestions of the effect of HPH on the inactivation of *E. coli*: in the case of apple and carrot juice, at 50 to 350 MPa at 25 °C (Ti), almost complete inactivation was achieved above 250 MPa of an initial load of 6 to 7 log CFU/mL [12,13]; in orange juice, a lethality of ~3.5 log CFU/mL was achieved at 300 MPa with no significant difference between 6 and 20 °C (Ti) [14]. However, there is an absence of studies on *E. coli* at inlet temperatures above 25 °C and there is a consensus on the need for studies in a specific food matrix, a factor that influences the inactivation efficacy of HPH.

In this sense, the main objective of this study was to explore the efficacy of high-pressure homogenization processing, by combining inlet pressure and temperature, for the inactivation of the *E. coli* strain, previously inoculated in Brazil nut beverages, and to develop an alternative technological process for the cold preservation and maintenance of Brazil nut beverage quality.

2. Materials and Methods

2.1. Plant Material

Brazil nuts (dry seeds and shelled (without woody tegument)) were purchased from a local market in Madrid (Spain). The proximate composition was 2.2%, total lipid 66.1%, protein 17.3%, carbohydrate 10.9%, and ash 3.4% of wet weight. Vacuum-packaged Brazil nuts were stored under refrigeration to 4 °C until their processing and assaying.

2.2. Solvents and Reagents

Ultra-pure water (Mili-Q) was obtained from a Milipak® Express 40 system (Merk-Milipore, Germany); brain heart infusion (BHI) and agar from BactoTM (Bactom Dickinson and Company, Lepont de Claix, France); buffered sodium and chloride–peptone solution (BSCP) from Panreac Quimica (Barcelona, Spain), Brillance *E. coli*/Coliform-selective medium; one broth Salmonella and Brilliance Salmonella agar from Oxoid LTD (Hampshire, UK); rose bengal agar + Dichloran + Cloramphenicol (DRBC) from Conda Promadisa (Madrid, Spain); plate count agar (PCA) from VWR Chemicals (Leuven, Belgium); trichloroacetic acid (PanReac AppliChem ITW Reagents, Barcelona, Spain); and 2-thiobarbituric acid (≥98%), hydrochloric acid 37%, and malonaldehyde bis (diethyl acetal) (≥96%) from Sigma-Aldrich (Steinheim, Germany).

2.3. Production of Brazil Nut Beverages

The production of Brazil nut beverages was achieved as reported Vasquez-Rojas et al. [1] with some modifications. Briefly, Brazil nuts were previously ground to a particle size lower than 2 mm and then homogenized at 10,000 rpm in a T-25 Ultraturrax (IKA-Werke GmbH & Co., KG, Staufen, Germany) with water at 75 °C in a 7:1 ratio (water: raw material, v/w) for five minutes. Then, this solution was immediately filtered with a filter cloth ($\leq$1 mm) to obtain the hot aqueous extract and subsequently cooled to 5 °C in ice bath. Brazil nut beverages have a high level of lipids, higher than 5% [1], so partial defatting strategies were carried out to bring them to a reasonable range of 2.5 to 3%, which usual in analogous beverages. Three methods were selected to obtain the partially defatted Brazil nut beverage (BNB): through centrifugation (7000× g, 10 min, 5 °C), obtaining the aqueous phase and reincorporation of 30% of the cream, or through resting for 5 and 15 h to obtain the intermediate phase (separated from the sediment and cream). Table 1 shows a short description of the three Brazil nut beverages. The proximate composition of the BNB obtained was analyzed (Supplementary material, Table S1). The beverage obtained by resting for 5 h had a fat content of 3.4% and those obtained by centrifugation/fat reincorporation and resting for 15 h showed a similar total lipid content of ~2.85%. Therefore, for practicality and lower variability, the standardized Brazil nut beverage was obtained with the second method, resting under 15 h, and selected for the HPH validation assays.

Table 1. Description of procedures to obtain the Brazil nut beverages.

Procedure	Description
Preliminary step	Brazil nuts were ground and then homogenized at 10,000 rpm with water at 75 °C, in a 7:1 (water: raw material, v/w) ratio for 5 min. They were then filtered to obtain the hot aqueous extract which was immediately cooled to 5 °C in an ice bath.
Partial Defatting Method	
BNB 1	The extract was centrifuged (7000× g, 10 min, 5 °C), obtaining three phases (sediment, aqueous phase, and cream). The cream phase (upper) was separated manually with a spatula. The aqueous and sediment phases were then separated by transferring. Finally, a **30%** amount of the **cream** was mixed (homogenizer) with the aqueous phase.
BNB 2	The extract was kept for **5 h** in a refrigerator at **5 °C**. The cream was separated manually and the sediment via the transfer of the supernatant. The aqueous phase (supernatant) was used for the assay.
BNB 3	The extract was kept for **15 h** in a refrigerator at **5 °C**. The cream was separated manually and the sediment via the transfer of the supernatant. The aqueous phase (supernatant) was used for the assay.

2.4. Bacterial Strains and Inoculation

The *E. coli* (CECT 434 strain (cryopreserved at −80 °C in 25% glycerol)) was inoculated with a needle in the brain heart infusion medium and incubated at 37 °C for 20 h to obtain an enrichment of ~10^9 CFU/mL. After incubation, to verify the purity of the strain, it was seeded on an agar medium (Bacto) and incubated at 37 °C for 24 h, proving the suitability for assays. Then, the inoculation of *E. coli*-enriched broth was performed by adding 2% (v/v) into the BNB. The final concentration of cells in the BNB was ~10^8 CFU/mL.

2.5. HPH Treatment and Thermal Pasteurization of Brazil Nut Beverage

As the first part of the study, the level of *E. coli* reduction (inoculated in the BNB) by HPH and thermal processes was evaluated. HPH treatments were applied using a high-pressure homogenizer (Panda Plus 2000, GEA Niro Soavi, Parma, Italy) with a maximum capacity of 200 MPa. Pressures of 50, 100, 150, and 180 MPa and inlet temperatures of 25,

55, and 75 °C were tested. The inlet temperatures were obtained using a hot water bath and the HPH-treated beverage was immediately collected in sterile falcon tubes and cooled to 5 °C using an ice bath. As a control of traditional thermal treatment, pasteurization was conducted at 63 °C for 20 min, with a posterior cooling of the beverage to 5 °C using the ice bath which was then collected in sterile falcon tubes.

In the second part of the study, the best HPH conditions for *E. coli* reduction (≥5 log CFU/mL) were selected to study the stability of the beverage under cold storage, up to 21 days at 5 °C, evaluating the physical, microbiological, physicochemical and sensory aspects; the pasteurized beverage was used as a control.

2.6. Physicochemical Analysis

The centesimal composition of the beverages was determined using the following methods: ash, moisture, and dry matter were analyzed following the AOAC-standardized methods [15]; the total protein content was determined with an elemental chemical analysis of nitrogen (conversion factor: 6.25); the total lipid content was determined with the method of Folch [16]; and the total carbohydrate was calculated using the difference. In the physicochemical properties, the pH was determined by direct reading on a digital potentiometer (Metrohm 827 pH Meter, Metrohm, Herisau, Switzerland), the total acidity concentration in the samples was calculated using potentiometric titration (expressed as %, g citric acid/100 mL) [15], and the °Brix (soluble solids) was measured with a digital refractometer (PR-32α, ATAGO™, Tokyo, Japan).

2.7. Microbiological Analysis

The previous step of preparation of the samples at different concentrations was performed via dilution using peptone solution (BSCP) in water. For the enumeration of mesophilic aerobes, based on ISO 4833-1:2003, the seeding technique was by depth on agar (PCA) incubated at 30 °C for 72 h. The molds and yeasts were analyzed following the ISO 21527-1:2008 reference method, seeded by surface, enumerated on an agar medium (DRBC) and incubated at 25 °C for 4 days. The *E. coli* and fecal coliform counts were performed according to Frampton et al. [17] and seeded by depth, enumerated on Brilliance *E. coli*/coliform-selective agar, and incubated at 30 °C for 24 h. The detection and identification of *Salmonella* were performed based on ISO 6579:2017; a pre-enrichment in liquid medium was performed, mixing 25 mL of the BNB in 125 mL of One Broth Salmonella in a sterile bag, and incubated at 42 °C for 20 h. Then, with a plastic loop, a sample of the cultured medium was taken and sowed superficially on plates with Brilliance *Salmonella* Agar and then incubated at 37 °C for 24 h. The colonies were counted with a colony counter (Cole-Parmer "Stuart"—colony counter, Surrey, UK) and the microbial reduction log (N/N_0) was calculated, where N_0 is the number initial of cells in the untreated samples and N is the microorganism counts after the treatments.

2.8. Color Measurement

Color measurements were performed in a colorimeter (SPECORD 210 PLUS, Analytik Jena, Germany). The data were acquired in CIELab color space—L* (luminosity), a* (red-green), and b* (blue-yellow)—with an illuminant of D65 and a standard viewing angle of 10. The whiteness index (WI) was calculated using the parameters L*, a*, and b* (Equation (1)). The total color differences ΔE (Equation (2)) were calculated by taking into account the BNB (untreated) as the reference.

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

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

2.9. Lipid Oxidation Analysis

The oxidative stability of the beverage samples was evaluated by measuring the value of 2-thiobarbituric acid (TBA) during storage, following the reference protocol of Pakzadeh et al. [18] with minor modifications. The TBA solution was prepared by dissolving 15 g of trichloroacetic acid, 0.375 g of TBA, and 1.76 mL of 12 M HCl diluted in water in a 100 mL volumetric flask. An amount of 2 g of the beverage was mixed with 4 mL of the TBA solution and placed in a boiling water bath for 30 min, then rapidly cooled with an ice bath to room temperature, and centrifuged at 10,000× *g* for 30 min. The absorbance of the clear phase and the TBA solution (as a control) was recorded at 532 nm using a spectrophotometer (Gen10S UV-Vis, Thermo Fisher Scientific, Madison, WI, USA). The TBA value was determined using an equation obtained from the standard curve of concentrations of malonaldehyde bis (diethyl acetal), an equivalent of malonaldehyde (MDA), in the TBA solution and the results were expressed as μg MDA/mL beverage.

2.10. Microstructure

The microstructure of the Brazil nut processed beverages was observed using an optical microscope (Leica DM2500, Wetzlar, Germany) under 40× magnification. One drop of the samples was carefully placed on a glass slide and covered with a coverslip. Images were captured at least in triplicate for each sample with a Leica DFC295 camera system (Leica Microsystems, Wetzlar, Germany) mounted on a microscope, and images were processed with the device software LAS Version 4.13.0 (Leica).

2.11. Analysis of Particle Size and Electrical Charge

The particle size/distribution and the zeta potential of the samples were measured using dynamic light scattering (DLS) and electrophoretic light scattering (ELS), respectively. A Zetasizer Pro apparatus (Malvern Instruments Ltd., Worcestershire, UK), with a measurement range from 0.3 nm to 10 μm, was used. An aliquot of the 100 μL from the intermediate part (beverage container) of each sample was taken for analysis after it was diluted with 10 mL water Mili-Q homogenizing with a vortex and transferred to a disposable cuvette. Then, the cuvettes were placed in the measuring chamber, where the samples were equilibrated for 120 s at 25 °C. The analysis was conducted at a scattering angle of 174° and a refractive index of 1.45. Each experiment was measured in triplicate.

2.12. Sensory Evaluation

The evaluation was performed following the reference procedure of Poliseli-Scopel et al. [19], with some modifications. A semitrained panel of twelve people participated in the sensorial test; we considered and preselected only those with previous experience in sensorial evaluation, who habitually consumed plant-based milk and were adults (between 18 and 40 years old), without gender discrimination. The sensory evaluation of the beverages was carried out at days 1, 5, 9, 15, and 21 of cold storage at 5 °C. The place of evaluation was in a laboratory with private cabins, each of them conditioned with light, without disturbance by strange odors or noise. Brazil nut beverages (~30 mL of each sample), in no order (completely randomized), were presented to the panelists at the usual temperature of consumption of these products (~10 °C) in transparent glasses with a random 3-digit code each day of evaluation. At day 1, the BNB, PAS, T1, T2, and T3 beverages were evaluated by each panelist; on the following days, just PAS, T1, T2, and T3 were evaluated. The evaluation consisted of a descriptive test, in which six attributes were evaluated: color (whiteness), thickness, nutty flavor, nutty flavor, rancid flavor, and graininess (mouthfeel), recorded on an intensity scale from 1 (not intense) to 5 (extremely intense points). The overall acceptability was evaluated on a hedonic scale of 1 (hated), 2 (disliked), 3 (indifferent), 4 (liked), and 5 (loved) points.

2.13. Statistical Analyses

The analyses were in triplicate and expressed as mean and standard deviation. The obtained results were evaluated with a variance analysis (ANOVA) and the differences between means were evaluated using Tukey's test. The significant statistical differences were calculated at a $p < 0.05$ level. The statistical software employed was IBM SPSS Statistic 20.0.

3. Results and Discussion

3.1. Physicochemical and Microbiological Characteristics of the Brazil Nut Beverage

The high fat content of the Brazil nut (66.1%) leads to a high level of lipids in Brazil nut beverages (between 5 and 10%) [1,20,21] compared to other vegetable beverages such as almond, soybean, rice, or others (between 1 and 3.4%) [22]. The partially defatted standardized Brazil nut beverage (BNB) showed a nutritional composition of: energy—35.42 Kcal/100 mL, moisture content—94.3%, total lipids—2.9%, protein—1.27%, carbohydrate—1.06%, and ash—0.14%, a profile which is within the range of most commercial vegetable milks in Spain, such as soybean and almond beverages [22,23]. Due to their relevant nutritional composition, solid soluble content (2.2 °Brix), and low acidity (pH 6.6 and an acidity of 0.03%), BNBs represent an ideal medium for the growth of food spoilage microorganisms that could diminish their shelf life. The composition of the standardized Brazil nut beverage is showed in Table S1 (Supplementary Material).

The BNB (untreated beverage, control) had a low microbiological load of viable mesophilic aerobes (2.1 log UFC/mL), an absence of *E. coli*/coliforms, and an absence of *Salmonella*, molds, and yeasts (<2 log UFC/mL), values below the maximum limit according to the microbiological criteria required in the regulation for unpasteurized fruit and vegetable juices (CE n° 2073/2005) and pasteurized horchata (CE n° 2073/2005). These results show that the raw material had good microbiological quality and the beverage process did not generate biological contamination. Another factor that may have reduced and inactivated microorganisms was the temperature of the high-speed homogenization (75 °C) (by Ultraturrax) process. Although many microbial spores are heat-resistant, most nonsporing food pathogens (such as *Listeria*, *E. coli,* and *Salmonella* ssp.) and enterococci are not resistant to temperatures above 72 °C [24]. Considering the above, the study aimed to select and inoculate the beverage with a recognized food safety risk strain such as *E. coli*. and evaluate the reduction effect by HPH.

3.2. Effect by HPH on Inoculated E. coli in Brazil Nut Beverage

In the HPH process, pressure and temperature are considered to be the main factors in microbial inactivation [2]. The effect of inlet temperature (Ti) and pressure by HPH on the reduction in the initial inoculated *E. coli* strain (~8.2 log CFU/mL) in the BNB is shown in Figure 1 (data from Table S2, Supplementary Material). It can be observed that at a constant temperature, the *E. coli* inactivation increased with increasing pressure, and at constant pressure, the *E. coli* inactivation increased with increasing temperature, with the exception of the treatments conducted at 75 °C (Ti) without any difference ($p > 0.05$) between the employed pressures. This fact could be due to the *E. coli*-lethal temperature, at which a complete reduction in *E. coli* was observed. Previous studies about *E. coli* inactivation showed that this microorganism has thermolabile cells that showed a mean D-value of 39 s at 60 °C [24–26]. Therefore, at a Ti of 25 to 55 °C, the greatest reduction in *E. coli* cells was achieved at 180 MPa with a complete inactivation (~8.2 log CFU/mL), similar to the *E. coli* inactivation using a pasteurization treatment (~8.2 log CFU/mL) in Figure 1. The results were consistent with those obtained by other researchers about susceptibility of *E. coli* when the pressure of the HPH process was increased [25,26]. In the studies about the use of HPH to pasteurize fruit and vegetable juices, treatment with 25 °C (Ti) and 250 MPa was necessary to inactivate *E. coli* (e.g., inoculated to 6 log CFU/mL in apple and carrot juice [12] and 7.5 log CFU/mL in apple juice [13]). However, there are also studies reporting a minor effect on *E. coli* inactivation, e.g., a reduction of only ~3 log CFU/mL

in soy milk inoculated with 8 log CFU/mL and treated with 300 MPa/25 °C (Ti) [25]. In previous studies, a positive correlation between homogenization pressure and valve temperature was observed, reaching ~70 °C up to 250 MPa, where the nonthermal factor of *E. coli* inactivation predominated [12,13,26]. Several possible mechanisms of cell rupture by pressure homogenization are exposed in the literature, mainly the rapid pressure drop, turbulence, shear and stress caused by the impact of high velocity, and the cavitation of collapsing bubbles due to the pressure difference [13]. Morphological and intracellular changes can occur at pressures over 100 MPa with a Ti of 20 °C, with the occurrence of roughness and folding up to cell collapse and the fragmentation of *E. coli* cells; the magnitude is variable depending on a combination of factors such as the matrix type (beverage composition), valve design, the physical strength of the cells, and fluid dynamic stress mechanisms that occur in the homogenization valve [27,28].

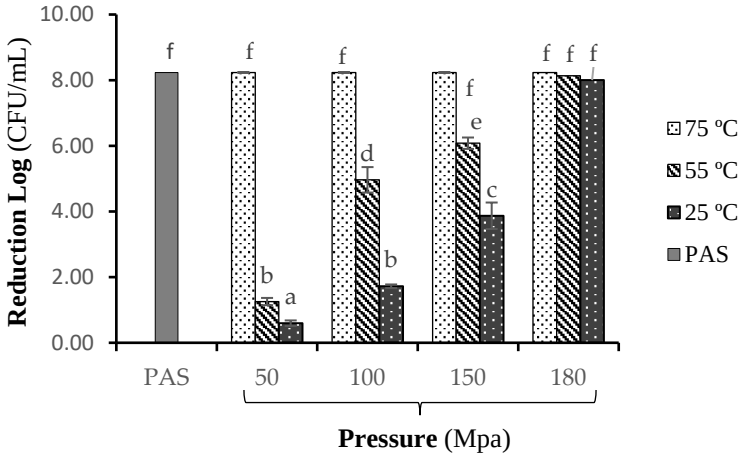

Figure 1. Inactivation of *E. coli* (CECT 434) (8.2 log CFU/mL) inoculated in Brazil nut beverage submitted to pasteurization (PAS) (63 °C, 20 min) and high-pressure homogenization process (HPH). Bars with different letters, lowercase in column or uppercase in row (day 0), are different ($p < 0.05$).

The evaluation of inlet temperature on *E. coli* inactivation in plant-based milks has not been reported, but consistent with our results (the influence of temperature), it was reported that a smoothie (mixed milk and apple juice) treated at 200 MPa showed a significant difference in the *E. coli* inactivation among treatments with 20 °C and 40 °C inlet temperatures, achieving a reduction of around 1 and 3.5 log UFC/mL, respectively [28]. Similarly, *E. coli* inoculated in PBS buffer had a reduction with an increase in the process inlet temperature ranging from 5 to 50 °C at a specific pressure (100 to 300 MPa), achieving a complete inactivation (~8 log CFU/mL) when it was treated at 250 to 300 MPa/50 °C [29]. It is well known that the effect of pressure can cause an increase in the beverage temperature, around 2.5 °C/10 MPa [2], which means that treatment at 180 MPa and inlet temperatures from 25 to 75 °C can heat the beverage at a valve temperature of around 70 °C to 120 °C, respectively. For this reason, temperature was the most important parameter to inactivate *E. coli* and this fact could explain why no differences were found between HPH treatments with different inlet temperatures, because the observed effect was the equivalent real temperature of the samples when they passed through the pressurization valve. In this sense, several studies have estimated this equivalent thermal inactivation of *E. coli*, suggesting that when the valve temperature (resulting from the combination of pressure and Ti) was below ~70 °C, the *E. coli* inactivation was minimal, but at valve temperatures above ~70 °C, the thermal inactivation was the predominant and significant effect, even with very short residence times (a maximum of 2 s) [12,13].

Considering the obtained results, the combinations of temperature and pressure of 50 to 180 MPa/75 °C, 100 to 180 MPa/55° C, and 180 MPa/25 °C produced a good inactivation performance of *E. coli* cells, above the minimum 5 log reduction in target bacterium as required by HACCP regulations [11]. For this reason, three treatments were selected:

50 MPa/75 °C, 150 MPa/55 °C, and 180 MPa/25 °C, parameters with the minimum homogenization pressure for a sufficient *E. coli* reduction at each inlet temperature for the Brazil nut HPH-treated beverage stability studies conducted at 5 °C for 21 days.

3.3. Stability of the HPH-Treated Beverage in Cold Storage

3.3.1. Microbiological Stability

Mesophilic aerobes (MAs) are indicator microorganisms used to verify the microbiological quality of food, as they provide valuable information about the sanitary and hygienic conditions of processing and the possible presence of pathogenic microorganisms. In this sense, as shown in Table 2, the presence of MA microorganisms in the HPH-treated beverage for a period of 21 days compared with the conventional pasteurization (63 °C, 20 min) was evaluated. At day 0, the T1 (HPH at 50 MPa/75 °C) allowed the greatest reduction in MAs (1.9 log CFU/mL), followed by the pasteurization process (PAS) with 1.2 log CFU/mL and T2 (HPH at 150 MPa/55 °C) with 0.8 log CFU/mL. However, the T3 sample (HPH at 180 MPa/25 °C) showed a MA count of 3.5 log CFU/mL, which was not significantly different to the control sample (untreated BNB). Consistent with the inactivation of *E. coli* (the previous section), the inlet temperature of T1 had a greater microbial reduction effect than the pressure (T2 and T3). Although the selected HPH treatments could completely inactivate the *E. coli* cells, the inactivation of Mas was not complete, suggesting the probable presence of sporulated species resistant to pressure and the thermic process, such as *Bacillus sporothermodurans* which is capable of resisting temperatures up to 120 °C [24]. Previous reports on other vegetable beverages usually use inlet temperatures of 40 to 75 °C and 200 to 300 MPa, where the intensity of these parameters on the reduction in MA count was also verified [6,9,30] and complete inactivation was only achieved at 200 MPa/75 °C and 300 MPa/55 to 75 °C in almond milk [9].

Table 2. Total count of mesophilic aerobes of the Brazil nut beverage (BNB) treated with high-pressure homogenization (HPH) and pasteurization (PAS) and stored at 5 °C for 21 days.

Samples/Treatment	Log CFU/Ml *					
	Storage at 5 °C					
	0 Day	2 Day	5 Day	9 Day	15 Day	21 Day
BNB (untreated)	3.5 ± 0.0^{D}	ND	ND	ND	ND	ND
PAS (Pasteurized)	2.3 ± 0.0^{aB}	2.4 ± 0.0^{a}	2.4 ± 0.1^{a}	2.2 ± 0.1^{a}	2.4 ± 0.3^{a}	2.3 ± 0.1^{a}
T1 (50 MPa/75 °C)	1.6 ± 0.1^{dA}	1.5 ± 0.0^{bcd}	1.6 ± 0.1^{cd}	1.0 ± 0.0^{a}	1.2 ± 0.2^{abc}	1.2 ± 0.3^{ab}
T2 (150 MPa/55 °C)	2.7 ± 0.1^{aC}	2.6 ± 0.0^{a}	2.7 ± 0.0^{a}	2.7 ± 0.1^{a}	2.6 ± 0.0^{a}	4.0 ± 0.1^{b}
T3 (180 MPa/25 °C)	3.5 ± 0.0^{aD}	3.2 ± 0.0^{a}	3.7 ± 0.0^{a}	3.9 ± 0.0^{a}	4.1 ± 0.1^{a}	5.1 ± 0.1^{b}

* The data are provided as mean value ± standard deviation (n = 3). Values with different letters, lowercase in row or uppercase in column (day 0), are different ($p < 0.05$). ND, not detectable.

The data are provided as the mean value ± standard deviation (n = 3). Values with different letters, lowercase in rows or uppercase in columns (day 0), are different ($p < 0.05$).

During the BNB storage at 5 °C, all samples remained stable without significant changes ($p > 0.05$) in the MA count, with the exception of treatments T2 and T3 which, at day 21, increased their MA count to 4 and 5.1 log CFU/mL, respectively (Table 1). Nevertheless, all MA count values were below the maximum limit (5.4 log CFU/mL) required for the elaboration and commercialization of an analog beverage (tiger nut milk) [5]. It is interesting to note the effect of the T3 treatment, despite not showing a reduction from the HPH process with respect to BNB, was stable until day 15, suggesting the sublethal damage effect of the pressure on the microbes. It is well known that the HPH process at pressures above 100 MPa contributes to the reduction in/inactivation of microbial load with structural modifications of the cell walls of microorganisms [2]. The HPH treatments (T1, T2, and T3) had a growth in MA counts between 0.4 and 1.6 log CFU/mL at 21 days, favorable values when compared to other studies of HPH-treated beverages. At a similar cold storage time, in HPH-treated soy milk, a growth of 0 and ~1.5 log CFU/mL was observed when subjected at 200 MPa/75 °C and 200 MPa/55 °C, respectively [31]; in almond milk, a growth of 0.9 and 3.9 log CFU/mL was reported at 200 MPa/75 °C and 200 MPa/55 °C, respectively [8].

However, storage times greater than 21 days and less intense HPH processes (for example, 200 MPa/40 °C and 55 °C) tend to rapidly increase microbiological levels compared to those more intense HPH processes (for example, 300 MPa/40 °C and 200 MPa/75 °C), as observed in soymilk [31] and tiger nut milk [6]—a characteristic consistent with the results, where T2 and T3 increased after day 15 and T1 remained stable.

3.3.2. Physicochemical and Oxidative Stability

Figure 2 and Table S3 (Supplementary Material) show the effect of the treatments (HPH and pasteurization) on the physicochemical properties (°Brix, pH, and acidity) of the Brazil nut beverages and the evolution throughout the storage period (21 days to 5 °C). Regarding the effect of the treatments, on day 0, it can be seen that there were no differences in pH or acidity, but there were differences in soluble solids (°Brix). The pasteurized Brazil nut beverage (PAS) had a lower soluble solids content (°Brix) than the HPH-treated samples; this same behavior was also observed in a previous report of hazelnut milk HPH-treated from 25 to 150 MPa [32]. This fact could be due to the solubilization of proteins and the formation of an aggregate network of protein and lipid particles that contribute to the stability of the emulsion [33]. During the conservation period, there were no significant changes ($p > 0.05$) in the pH, the soluble solids (°Brix), or the acidity, with one exception—the pasteurized beverage (PAS)—which showed a slight increase in its acidity from 0.02 to 0.03 g citric acid/100 mL from day 0 to 21, respectively, but it was a negligible variation, considering that these are minimum values in the range (0.03 to 1%) of other analogous vegetable beverages [34]. Generally, the increase in acidity and decrease in pH in vegetable beverages and milk are correlated with microbial growth, such as lactic acid bacteria, but in this study, the microbiological stability of the samples in the period studied did not allow noticing such changes.

Lipid oxidation is one of the main causes of nutritional quality deterioration and reduces the shelf life of plant-based milks, which affects fatty acids, particularly polyunsaturated fatty acids. One of the main secondary products (volatile compounds) formed during lipid autoxidative degradation is malondialdehyde (MDA), which was determinate using the thiobarbituric acid (TBA) test in this work. According to Figure 3 (data from Table S4, Supplementary Material), there were no significant differences in the TBA values between the treated (pasteurization and HPH) and untreated samples, nor during the cold storage time at 5 °C, with values in a narrow range from 0.75 to 1.07 μgMDA/mL, suggesting the Brazil nut beverage was stable up to 21 days.

These TBA values were close to the reported ones for the "horchata" beverage (~0.46 μgMDA/mL) [35] and for the cow milk (~1.2 μgMDA/mL) [36]. The observed effect of HPH process was consistent with the published report of soy milk treated at 200 MPa/55 to 75 °C, where the total aldehyde levels (dominated by hexanal and pentanal) showed no differences with respect to the raw and pasteurized (95 °C, 30 s) soy milk.

However, there was an increase in TBA values when the higher pressure/temperature (300 MPa/80 °C) process was applied. This effect could be attributed to the extreme HPH conditions and time [7]. This same study reported that after the cold storage (4 °C), the HPH-treated soymilk at 200 MPa/55 to 75 °C showed several modifications of volatile compounds at 28 days, such as the decreased hexanal and pentanal, evidencing the activity of the oxidative process and the formation of new compounds [31]. Although HPH treatments break down the oil phase into small particles thus increasing the total interfacial area susceptible to oxidation, the formation of aggregates (protein-coated oil droplets), the cluster of protein denaturation, and its adsorption on the interface layer would offer a strong barrier against lipid oxidation [37]. In the present study, in addition to the above, diverse factors such as the composition of Brazil nut beverages' antioxidant phytochemicals and fatty acid profile may have contributed to their oxidative stabilities.

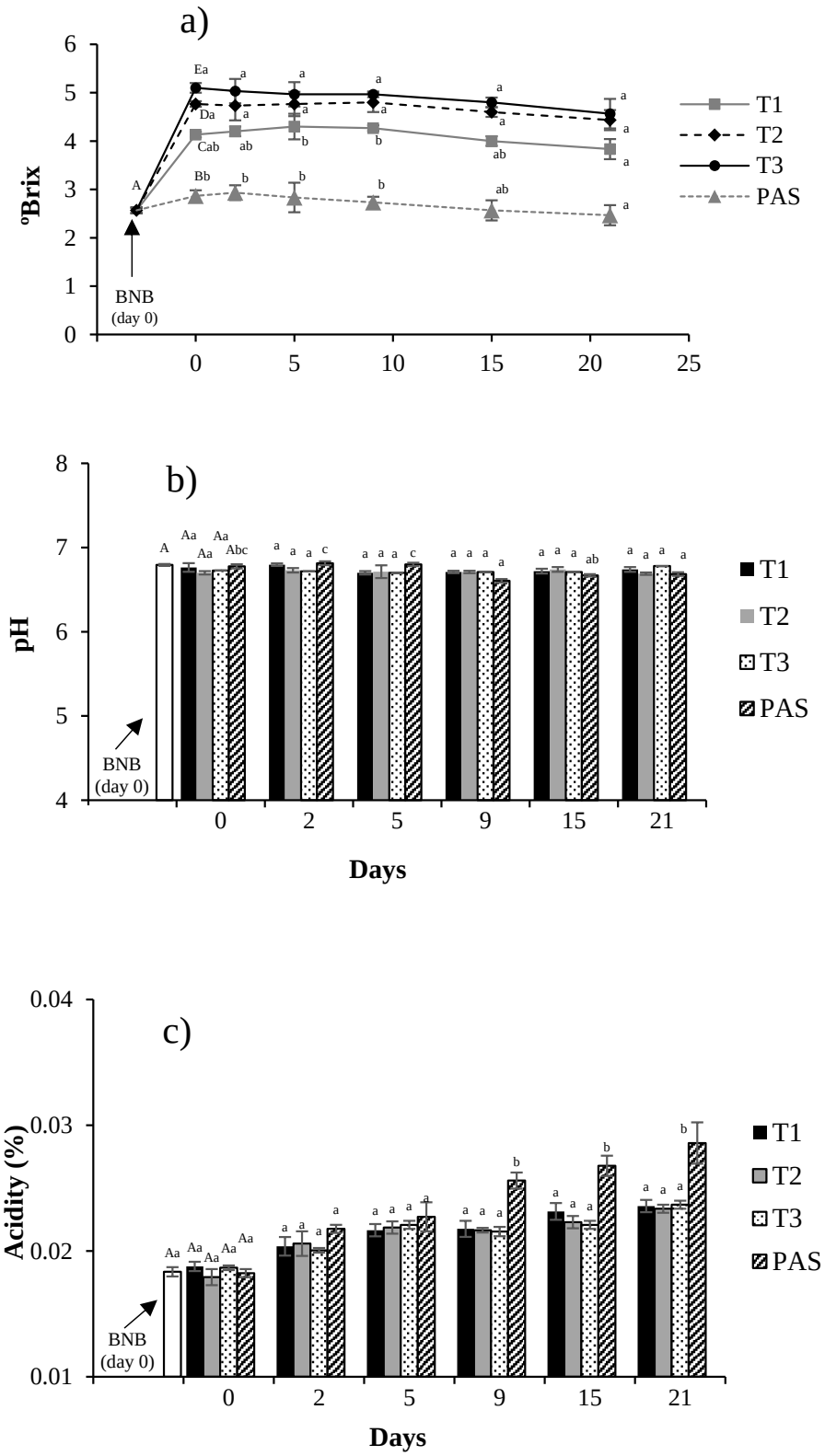

Figure 2. Physicochemical characteristics of the Brazil nut beverage (BNB) treated with high-pressure homogenization (HPH) and pasteurization (PAS) and stored and preserved at 5 °C for 21 days: (**a**) Soluble solids (°Brix); (**b**) pH and (**c**) acidity (%). Data were expressed as mean ± standard deviation (n = 3). Different superscript capital letters in-dicate statistically significant differences ($p \leq 0.05$) between treatments in day 0. Different super-script lower-case letters indicate statistically significant differences ($p \leq 0.05$) between days of cold storage within the same treatment.

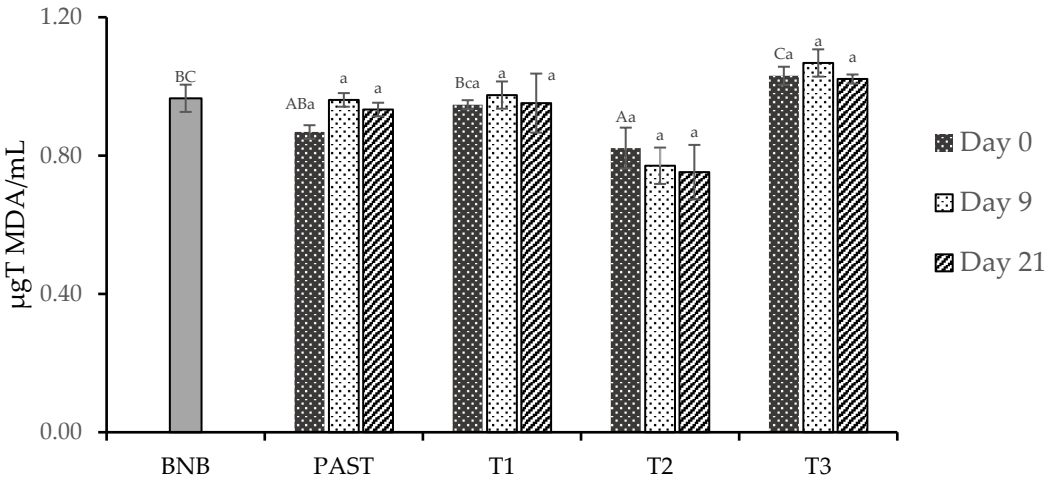

Figure 3. TBA values (µgMDA/mL) of the Brazil nut beverage (BNB) treated with HPH (T1, T2, and T3) and pasteurization (PAS) and stored at 5 °C for 21 days. Data were expressed as mean ± standard deviation (n = 3). Different superscript capital letters in-dicate statistically significant differences ($p \leq 0.05$) between treatments in day 0. Different super-script lower-case letters indicate statistically significant differences ($p \leq 0.05$) between days of cold storage within the same treatment.

3.3.3. Physical Properties of Processed Brazil Nut Beverage

Color

Color is a factor that influences the acceptability of food to the consumer, and in vegetable beverages, it has relevant importance because it is considered an alternative food to cow milk. According to Table 3, at day 0 (just processed beverages), the HPH-treated samples showed higher values of luminosity (L*) and whiteness index (WI) than the pasteurized and untreated sample (control), associated with a whiter and brighter appearance; these parameters had the greatest influence on the total color change (ΔE) [8]. At day 0, the HPH-treated samples showed a greater ΔE (8.8 to 9.5) than the PAS sample (ΔE: 1.8). These results are in agreement with previous studies carried out on tiger nut milk [6], hazelnut milk [38], and almond milk [8], where they state that lightness and whiteness increase with the HPH process, associated with particle distribution and fragmentation to smaller particles with a higher ability to scatter light and reflection. The a* and b* parameters in all samples had positive values, indicating that red and yellow, respectively, were the primary color parameters which contributed to the color of Brazil nut beverages, with a predominance of the yellow color. A significant increase in redness (a*) and a decrease in yellowness (b*) were observed in all the samples from day 0 which were only treated (PAS and HPH). This modification of color parameters could be due to the Maillard reaction and the formation of browning compounds, mainly when the beverages are processed by pasteurization and HPH with high temperatures [3,33].

In general, during cold storage, the HPH-treated beverages showed a great stability in their optical color characteristics, without any significant changes ($p \geq 0.05$). However, the PAS sample showed some changes in all color parameters after day 2, attributable to the physical instability of the beverage (cream formation, sedimentation, and an increase in the particle size). This effect was more noticeable in the total color change value, across all the cold storage time, where the PAS beverage showed a high variation from ΔE 1.8 (day 0) to ΔE ~ 12.5 (from day 2). Meanwhile, the HPH-treated beverages did not show any variation in this parameter according to storage time. These results were in agreement with previously reported studies on HPH-processed tiger nut milk [6] and almond milk [8], which did not show significant changes in color parameters when stored in similar conditions (time and temperature). In addition, the color stability of processed beverages can be rapidly affected by microbial growth during cold storage, forming new particle aggregates and modifying the diffraction of light from the food matrix, as was observed in

tiger nut milk, where L* and WI decreased with a high increase in the microbial population (aerobic mesophilic) [6]. Moreover, longer storage times can also cause changes in color due to the alteration of the concentration and particle size, which affect the scattering and absorption of light, as observed in the HPH-treated soy milk, which had a noticeable change in lightness after the third week of cold storage [39]. Thus, the microbial inactivation (seen in the previous section) and the relatively short storage time allowed maintaining the color stability of the HPH-treated Brazil nut beverage.

Table 3. Color properties of the BNB treated with HPH and pasteurization conserved at 5 °C for 21 days.

Parameter	Day	BNB	PAS	T1	T2	T3
L*	0	80.7 ± 0.3^{A}	81.4 ± 0.3^{bB}	89.2 ± 0.3^{aC}	89.8 ± 0.2^{aCD}	90.0 ± 0.2^{aD}
	2		68.9 ± 0.7^{a}	88.9 ± 0.4^{a}	90.0 ± 0.2^{a}	90.3 ± 0.2^{a}
	5		69.2 ± 0.2^{a}	89.2 ± 0.1^{a}	90.9 ± 0.3^{a}	91.0 ± 0.1^{b}
	9		69.7 ± 1.1^{a}	88.4 ± 0.7^{a}	90.2 ± 0.7^{a}	90.1 ± 0.1^{a}
	15		69.2 ± 1.4^{a}	88.2 ± 0.2^{a}	90.5 ± 0.0^{a}	90.3 ± 0.3^{a}
	21		69.0 ± 0.7^{a}	88.9 ± 0.3^{a}	89.8 ± 0.4^{a}	90.2 ± 0.4^{a}
a*	0	0.5 ± 0.1^{A}	1.24 ± 0.3^{bAB}	2.43 ± 0.2^{bD}	2.30 ± 0.3^{cCD}	1.64 ± 0.4^{aBC}
	2		0.53 ± 0.0^{a}	2.12 ± 0.2^{a}	1.92 ± 0.2^{ab}	1.81 ± 0.1^{a}
	5		0.63 ± 0.1^{a}	2.27 ± 0.5^{ab}	1.99 ± 0.3^{a}	1.82 ± 0.3^{a}
	9		0.45 ± 0.1^{a}	2.13 ± 0.2^{ab}	1.87 ± 0.4^{ab}	1.76 ± 0.1^{a}
	15		0.47 ± 0.1^{a}	2.36 ± 0.3^{ab}	2.18 ± 0.1^{bc}	1.75 ± 0.1^{a}
	21		0.59 ± 0.2^{a}	2.42 ± 0.7^{ab}	2.10 ± 0.3^{ab}	1.85 ± 0.4^{a}
b*	0	11.3 ± 0.5^{B}	9.9 ± 0.3^{bA}	10.2 ± 0.6^{aA}	10.2 ± 0.4^{aA}	9.6 ± 0.3^{aA}
	2		6.4 ± 0.5^{a}	10.3 ± 0.4^{a}	12.9 ± 1.3^{a}	8.6 ± 2.0^{a}
	5		4.7 ± 0.1^{a}	10.8 ± 1.5^{a}	11.4 ± 1.1^{a}	9.2 ± 1.5^{a}
	9		6.7 ± 1.2^{a}	11.0 ± 0.4^{a}	11.0 ± 0.6^{a}	9.7 ± 2.3^{a}
	15		6.4 ± 0.4^{a}	9.9 ± 0.5^{a}	8.5 ± 1.4^{a}	9.4 ± 0.2^{a}
	21		6.6 ± 1.6^{a}	10.2 ± 1.6^{a}	8.9 ± 0.9^{a}	8.4 ± 1.1^{a}
WI	0	77.6 ± 0.4^{A}	78.9 ± 0.3^{bA}	84.9 ± 0.4^{aB}	85.4 ± 0.9^{aB}	86.0 ± 0.3^{aB}
	2		68.2 ± 0.8^{a}	84.7 ± 0.5^{a}	83.6 ± 1.7^{a}	86.9 ± 1.2^{a}
	5		68.9 ± 0.2^{a}	84.5 ± 1.0^{a}	85.2 ± 1.6^{a}	87.0 ± 1.1^{a}
	9		68.9 ± 1.0^{a}	83.9 ± 0.3^{a}	85.1 ± 1.4^{a}	86.0 ± 1.6^{a}
	15		68.5 ± 1.3^{a}	84.4 ± 0.4^{a}	87.0 ± 0.9^{a}	86.3 ± 0.3^{a}
	21		68.3 ± 1.0^{a}	84.7 ± 0.9^{a}	86.3 ± 0.3^{a}	87.0 ± 0.4^{a}
ΔE	0	ND	1.8 ± 0.8^{aA}	8.8 ± 0.3^{aB}	9.5 ± 0.3^{aB}	9.5 ± 0.3^{aB}
	2		12.8 ± 0.6^{b}	8.5 ± 0.4^{a}	9.8 ± 0.5^{a}	10.3 ± 0.5^{a}
	5		13.0 ± 0.8^{b}	8.8 ± 0.3^{a}	10.3 ± 0.6^{a}	10.7 ± 0.4^{a}
	9		11.9 ± 1.3^{b}	7.9 ± 0.7^{a}	7.9 ± 0.7^{a}	9.8 ± 0.3^{a}
	15		12.5 ± 1.4^{b}	7.9 ± 0.4^{a}	10.4 ± 0.4^{a}	9.9 ± 0.3^{a}
	21		12.6 ± 0.2^{b}	8.6 ± 0.2^{a}	9.6 ± 0.1^{a}	10.1 ± 0.0^{a}

The data are provided as mean value ± standard deviation (n = 3). Values with different letters, lowercase in column or uppercase in row (day 0), are different ($p < 0.05$). ND: not detectable.

Particle Size and Electrical Charge

Table 3 shows the effect of the HPH process (day 0) on the mean particle size of the Brazil nut beverages, with values of ~300 nm. These mean particle data were smaller than those observed for the pasteurized (PAS) beverage (1780.3 nm) and the untreated beverage (8227.5 nm). These lower particle size values of the HPH-treated samples were favorable for the gravitational separation of the phases of the emulsion, since values $\leq$ 300 nm could contribute to better physical stability [40]. The HPH-treated beverages showed no changes ($p > 0.05$) in their mean particle size values over all of the cold storage time. On the other hand, the pasteurized Brazil nut beverage had an increasing trend in the particle size values up to day 9, attributable to the aggregation of fat globules and gravitational separation. However, after day 9, there was a decrease in the particle size, possibly due to the sampling

method, in which an aliquot was taken from the middle part of the sample container, which was more stable (with smaller fat droplets) than the upper phase, due to the occurring phase separation observed at this time of cold storage (Figures 4 and 5). Regarding particle distribution (Figure 4a), a decrease in particle size (peaks of the curve) was observed when the homogenization pressure increased (independent of the temperature), a common effect reported in other HPH-treated beverages [33,41]. However, over the course of the cold storage time, the curves of the size distribution and particle size range of the HPH-treated beverages tended to overlap, suggesting that a colloidal equilibrium of the aggregate products took place. Usually, untreated (control) and pasteurized vegetable beverages show a bimodal and polydisperse distribution. However, in the present study about Brazil nut beverages, a monomodal distribution was observed (Figure 4). This fact could be due to the low detection limit (max. 10 μm) of the employed analytical equipment and to the fat globule size in vegetable beverages which are usually higher, as reported in hazelnut milk and almond milk [32,33]. The particle distribution of the HPH-treated beverages was bimodal along the storage time and distinguished in two clear ranges: first, a peak with particle sizes $\leq$ 1000 nm, probably mostly fat globules; second, bigger-sized particles in the range of 4000 to 6000 nm. A similar effect has been observed in previous studies, such as soy milk, almond milk, and horchata treated at 200 MPa and 40 to 75 °C (Ti) [7,9,42], with the formation of aggregates with particle sizes greater than 4000 nm, linked to the denaturation of proteins and their interaction with fat globules.

In practice, the zeta potential is used to give an approximation value of the electrostatic properties of a colloid particle, indicating the degree of repulsion between the charged particles in the dispersion [43]. In general terms, when the zeta potential is farther away from zero, the stability is better, preventing the aggregation of particles by electrical repulsion. According to Table 4, at day 0, all samples showed a similar zeta potential ($p > 0.05$), with a value of about −35 mV. The high negative charge of particles seen in the results indicates the predominance of negative charge over positive charge in Brazil nut beverages. This characteristic is related to the isoelectric point (pI) (~5) of the major proteins of Brazil nut [44] and the pH (6.6) of Brazil nut beverages; at a pH above the pI, the protein exhibits negative charge [33]. A zeta potential of about ±30 mV is considered optimal to attain better physical colloidal stability [43]. Considering the above, all treated beverages had good performance in terms of colloidal system stability compared to other beverages, e.g., 21 mV in soy milk, −16 mV in coconut milk, and −18 to −22 mV in almond and hazelnut milk [43]. Is common that untreated (control) and pasteurized vegetable beverages have less absolute zeta potential than HPH-treated ones [33,41], but in the present work, no significant differences were observed. This high zeta potential of pasteurized beverages (−39.1 to −47.9 mV in stored samples) may be due to the above-mentioned sampling of the intermediate fraction in the sample containers, which is more stable than the upper fraction (a higher quantity of fat aggregate particles; see the next section). In concordance with other reports [33,41], the HPH process improved the zeta potential due to the rearrangement of components that occur in the dispersed phase, with a major interfacial adsorption of proteins and an increase in the surface charge of the dispersed oil droplets, but the denaturation at a high pressure/temperature could be the cause of an absolute reduction in the zeta potential [33,41]. During the storage time, the zeta potential of the HPH-treated Brazil nut beverages and the pasteurized one did not show differences, indicating good physical stability with a weak aggregate formation capacity.

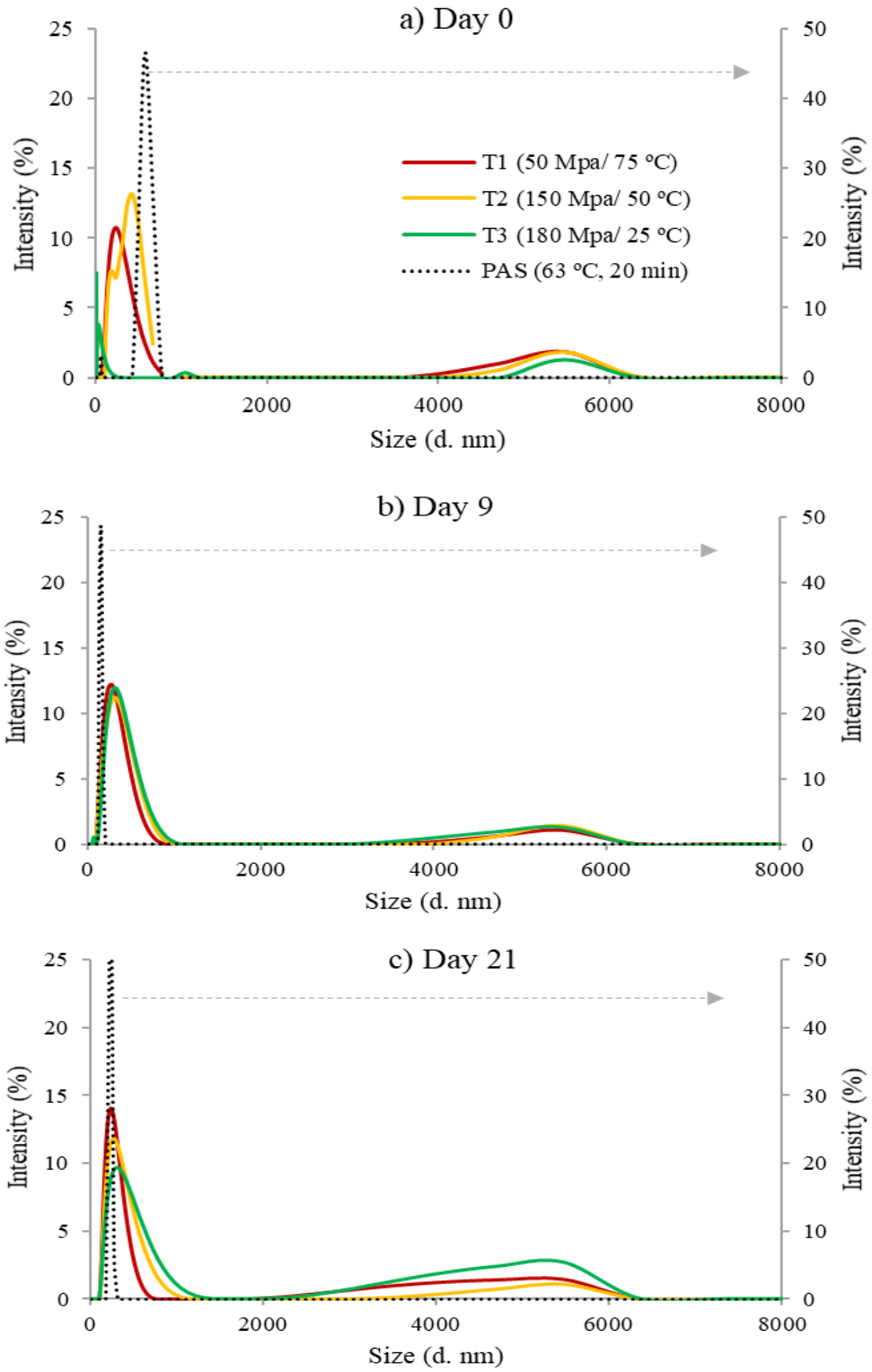

Figure 4. Particle size distribution of Brazil nut beverage treated with HPH and pasteurized and analyzed at (**a**) day 0, (**b**) day 9, and (**c**) day 21 of cold storage.

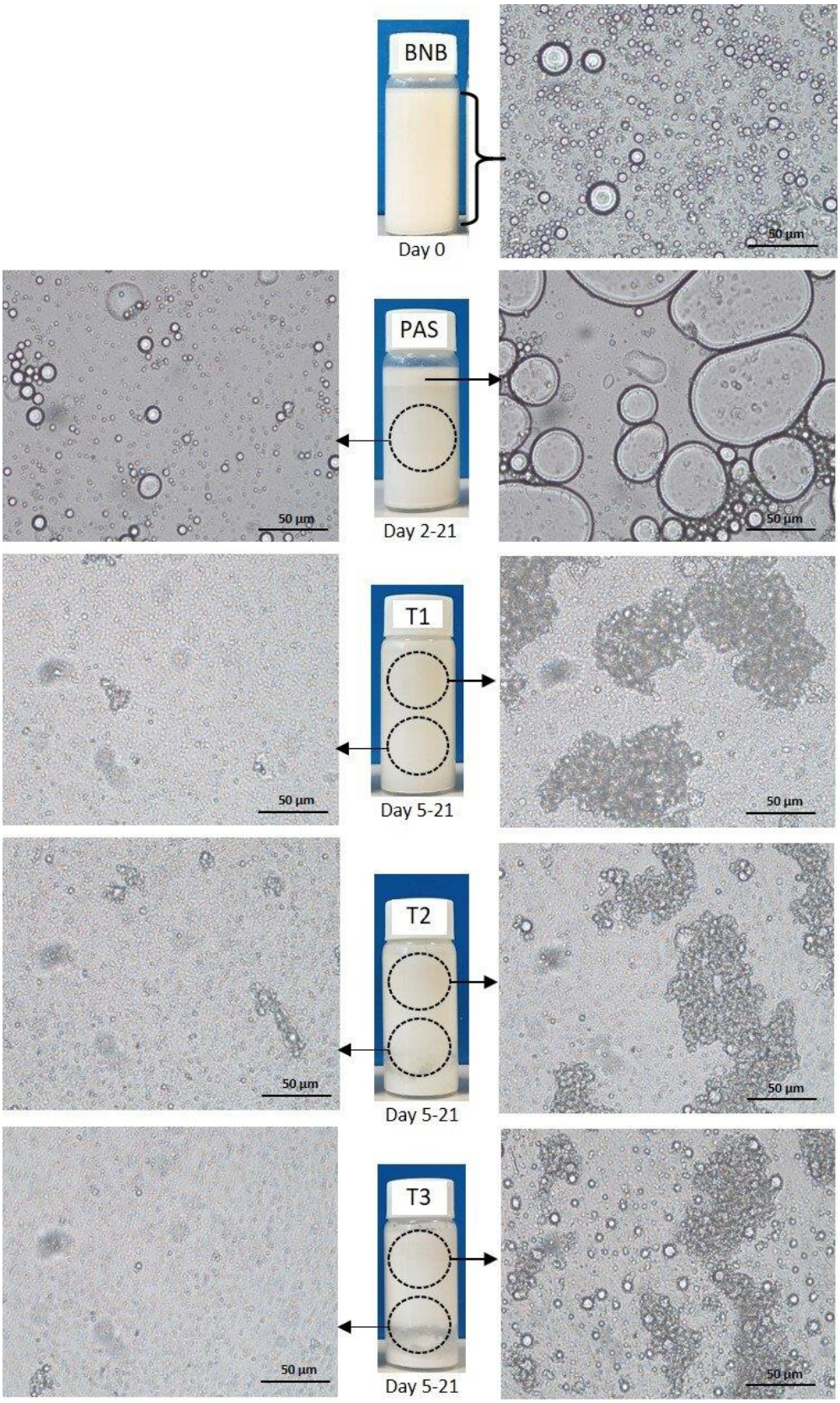

Figure 5. Visual appreciation and optical microscopy of the Brazil nut beverage (BNB) at day 0 and treated with high-pressure homogenization (HPH) and pasteurization (PAS) and stored and preserved at 5 °C for 21 days.

Table 4. Mean particle size and zeta potential of HPH-treated, pasteurized, and untreated Brazil nut beverages cold-stored for 21 days.

Sample	Particle Size (nm)					
	Day 0	**Day 2**	**Day 5**	**Day 9**	**Day 15**	**Day 21**
BNB	8227.5 ± 782.5^{A}	-	-	-	-	-
PAS	1780.3 ± 28.7^{Ba}	2119.2 ± 87^{b}	2813 ± 69.4^{c}	3173.8 ± 81.1^{d}	2755 ± 109.3^{c}	1697 ± 36.7^{a}
T1	261.2 ± 13.1^{Ca}	290.8 ± 12^{a}	353.6 ± 106.3^{a}	300.9 ± 25^{a}	248.2 ± 5^{a}	270.2 ± 21^{a}
T2	355.8 ± 51.5^{Ca}	350.4 ± 17.2^{a}	603.7 ± 104.7^{b}	344.2 ± 10.1^{a}	325 ± 33.3^{a}	283.9 ± 11.3^{a}
T3	345.3 ± 21.3^{Ca}	387.4 ± 24.9^{a}	620 ± 97.3^{b}	531.2 ± 66.6^{b}	295.95 ± 2^{a}	310.3 ± 24.9^{a}
	Zeta potential (mV)					
BNB	-38.6 ± 6.3^{A}	-	-	-	-	-
PAS	-39.1 ± 0.1^{Aa}	-47.9 ± 1.8^{b}	-44.1 ± 1.3^{a}	-40.5 ± 1.3^{a}	-38.3 ± 4.2^{a}	-40.9 ± 0.8^{a}
T1	-36 ± 0.7^{Aa}	-34.9 ± 1.1^{a}	-35.3 ± 0.5^{a}	-34.6 ± 1.2^{a}	-39.5 ± 0.3^{a}	-35.21 ± 0.6^{a}
T2	-35.8 ± 1.7^{Aa}	-36.4 ± 0.9^{a}	-37.1 ± 1.9^{a}	-36.5 ± 1^{a}	-37.7 ± 1.3^{a}	-35.9 ± 1.2^{a}
T3	-32.3 ± 6^{Aa}	-35.7 ± 1.7^{a}	-33.6 ± 1.7^{a}	-37.8 ± 0.9^{a}	-34.8 ± 2.1^{a}	-35.8 ± 1.1^{a}

Data were expressed as mean ± standard deviation (n = 3). Different superscript capital letters indicate statistically significant differences ($p \leq 0.05$) between treatments in day 0. Different superscript lowercase letters indicate statistically significant differences ($p \leq 0.05$) between days of conservation within the same treatment.

Visual Stability and Microstructure

As shown in Figure 5, the optical microscopy of the untreated Brazil nut beverage (day 0) showed large particles of fat droplets, mostly single and spherical, similar to what was observed in the pasteurized beverage (images not shown). During cold storage, the pasteurized beverage suffered an increase in particle size from day 2, produced by the formation of the cream and the appearance of a sedimentation layer, which was visually observed (Figure 5). This breakdown of the colloidal system can be caused mainly by physical mechanisms, including gravitational separation (creaming and/or settling) and the flocculation/coalescence of fat globules [40].

In contrast, HPH treatment can effectively reduce the size of fat globules and other dispersed particles, but it can also cause protein denaturation and the formation of particle aggregates, as found in this study. The HPH-treated Brazil nut beverages had a reduction in their fat globule size, but at the same time, the formation of aggregates (flake-shaped particles) of high structural heterogeneity as well as large agglomerates (cluster) was observed; it was optical microscopically noticeable from day 0, and was visually perceived from day 5 of storage through the formation of phases suspended in the upper half of the container (Figure 5). This type of aggregate formed by the HPH process was also previously reported in the hazelnut milk processed using HPH from 50 to 150 MPa MPa/15 °C (Ti) [32] and in the peanut milk at 150 and 300 MPa [45].

This cluster of aggregates (protein and fat aggregates) can be explained by heat-denatured proteins which migrate to the oil–water interface and that are absorbed onto the fat surface, creating protein bridges with other fat droplets, forming new clusters of particles [42]. Visually, during cold storage, the HPH-treated beverages had better physical stability than the pasteurized one, but from day 5, the sample T3 (180 MPa/25 °C) and T2 (150/55 °C) began to show bigger instabilities (the formation of an aqueous phase in the base) than sample T1 (50 MPa/75 °C) (Figure 5), mainly linked to the gravitational separation caused by the density of the particles [40].

3.3.4. Sensorial Quality

Relevant attributes were included in the sensory evaluation of the Brazil nut beverages to obtain a perception of their sensory profiles (Figure 6), based on the raw material characteristics, process type, and commercial suitability. Regarding whiteness (color), the score close to "remarkably intense" at day 1 did not differ between all beverages ($p > 0.05$), i.e., the panelists were not able to detect any differences among the HPH and PAS Brazil nut

beverages. However, at day 9 and 21 of cold storage, there was a clear decrease in whiteness in the pasteurized beverage, with this being effect verified by the colorimetric analysis, visual stability, and optical microscopy, concluding that the emulsion of the pasteurized beverage was less stable than the HPH-treated ones.

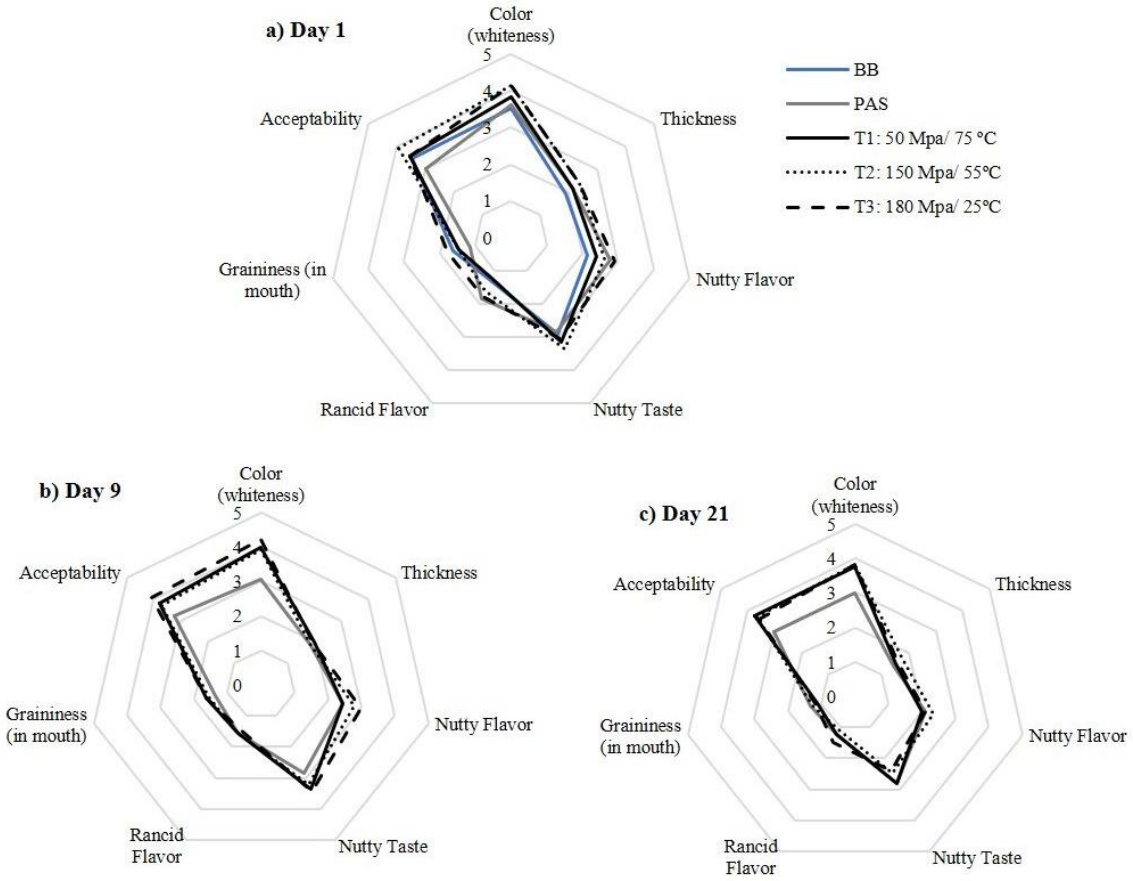

Figure 6. Sensorial control of the Brazil nut beverage (BNB) treated with high-pressure homogenization (HPH) and pasteurization (PAS) and stored at 5 °C for 21 days.

These higher whiteness values obtained in the HPH-treated samples were similar to those previously reported for other nut beverages, e.g., in tiger nut [6], almond, and hazelnut milk beverages [33], linked to the effect of the HPH process on the number and particle size of beverages, which affects the light reflection. Despite the formation of aggregates (seen in Visual and Microstructure Section 3.3.3) in the HPH-treated beverages, there was no difference in graininess (in the mouth) compared to the pasteurized one; this change was almost not perceived by the panelists (with a score close to "nothing intense") along the storage time. The sensory attributes of rancidity and nutty taste were almost unnoticeable and intense, respectively, along the storage time in all the Brazil nut beverages (Figure 6). However, the nutty flavor characteristic value of all the treated samples showed a slight decrease in intensity at the end of the storage time (day 21). The occurrence of rancidity and aroma modification in vegetable beverages is usually associated with lipid oxidation processes, the formation of volatile compounds, defective odors caused by microbial spoilage, and chemical reactions during storage, but at the sensory level, it may be difficult for panelists to detect these modifications, as observed in HPH-treated soy milk [46], where chemical changes (volatile compounds) during storage did not impact the organoleptic characteristics (flavor as well as grassy and oxidized aromas). The results of the present study are consistent with the oxidative stability (TBA) and physicochemical characteristics (pH, acidity, and °Brix) discussed in previous sections, but the slight reduction noted in the nutty flavor intensity at day 21 in all samples suggests the

possible existence of certain chemical modifications during cold storage linked to volatile compounds (not analyzed). For overall acceptability, the HPH-treated beverages had a good (with "like") score and this fact was consistent during the storage time compared to the pasteurized beverage, which was assigned an "indifferent" score from day 1, an aspect that may be mainly influenced by the lower "whiteness". In general, the HPH-treated Brazil nut beverages had positive sensory values and great acceptability values ranging from 3.5 to 4.1 (liked samples) at the end of the cold storage (21 days at 5 °C), associated with the decrease in the droplet size of the emulsion and better droplet distribution, which improved the color, appearance, and mouthfeel. It should be noted that the general preference in the consumption of nuts favors the acceptability of the derived beverage.

4. Conclusions

HPH treatment was an effective process for the inactivation of the microbial population in Brazil nut beverages. The combination of pressure and inlet temperature had a strong effect that could achieve the complete inactivation of the inoculation of *E. coli* cells in the validation assay. In the Brazil nut beverages, the application of 50 to 180 MPa/75 °C, 100 to 180 MPa/55 °C, and 180 MPa/25 °C made it possible to achieve a reduction in *E coli* of $\geq$5 log CFU/mL, the minimum target bacterium level for process validation required by food health regulations. In terms of the beverages' sensorial quality during cold storage (5 °C), HPH process represented an alternative to pasteurization treatment due to its good performance in microbial inactivation, physical stability, and the sensorial characteristics of the processed beverage. Despite the occurrence of protein denaturation observed in HPH-treated beverages and the formation of complex aggregates, they had better colloidal stability than the PAS one. Furthermore, the employed HPH conditions in this study did not show any effect on the lipid oxidation components (TBA values) or on the physicochemical properties (acidity, pH, and soluble solids), with their values being similar to those obtained for the nontreated Brazil nut beverage (control) and for the pasteurized (PAS) one.

HPH technology was able to produce a Brazil nut beverage with high sensorial quality stored under cold conditions (5 °C) for up to 21 days. Further studies must be conducted to investigate the effect of HPH treatments on the phytochemical compounds, antioxidant activity, and digestibility of treated Brazil nut beverages and to assess the complete composition of an HPH Brazil nut beverage without the use of any additives, from the perspective of functional food development.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages9010022/s1, Table S1: Centesimal compositional and physicochemical characteristic of Brazil nut beverage; Table S2: Inactivation of E. coli (CECT 434) (~108 CFU/ml) by HPH of the B; Table S3: Physicochemical characteristics of the Brazil nut beverage (BBN) treated by high-pressure homogenization (HPH) and pasteurization (PAS) and stored preserved; Table S4: TBA values (µg MDA/ mL) of the Brazil nut beverage treated by HPH (T1, T2 and T3) and pasteurization (PAS) and stored at 5°C for 21 days.

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