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Article

Valorization of Brewer's Spent Grain Liquid Fraction for the Development of a Pasteurized Strawberry-Based Blend Juice

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Abstract

Brewer's spent grain (BSG), a by-product of the brewing industry with 70–80% moisture, produces a liquid fraction during dewatering that can be combined with strawberry pulp (SP) to create a new food product. This study evaluated the use of BSG liquid fraction as a dilution medium for SP to develop a novel blend juice (BJ) and assess its shelf life after pasteurization. The optimal formulation consisted of 70% BSG liquid fraction and 30% SP, pasteurized at 95 °C for 5 min (BJ5) and 10 min (BJ10), and stored for 8 weeks at 25 °C. Pasteurization caused no significant ($p > 0.05$) changes in moisture, color, chemical composition, microbiological quality, or sensory properties. Both BJ5 and BJ10 showed sensory attributes, overall acceptance, and purchase intent comparable to two commercial Chilean blend juices. During storage, color, chemical, and microbiological parameters remained stable, though moisture, soluble solids, pH, and total polyphenol content were significantly affected ($p < 0.05$). Both pasteurization conditions ensured microbiological stability throughout the 8-week period. Overall, the pasteurized BSG–strawberry blend juice demonstrated good preservation, sensory quality, and consumer acceptance, suggesting strong potential as a sustainable and appealing product for the food and gastronomy industries.

Keywords: Brewers' spent grain; dewatering; liquor; thermal treatment; sensory juice

Highlights

- The liquid fraction from BSG can be used to formulate a novel blend juice.
- SP contributes with its physical and chemical properties to create a novel blend of juice.
- Pasteurization enables the preservation of BSG–strawberry blend juice.

BSG–strawberry blend juice presents natural sugars.

Sensory of BSG–SP blend juice is comparable to commercial Chilean blend juices.

1. Introduction

Brewer's spent grain (BSG) is a by-product of brewery industry, composed of husks of barley malt grain and its annual worldwide production is around 37 million tons, usually discarded or used for animal feed [1–3].

BSG is rich on dietary fiber and protein, but contain others important nutrients and bioactive compounds, such as lipids, starch, sugars, minerals, vitamins and polyphenolic compounds [1,2,4].

With a moisture content of 70–80%, BSG often undergoes dewatering to stabilize the solid fraction [5–8]. The resulting liquid fraction has shown potential for various applications, including the development of plant-based yogurt alternatives [9] and serving as a fermentation medium [10,11].

Finley et al. [5] obtained 42.1% of press water, which contained 3% solids, when wet BSG (from malt and corn) was dewatered using a Type 3A Davenport press. El-Shafey et al. [6] reported a decrease in the moisture of BSG cakes from 80 to 51% by filtration stage with hot-water squeezing, and finally to 20–30% with the application of vacuum over hot-squeezed cakes. Using a similar method, Machado et al. [7] were able to remove up to 95% of the water present in the BSG, decreasing its moisture content from 75 to 15%. Bjerregaard et al. [12] used a new type of continuous rotary drum press to produce a liquid filtrate that comprised 50% of the hot BSG mass. BSG treated without and with ultrasound and subsequently pressed with manual press of a 2 L stainless steel container, yielded 47.2 and 53.7 mL of liquid fraction per 100 g of BSG, respectively, and the BSG moisture decreased from 72.4–71.2 to 59.8–59.1% [8]. Akermann et al. [10] reported a yield of ~17.6 kg of liquid fraction (liquor) per ~34 kg of wet BSG, whose moisture content decreased from ~75 to ~65 wt%. The disposal or use of the liquid fraction of BSG should be studied to avoid adverse environmental effects [3,8].

The composition of BSG liquor obtained by a friction press (ENOL OP 20, Wein GmbH), from *Wheat bock*, *Wheat*, and *Helles* brewing recipe, includes sugars, protein, amino acids and minerals [10]. According to Shetty et al. [11], the liquid of BSG, produced using a rotary drum press equipped with a 300- μm filter, contained ca 12 g L⁻¹ of fermentable (maltose and glucose) and unfermentable (raffinose and maltodextrin) sugars, besides a small amount of lactic acid (1.2 g L⁻¹), acetic acid, and citric acid. Madsen et al. [9], using a filtration machine, obtained a 100 μm liquid fraction from BSG that was blended with a commercial unsweetened soy drink in a ratio of 20:80, and was chemically characterized by the presence of sugars, mannitol, pectin, acids, and ethanol, which was used to develop a product similar to yogurt. The main sugars found in the water extract of BSG were D-erythrose, fructose, D-tagatofuranose, xylose, glucopyranose, maltose, D-turanose, and cellobiose [13].

Strawberry pulp is a popular ingredient used in juices, yogurts, jams, jellies, and bakery products due to its bioactive compounds and sensory characteristics, primarily its color and aroma components [14–16]. Blends of different juices, pulp, puree or extracts, including those obtained from strawberry, have been studied for changes in their physical, chemical and sensory characteristics and to become more attractive to consumers that seek for bioactive compounds [17–21].

Recently, two processes for brewer's spent grain into powders have been proposed, using pressing to physically separate the liquid and solid fractions for potential use in food applications [8]. Building on this concept, we considered that the liquid fraction obtained from BSG dewatering, when combined with strawberry pulp, could contribute to its physical and chemical properties to create a novel blend juice that can be effectively preserved through pasteurization. The objective of this study was to evaluate the use of the BSG liquid fraction as a dilution medium for strawberry pulp to produce a new blend juice and to assess its shelf-life stability following pasteurization.

2. Materials and Methods

2.1. BSG Liquid Fraction, Strawberry Pulp and Blend Juice

The wet BSG, with a moisture content of 72.4% weight base, was generously provided by Birrell Ltda., a craft brewery located in Villarrica, Chile, in the Araucanía Region. The BSG was portioned into 1,010 g samples, packaged in Ziploc® bags (26.8 × 27.3 cm, Racine, WI, USA), and immediately frozen at -18 °C. The additional 10 g in each sample was allocated for moisture measurement in each of the experimental replicates after the BSG was thawed. This step was taken to ensure the accuracy of the measurements required for the study. Each bag was thawed in a refrigerator for 24 h at a temperature of 4–5 °C. Once thawed, the BSG was pressing in a manual press equipped with a 2 L stainless steel container and a mechanical screw. In each repetition, 1,000 g of wet original BSG was pressed to produce two fractions: liquid and solid. The BSG liquid fraction (BSG-LF) was stored in Schott glass bottles and frozen at -18 °C [8].

The strawberry pulp (SP) was obtained as frozen product from Varfel (<https://www.varfel.cl/>), located in Temuco, Chile, which is a producer of natural and commercial frozen pulp from various fruits and vegetables.

Before to preparing the blend juice, both frozen components were thawed in a refrigerator for 24 h at a temperature of 4–5 °C.

The blend juice (BJ) was established during preliminary experiments to define the proportion of 70% BSG liquid fraction and 30% strawberry pulp.

2.2. Blend Juice Pasteurization

The pasteurization was performed using a 20 mL test screw cap tube (N° 9825, Pyrex®, México) with 10 mL of BJ. For this thermal treatment, a thermoregulated water bath equipment (WNB 14, Memmert, Germany) was used, fixed at a hot water temperature of 95 °C. The temperature of the bath water and juice blend during pasteurization was monitored with thermocouples (TM-906A, Lutron Electronic Enterprise Co., Taiwan) placed inside the bath equipment and in the center of the BJ volume in the test tube (at the central geometrical point), respectively. Heating of pasteurization was kept for 5 (BJ5) and 10 (BJ10) min. Afterwards, the blend juice in the test tube with a screw cap was cooled using a mixture of water and ice (~2°C), and the temperature of which was also monitored. Twenty-five tubes with blend juice were placed in a rack and used for each pasteurization at 5 and 10 min. Pasteurization temperature and time were selected based on the study by Xue et al. [22].

2.3. Shelf-Life of Pasteurized Blend Juice

After pasteurization, twenty tubes with screw cap containing each one 10 mL of blend juice of 70% BSG-LF – 30% SP, of each thermal treatment (5 and 10 min), were stored at 25 °C during eight weeks using a temperature-controlled chamber (Archiclíma, Chile). At the end of the first, second, fourth and eighth weeks, moisture, color, chemical and microbiological characteristics were evaluated. The experiment was carried out in triplicate.

2.4. Analysis

2.4.1. Moisture Content

Between 4 and 5 g of samples were used to determine the moisture content in an oven at 105 °C for 2 h, and then weighed. They were kept at 105 °C until a constant weight [8,23]. The results are reported on a wet basis (w.b.).

2.4.2. Instrumental Color

The instrumental color was measured using a Minolta Chromameter CR-200b colorimeter (Japan) with the CIE L*a*b* system, as described by Ruíz et al. [8] and Ihl et al. [24]. The instrument was first calibrated with a white standard tile ($Y = 93.1$, $x = 0.3140$, and $y = 0.3212$) under illuminant condition C (6774 K). The L* variable lightness index ranges in the scale from 0 for black to 100 for white. The a* scale measures the degree of red (+a*) or green (-a*) colors, and the b* scale measures the degree of yellow (+b*) or blue (-b*) colors.

For color measurement, each sample (20 mL) was homogeneously distributed in a 7 cm diameter Petri dish. Readings were taken by placing the instrument on the surface at five different points of each sample.

The ΔE , which quantifies total color differences [25], was determined by Equation (1), following the analytical classification of Adekunle et al. [26], where values indicate very different colors ($\Delta E > 3$), medium differences in colors ($1.5 < \Delta E < 3$) and small differences in colors ($\Delta E < 1.5$).

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

2.4.3. Soluble Solids

Samples were homogenized and measured with a refractometer (model 10430, Reichert-Jung, USA) as °Brix [27].

2.4.4. Reducing Sugar Content

The reducing sugar content was determined by the Miller method using 3,5-dinitrosalicylic acid (DNS) [13,28]. Previously, all samples were centrifuged at 10,000 rpm (10 min) using a centrifuge (GS-15, Beckman, USA). First, each supernatant sample was diluted to 1/150 with distilled water. Then 0.5 mL of the diluted sample and 1.5 mL of DNS reagent were added to a test tube. It was heated to 100 °C in a water bath for 5 min. Next, the test tube was chilled in an ice bath for 3 min. Finally, 7.5 mL of distilled water was added, and the absorbance was measured at 540 nm. The concentration of reducing sugar in the samples was estimated from the standard glucose curve, and the results were expressed as g per 100 g dry matter (d.m.).

2.4.5. pH

The pH was determined by direct immersing the electrode of a pH meter into the homogeneous mass. The pH meter was calibrated using buffered standards at pH levels of 4.01 and 7.00 [27].

2.4.6. Titratable Acidity (TA)

The TA was determined by potentiometric titration as described for Xue et al. [22]. To perform this analysis, a 5 mL sample was titrated with 1 mol L⁻¹ sodium hydroxide solution until the pH reached 8.2 (± 0.1). Subsequently, the TA content was calculated using Equation (2) and expressed in terms of citric acid equivalent.

$$TA (\%) = \frac{C_{NaOH} \cdot V_{NaOH} \cdot K}{W} \cdot 100 \% \quad (2)$$

where C_{NaOH} is the concentration of sodium hydroxide (1 mol L⁻¹), V_{NaOH} is the volume of sodium hydroxide (mL), K is the conversion factor for citric acid (0.064) and W is the mass of the measured sample (g).

2.4.7. Total Polyphenol Content (TPC)

The Folin-Ciocalteu method was used for TPC determination. Samples were previously centrifuged at 10,864 x g (F0685 fixed-angle rotor for 10 min) using a centrifuge (GS-15, Beckman, USA). A 40- μ L aliquot of each supernatant sample was mixed with distilled water (3.16 mL), added

with 200 μL of Folin–Ciocalteu reagent, and, after 5 min, added with 600 μL of 20% Na_2CO_3 solution. Samples were kept at 20 °C for 120 min in the dark. The absorbance was measured at 765 nm using a spectrophotometer (Spectronic Genesys 10, Sweden), and the results were expressed as mg of gallic acid equivalents (GAE) per 100 g dry matter (d.m.) using a calibration curve [29].

2.4.8. Ascorbic Acid Content

Samples were previously centrifuged at $10,864 \times g$ (F0685 fixed-angle rotor) for 10 min using a centrifuge (GS-15, Beckman, USA). Subsequently, the ascorbic acid reflectometric test strip (Merck, Germany) was immersed for 20 seconds in the supernatant, the liquid excess on test strip was removed, and the concentration was determined, introducing the strip for 10 seconds into Merck RQ Flex at $570/657 \pm 10$ nm [24]. The results were expressed as mg ascorbic acid per 100 g dry matter (d.m.).

2.4.9. Microbiology Determination

The aerobic mesophilic bacteria (AMB) and the yeast and mold (YM) were determined according to the Instituto de Salud Pública de Chile [30] and Choo et al. [31]. AMB count was examined using plate count agar (PCA), and YM counts were analysed using potato dextrose agar (PDA) with the addition of 10% tartaric acid. The PCA plate was incubated at 37 °C for 1-2 days, whereas the PDA plate was incubated at 25 °C for 3-5 days. Results were expressed as log colony-forming units (CFU) per mL of sample.

2.5. Sugar Characterization by HPLC

High-performance liquid chromatography (HPLC) analysis of sugars was conducted using a Shimadzu LC-20AT system (Kyoto, Japan) equipped with a refractive index detector (RID) and a Shim-pack GIST NH2 column (4.6 mm \times 250 mm, 5 μm). Chromatographic separation was performed under isocratic conditions with a mobile phase of acetonitrile and water (75:25, v/v) at a flow rate of 1.0 mL/min. Prior to injection, all five samples (Table 3) were centrifuged, and the resulting supernatants were diluted 1:1 with the mobile phase. The samples were subsequently filtered through 0.22 μm membrane filters, and 20 μL aliquots were injected into the HPLC system in triplicate. The authentic standards of sugars used in the analysis were cellobiose, xylose, maltose, glucose, fructose, sucrose, raffinose, nystose, kestose, and turanose. Sugar identification was achieved by comparing sample retention times with those of authentic standards, and quantification was performed using calibration curves prepared from standard solutions.

2.6. Sensory Evaluation

It was performed according Lepaus et al. [32] and Cendrowski et al. [33] for blend juice prepared with 70% of BSG-LF and 30% of SP, before and after pasteurization, and two commercial blend juices. The samples were subjected to sensory evaluation by a group of 26 untrained panelists selected from staff and students of Chemical Engineering Department, Universidad de La Frontera, Temuco, Chile. The panelist rated the products based on a nine-point Hedonic score ranging from 1 (dislike extremely) to 9 (like extremely) for attributes color, appearance, consistency, aroma, taste, overall acceptance and purchase intent. Panelist received 30 mL of each sample in transparent plastic cups coded with three random digits, in random order. Participants also received a glass of water to clean their palates before each assessment.

2.7. Statistical Analysis

The data were subjected to analysis of variance for experiments described in 2.1., 2.2. and 2.3., which carried out in triplicate. For significant differences, Tukey's honestly and t-Student post-hoc tests were applied to determine significant differences between means. Mean values were considered

significantly different at $p < 0.05$. Minitab® Statistical Software 21.0.3.1.0 (Chicago, USA) was used for data analysis.

3. Results and Discussion

3.1. Characterization of BSG Liquid Fraction, Strawberry Pulp and Blend Juice

Table 1 presents the characterization of BSG-LF, SP and BJ with respect to their moisture, color, chemical parameters, aerobic mesophilic bacteria count, and yeast and mold count. In general, the characteristics of BJ corresponded to a proportion of 70% and 30% of BSG-LF and SP, respectively.

Table 1. Moisture, color, chemical and microbiological characteristics of BSG liquid fraction, strawberry pulp and blend juice.

Parameter	BSG-LF	SP	BJ
Moisture content (% w.b.)	87.0 ± 0.1 ^a	69.2 ± 0.1 ^c	82.4 ± 0.1 ^b
Color			
L*	43.7 ± 4.3 ^a	30.1 ± 0.6 ^b	34.4 ± 0.4 ^b
a*	0.2 ± 0.3 ^c	15.9 ± 0.8 ^a	8.9 ± 0.1 ^b
b*	12.9 ± 0.2 ^a	11.4 ± 0.4 ^b	10.6 ± 0.5 ^b
ΔE	13.2 ± 2.9 ^a	8.4 ± 0.4 ^a	-
Soluble solid (° Brix)	13.5 ± 0.1 ^c	29.1 ± 0.1 ^a	17.2 ± 0.2 ^b
Reducing sugar (g 100 g ⁻¹ d.m.)	65.6 ± 1.3 ^a	20.9 ± 0.1 ^c	47.4 ± 0.8 ^b
pH	6.26 ± 0.09 ^a	3.64 ± 0.16 ^c	4.28 ± 0.03 ^c
Titratable acidity (%)	0.065 ± 0.007 ^c	0.663 ± 0.067 ^a	0.243 ± 0.002 ^b
TPC (mg GAE 100 g ⁻¹ d.m.)	169.4 ± 13.2 ^b	256.6 ± 10.3 ^a	181.2 ± 3.3 ^b
Ascorbic acid (mg 100 g ⁻¹ d.m.)	< 16,7	30.9 ± 3.2 ^a	23.2 ± 1.5 ^b
Microbiology			
AMB (log CFU mL ⁻¹)	4.8 ± 0.1 ^a	Absent	3.5 ± 0.0 ^b
YM (log CFU mL ⁻¹)	Absent	Absent	Absent

BSG-LF: BSG liquid fraction; SP: Strawberry pulp; BJ: blend juice (70% BSG-LF - 30% SP) before pasteurization; w.b.: wet basis; d.m.: dry matter; AMB: aerobic mesophilic bacteria; YM: yeast and mold; CFU: colony forming unit. In each row, different letters indicate significant differences by Tukey's test at $p < 0.05$ or by the t-Student test at $p < 0.05$ when two data were compared. Data are presented as mean ± standard deviation.

The moisture content (Table 1) of the BSG-LF obtained by pressing in this study was 87.0% (w.b.). Although no previous reports were found on the moisture content of liquor or press water, Finley et al. [5] reported a ratio of 10,008 lb of water to 690 lb of solids after centrifugation of 10,698 lb of BSG press liquid, which corresponds to approximately 93.6% moisture. Bjerregaard et al. [12] reported dry weights of 9.45% and 10.3% in a liquid filtrate obtained from hot BSG by pressing using 100 and 300 μm pore size filters on a continuous rotary drum, respectively.

Color values L* (43.7), a* (0.2), and b* (12.9) of BSG-LF are similar or lower values than those informed by Ruiz et al. [8] for wet and press solid BSG, which were L* 44.1 and 45.0, a* 5.3 and 4.9, and b* 22.5 and 22.6, respectively. According to Adekunte et al. [26] classification, which considered $\Delta E > 3$ as very different colors, the ΔE (13.2) of BSG-LF, determined using the BJ color parameters as a reference, indicates a strong change in color after blending BSG-LF with SP, as expected.

According to some studies [10,11,13], the liquid obtained from BSG for different methods included several sugars in its composition, which are represented in levels of soluble solid (13.5 °Brix) and reducing sugar (65.6 g 100 g⁻¹ d.m.) determined for BSG-LF (Tables 1 and 3).

The pH (6.26) of BSG-LF is slightly higher than the values (5.13 - 5.98) reported for Akermann et al. [10] for BSG liquor obtained by pressing from BSG, namely *Wheat bock*, *Wheat*, and *Helles* (Barley).

The low titratable acidity (%) is in accordance with the small amount of lactic acid (1.2 g L^{-1}), acetic acid, and citric acid reported by Shetty et al. [11].

The TPC in the BSG-LF ($169.4 \text{ mg GAE } 100 \text{ g}^{-1} \text{ d.m.}$) shows that polyphenols present in wet BSG [1,2,4] are transferred to the liquid fraction by the pressing operation according to Ruíz et al. [8], who reported TPC of $304\text{--}305 \text{ mg GAE } 100 \text{ g}^{-1} \text{ d.m.}$ for powder of BSG. Previously, the TPC of BSG was reported by Carciochi et al. [34] (2018), whose values ranged from 159 to $357 \text{ mg GAE } 100 \text{ g}^{-1} \text{ BSG d.m.}$, and Meneses et al. [35] and Bonifácio-Lopes et al. [36], who determined 713 and $1300 \text{ mg GAE } 100 \text{ g}^{-1} \text{ BSG d.m.}$, respectively. However, TPC values of BSG liquid or liquor have not been reported before. The *Hibiscus sabdariffa* extract, used to develop blends with different fruit juices, showed a phenolic content of $1,496 \text{ mg GAE } 100 \text{ g}^{-1}$ [37], which is 8.8 times higher than the TPC of BSG-LF. Defective coffee green beans and coffee silver skins as by-products, treated with 5 mL of 95% (v/v) methanol, showed a TPC of $2,722$ and $536 \text{ mg GAE } 100 \text{ g}^{-1} \text{ d.m.}$, respectively [38].

The method used to determine ascorbic acid can measure the range of $25\text{--}450 \text{ mg L}^{-1}$; however, BSG-LF presented a value below 25 mg L^{-1} , which is equivalent to a concentration less than $16,7 \text{ mg } 100 \text{ g}^{-1} \text{ d.m.}$

Aerobic mesophilic bacteria were present in $4.8 \text{ log CFU mL}^{-1}$, which is within the AMB counts ranging from 2.58 to $6.12 \text{ log CFU g}^{-1}$ of BSG immediately after lautering, reported by Robertson et al. [39]. They considered these values as microbiologically stable and within acceptable limits for food use. Pressing, described in 2.1. and, as noted by Ruíz et al. [8], could be an operation that contributes to transferring part of the AMB load from the original wet BSG obtained from the craft brewery to BSG-LF.

Characterization of strawberry pulp (Table 1) reveals a significantly lower moisture content ($p < 0.05$) compared to the value of BSG-LF; therefore, the latter allows the pulp to be diluted for consumption as juice. Strawberry juice presents a moisture of 91% according to Basiony et al. [40].

Color of concentrated and dried strawberry pulps have been reported with values of L^* of 26.9 , a^* of 19.7 and b^* of 7.3 [14] and L^* of $22\text{--}38$, a^* of $19\text{--}22$ and b^* of $2\text{--}10$ [16], respectively, which are similar to the color parameters determined in this work for SP. The ΔE (8.4) of SP, determined using the blend juice color parameters as a reference, evidences a strong change in color [26] after blending SP with BSG-LF.

Additionally, our results align with those reported by EL Moutaouakil et al. [41], who found pH levels ranging from 3.3 to 3.7 in several strawberry pulps, both before and after pasteurization. Regarding microbiological quality, our results agreed with the last-mentioned authors, who inform that after pasteurization ($85^\circ\text{C}/7.5\text{min}$), the finished products do not contain microbiological agents such as total aerobic mesophilic flora, yeasts, molds, and enterobacteria. However, the solid soluble (29.1°Brix) determined for the SP, which is a concentrated product, is 2.7 to 4.1 times higher than the values ($7\text{--}11^\circ\text{Brix}$) reported by the last-mentioned authors.

Typically, strawberry fruit, pulp and juice are sources of several acids, polyphenols, and ascorbic acid [15], compounds present in higher concentrations compared with BSG-LF.

Moisture content (82.4%) of BJ (Table 1), made with 70% of BSG-LF and 30% of SP, is below of the moisture ranging ($85.9\text{--}88.2\%$) for several proportion blends of pumpkin juice with orange, carrot and lemon juices, which individual values of moisture were 91.4 , 84.7 , 89.0 and 89.8% , respectively [19]. The moisture content determined for our blend agrees with the proportion volume used in its formulation, and it is significantly different from the values of LB-BSG and SP.

Soluble solids of blend juice (17.2°Brix) are higher than the total soluble solids of several pumpkin juice blends that range from 7 to 13.5 [19]; however, their pH values ($5.03\text{--}5.71$) are higher than that of the BJ (pH 4.28). Feng et al. [21] report values of total soluble solids of 7.8°Brix , pH 3.38 , total phenols of $877.69 \text{ mg GAE L}^{-1}$, ascorbic acid content of $2.78 \text{ mg } 100 \text{ mL}^{-1}$, total aerobic bacteria of $4.19 \text{ log CFU mL}^{-1}$ and YM of $4.21 \text{ log CFU mL}^{-1}$ for blended strawberry-apple-lemon juice.

In general, the values of parameters present in Table 1 show that there are significant differences ($p < 0.05$) among BSG-LF, SP, and BJ. Although strawberry pulp is the smallest proportion in the

blend juice formulation, both did not differ significantly ($p > 0.05$) for the parameters L^* , b^* , and pH, which would allow the BJ to be recognized as a strawberry product derivative.

3.2. Effect of Pasteurization on Blend Juice

Figures 1 and 2 illustrate the temperature profiles of the blend juice during pasteurization and subsequent cooling. The measurements were recorded at the central geometric point of the sample contained in a 20 mL screw-cap test tube, as well as in the water of the thermoregulated bath. The heating phase was conducted for 5 and 10 min, followed by the cooling step.

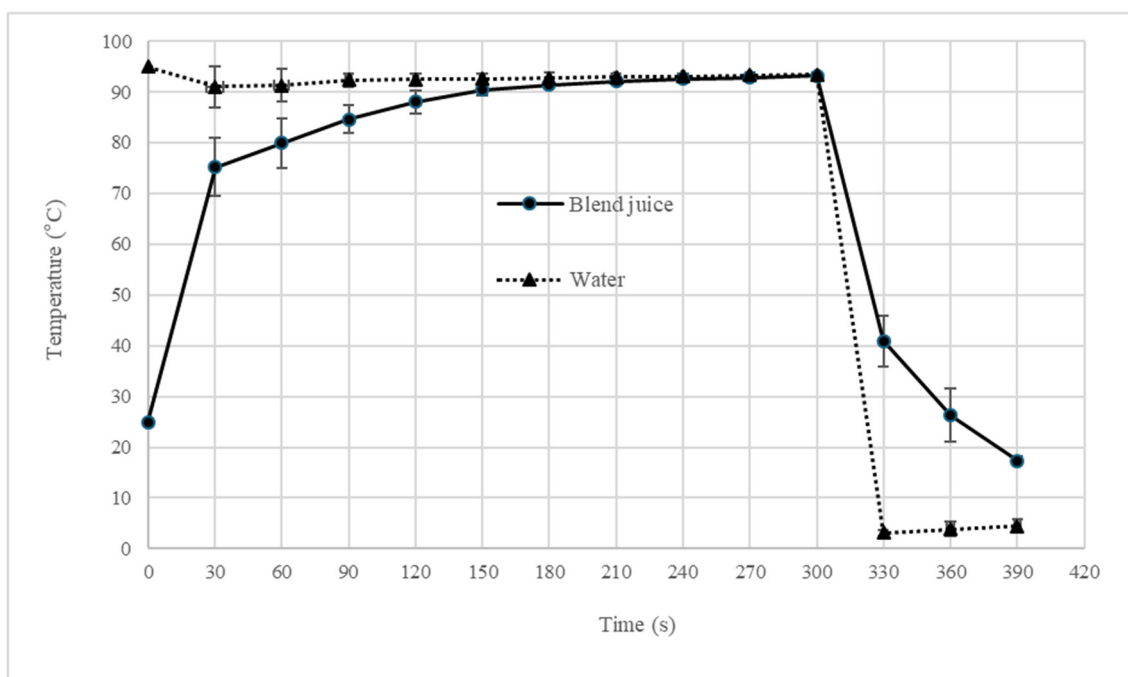


Figure 1. Temperature monitoring of the blend juice and bath water for pasteurization with a heating step of 5 min and subsequent cooling.

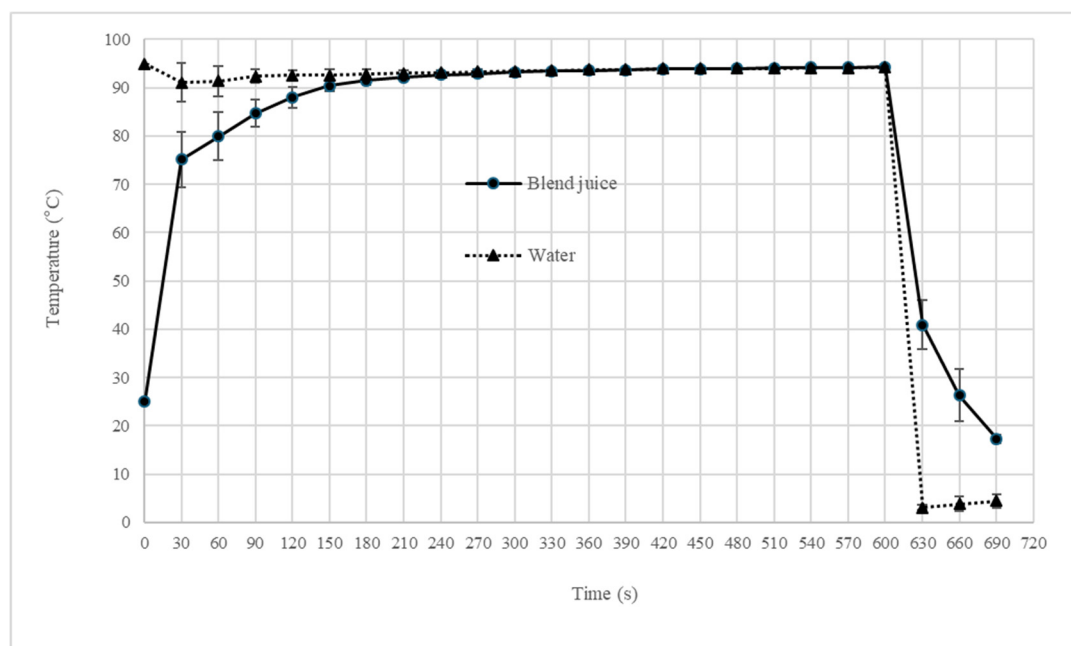


Figure 2. Temperature monitoring of the blend juice and bath water for pasteurization with a heating step of 10 min and subsequent cooling.

As mentioned in the methodology, the water temperature of the thermoregulated bath equipment was fixed at 95 °C. When the rack with 20 tubes with screw cap, containing each one 10 mL of blend juice of 70% BSG-LF – 30% SP, was introduced in the bath water, the blend juice's temperature increased, reaching the equilibrium with the water after 180 s at a temperature below 95 °C for both treatment pasteurization (Figures 1 and 2). Xue et al. [22] reported a thermal pasteurization of 95 °C for 2 min that completely inactivated the total plate count, coliforms, yeast and mold in bayberry juice. Pasteurization treatment at Figure 1 shows that the temperature stabilization, at a range of 90-95 °C in the blend juice, was for 2 min (180 to 300 s). However, in Figure 2 the period of temperature stabilization was 7 min (180 to 600 s) for the same range of 90-95 °C. Radziejewska-Kubzdela [42] evaluated the effect of ultrasonic, thermal and enzymatic treatment on yield and content of bioactive compounds in strawberry mash using a batch heated up to a temperature of 80 °C for 15 min and then held for 5 min at this temperature. The mash heat was equivalent to other treatments for preservation of ascorbic acid, phenolic compounds and antioxidant activity, which in the last case was significantly higher than samples of untreated mash.

Table 2 presents the sugar content determined by HPLC before and after pasteurization of the blend juice obtained from a mixture of 70% BSG-LF and 30% SP.

Table 2. Sugar content (mg mL⁻¹) in BSG liquid fraction, strawberry pulp and blend juice before and after pasteurization.

Sample	Fructose	Glucose	Maltose	Raffinose
BSG-LF	n.d.	12.16 ± 0.16 ^d	61.83 ± 0.72 ^a	17.14 ± 0.39 ^a
SP	28.50 ± 0.76 ^a	31.05 ± 1.12 ^a	n.d.	n.d.
BJ	11.22 ± 0.70 ^b	23.87 ± 0.54 ^b	34.58 ± 1.06 ^b	11.84 ± 1.20 ^b
BJ5	10.44 ± 0.42 ^b	19.26 ± 0.46 ^c	31.75 ± 0.96 ^c	10.92 ± 0.86 ^b
BJ10	11.19 ± 0.64 ^b	20.82 ± 1.02 ^c	34.50 ± 1.68 ^b	11.48 ± 0.61 ^b

BSG-LF: BSG liquid fraction; SP: Strawberry pulp; BJ: blend juice (70% BSG-LF - 30% SP) before pasteurization; BJ5 and BJ10: blend juice after pasteurization for 5 and 10 min at 95°C, respectively; n.d.: not detected. In each column, different letters indicate significant differences by Tukey's test at $p < 0.05$. Data are presented as mean ± standard deviation.

The sugars identified in BSG-LF in this study have also been reported by others (Table 2). Glucose, maltose, and raffinose were detected in BSG liquid fraction in the study by Madsen et al. [9], while maltose and glucose were identified in the liquid of BSG by Shetty et al. [11].

The addition of strawberry pulp was responsible for increasing the reducing sugar content in BJ. Since the BSG-LF did not contain fructose and exhibited lower glucose levels, its combination with strawberry pulp enhanced the concentrations of these monosaccharides, which may improve the palatability and energy value of the beverage. On the other hand, the BSG liquid fraction showed higher levels of the disaccharide maltose, composed of two glucose units, and the trisaccharide raffinose, composed of galactose, fructose, and glucose. Complex carbohydrates may contribute to the prebiotic potential of the beverage, as they are less readily digested and can promote beneficial gut microbiota activity [43].

Table 3 shows the sensory evaluation, including acceptance and purchase intent, of BJ before and after pasteurization for 5 and 10 min at 95 °C. For comparison, two recognized Chilean commercial blend juices were included in this study; they were purchased at a supermarket in Temuco.

Table 3. Sensory evaluation of the tested blend juice before and after pasteurization and two commercial fruit blend juices.

Sample	Color	Appearance	Consistency	Aroma	Taste	Overall Acceptance	Purchase Intent
BJ	7.2 ± 1.3 ^a	6.5 ± 1.4 ^a	6.9 ± 1.1 ^a	6.8 ± 1.4 ^a	7.2 ± 1.5 ^a	6.7 ± 1.5 ^a	6.7 ± 1.5 ^a
BJ5	7.0 ± 1.5 ^a	6.1 ± 1.8 ^a	6.5 ± 1.4 ^a	6.6 ± 1.6 ^a	6.9 ± 1.6 ^{ab}	6.7 ± 1.5 ^a	6.5 ± 1.3 ^a
BJ10	6.7 ± 1.6 ^{ab}	6.0 ± 2.0 ^a	6.7 ± 1.3 ^a	6.5 ± 1.4 ^a	6.5 ± 1.2 ^{ab}	6.6 ± 1.4 ^a	6.2 ± 1.7 ^a
FS	5.7 ± 1.7 ^b	5.8 ± 1.6 ^a	6.8 ± 1.6 ^a	6.5 ± 1.6 ^a	5.8 ± 1.8 ^b	6.2 ± 1.8 ^a	5.6 ± 2.3 ^a
AFE	6.4 ± 2.0 ^{ab}	6.2 ± 2.2 ^a	6.8 ± 1.5 ^a	6.2 ± 1.4 ^a	6.3 ± 1.6 ^{ab}	6.2 ± 1.7 ^a	5.7 ± 2.1 ^a

BJ: blend juice (70% BSG-LF - 30% SP) before pasteurization; BJ5 and BJ10: blend juice after pasteurization for 5 and 10 min at 95°C, respectively; FS: strawberry-cherry blend juice trademark Fundo Sofruco (<https://www.larosasofruco.cl>); AFE: apple-cherry blend juice trademark AFE (<https://www.jugoafe.cl>). In each column, different letters indicate significant differences by Tukey's test at $p < 0.05$. Data are presented as mean \pm standard deviation.

The panelists aged 20 to 62 years-old (mean age of 37.9 with 13.4 as standard deviation), with females and males in equal proportion, detected only a significant difference ($p < 0.05$) in color and taste attributes among all samples (Table 3). However, the pasteurization (BJ5 and BJ10) did not produce significant effect ($p > 0.05$) in all sensory attributes evaluated, overall acceptance and purchase intent respect to BJ. Our results show that the BJ5 and BJ10 sensory attributes are equivalent to those of two commercial blend juices (FS and AFE) sold throughout Chile.

The maintenance of sensory attributes (color, appearance, consistency, aroma, and taste) of BJ5 and BJ10 with respect to BJ as shown in Table 2, and physical and chemical characteristics presented in Table 4, agreed with the sugar composition. In contrast, Lepaus et al. [32] established a significant decrease ($p < 0.05$) in the evaluation of aroma, flavor, overall acceptance, and purchase intent by the panelists due to thermal treatment at 90 °C for 30 s in orange-carrot juice blend compared to the untreated one, associated with changes in aroma, flavor, and color compounds. A significant ($\alpha = 95\%$) effect was determined by Cendrowski et al. [33] on sensory preference (colour, aromatic odour, fruity odour, sweet taste, sour taste, tart taste, overall impression) of different mixed juices prepared with rose fruits (*Rosa rugosa*) and apple or strawberry, heat treated at 85 °C/15 min and then packed into 100 mL glass bottles. In our research, the panelists did not find a significant difference ($p > 0.05$) in sensory attributes (Table 3) among the pasteurized blend juices (BJ5 and BJ10), FS, and AFE, which are also thermally treated products

Table 4. Moisture, color, chemical and microbiological characteristics of the blend juice before and after pasteurization.

Parameter	BJ	BJ5	BJ10
Moisture content (% w.b.)	82.4 ± 0.1 ^a	81.9 ± 0.1 ^b	81.7 ± 0.1 ^b
Color			
L*	34.4 ± 0.4 ^a	33.5 ± 0.6 ^a	33.9 ± 1.0 ^a
a*	8.9 ± 0.1 ^a	8.7 ± 0.6 ^a	8.7 ± 0.7 ^a
b*	10.6 ± 0.5 ^a	9.4 ± 0.8 ^a	9.9 ± 0.7 ^a
ΔE	-	1.7 ± 0.5 ^a	1.2 ± 0.3 ^a
Soluble solid (° Brix)	17.2 ± 0.2 ^b	17.7 ± 0.2 ^a	18.0 ± 0.0 ^a
Reducing sugar (g 100 g ⁻¹ d.m.)	47.4 ± 0.8 ^a	48.9 ± 2.9 ^a	49.4 ± 3.4 ^a
pH	4.28 ± 0.03 ^a	4.18 ± 0.02 ^{ab}	4.14 ± 0.07 ^b
Titrateable acidity (%)	0.243 ± 0.002 ^a	0.259 ± 0.031 ^a	0.260 ± 0.030 ^a
TPC (mg GAE 100 g ⁻¹ d.m.)	181.2 ± 3.3 ^{ab}	172.8 ± 4.4 ^b	186.8 ± 1.8 ^a

Ascorbic acid (mg 100 g ⁻¹ d.m.)	23.2 ± 1.5 ^a	27.9 ± 2.5 ^a	26.7 ± 3.6 ^a
Microbiology			
AMB (log CFU mL ⁻¹)	3.5 ± 0.0	Absent	Absent
YM (log CFU mL ⁻¹)	Absent	Absent	Absent

BJ: blend juice (70% BSG-LF - 30% SP) before pasteurization; BJ5 and BJ10: blend juice after pasteurization for 5 and 10 min at 95°C, respectively; w.b.: wet basis; d.m.: dry matter; AMB: aerobic mesophilic bacteria; YM: yeast and mold; CFU: colony forming unit. In each row, different letters indicate significant differences by Tukey's test at $p < 0.05$. Data are presented as mean ± standard deviation.

Table 4 presents the effects of pasteurization at 95 °C for 5 and 10 min on blend juice (70% BSG-LF and 30% SP) in terms of its moisture, color, chemical parameters, aerobic mesophilic bacteria count, and yeast and mold count. No relevant changes were observed in most parameters, and the AMB load was eliminated by both heat treatments (Figures 1 and 2).

Moisture content (Table 4) of the blend juice decreased significantly ($p < 0.05$) after pasteurization (5 and 10 min). However, the level is still very high (81.9 and 81.7%, w.b.), which, together with the nutrient composition in blend juice, creates an optimal environment for the growth of spoilage microorganisms [22].

ΔE values (1.7 and 1.2), determined by comparing blend juices before and after pasteurization, showed medium and small differences in color, according to Adekunle et al. [26].

Soluble solids after the two conditions of pasteurization were higher ($p < 0.05$) than the value of blend juice before heat treatment, maybe due to the release of sugars and organic acids from the portion of pulp strawberry by breakdown of enzymes and temperature [31].

Only pH after pasteurization at 10 min decreased significantly ($p < 0.05$) compared to the value before submitting the blend juice to this thermal treatment. Choo et al. [31] and Xue et al. [22] did not observe significant changes in the quality of noni juice (90 °C for 1 min) and bayberry juice (95 °C for 2 min) after thermal pasteurization. This behavior aligns with the pasteurization of blended juice at 95 °C for 5 min, whose pH values did not differ significantly ($p > 0.05$) from those in its fresh condition.

Similar to BJ, Choo et al. [31] did not detect yeast and mold in fresh and pasteurized (90 °C for 1 min) noni juice.

3.3. Shelf Life of Pasteurized Blend Juice

Table 5 shows the evolution of shelf life at 25 °C of the BJ pasteurized at 95 °C for 5 and 10 min in terms of moisture, color, chemicals parameters, aerobic mesophilic bacteria count, and yeast and mold count. In general, the parameters evaluated remained stable up to 8 weeks, except for the total polyphenol content. Notably, AMB and YM stability was maintained during all period of storage.

Table 5. Shelf life at 25 °C of the blend juice after pasteurization at 90 °C by 5 and 10 min.

Parameter		Immediately after pasteurized	Storage time at 25 °C (Week)			
			1	2	4	8
Moisture content (% w.b.)	BJ5	81.9 ± 0.1 ^{aA}	81.6 ± 0.0 ^{abA}	81.3 ± 0.2 ^{bA}	81.7 ± 0.1 ^{abA}	81.4 ± 0.2 ^{bA}
	BJ10	81.7 ± 0.1 ^{aA}	80.7 ± 0.2 ^{cA}	80.9 ± 0.2 ^{bcA}	81.4 ± 0.0 ^{abA}	80.9 ± 0.0 ^{bcA}
Color L*	BJ5	33.5 ± 0.6 ^{aA}	36.1 ± 2.6 ^{aA}	34.3 ± 2.0 ^{aA}	34.9 ± 1.7 ^{aA}	34.6 ± 0.4 ^{aA}
	BJ10	33.9 ± 1.0 ^{bA}	37.3 ± 2.6 ^{aA}	33.9 ± 0.7 ^{bA}	33.6 ± 1.0 ^{bA}	33.4 ± 0.6 ^{bB}
a*	BJ5	8.7 ± 0.6 ^{aA}	8.5 ± 0.5 ^{aA}	7.3 ± 0.8 ^{bA}	6.5 ± 0.5 ^{bB}	6.8 ± 0.5 ^{bA}
	BJ10	8.7 ± 0.7 ^{abA}	9.0 ± 0.7 ^{aA}	7.6 ± 0.5 ^{bcA}	7.1 ± 0.4 ^{cA}	7.1 ± 0.6 ^{cA}
b*	BJ5	9.4 ± 0.8 ^{aA}	10.5 ± 2.3 ^{aA}	8.1 ± 0.5 ^{aA}	9.7 ± 2.2 ^{aA}	10.1 ± 1.6 ^{aA}
	BJ10	9.9 ± 0.7 ^{aA}	12.1 ± 2.6 ^{aA}	9.3 ± 1.2 ^{aA}	9.8 ± 1.7 ^{aA}	9.8 ± 0.4 ^{aA}

ΔE	BJ5	-	2.9 ± 2.5 ^{aA}	2.7 ± 1.4 ^{aA}	3.6 ± 2.1 ^{aA}	3.2 ± 0.7 ^{aA}
	BJ10	-	4.4 ± 2.6 ^{aA}	1.8 ± 0.9 ^{aA}	2.5 ± 0.7 ^{aA}	2.0 ± 1.0 ^{aA}
Soluble solid (° Brix)	BJ5	17.7 ± 0.2 ^{bcA}	17.5 ± 0.0 ^{cb}	18.0 ± 0.1 ^{abB}	18.2 ± 0.3 ^{aA}	18.3 ± 0.2 ^{ab}
	BJ10	18.0 ± 0.0 ^{dA}	19.1 ± 0.1 ^{aA}	18.9 ± 0.1 ^{bA}	18.5 ± 0.2 ^{cA}	19.0 ± 0.1 ^{abA}
Reductor sugar (g 100 g ⁻¹ d.m.)	BJ5	48.9 ± 2.9 ^{aA}	46.2 ± 0.4 ^{aA}	48.0 ± 5.2 ^{aA}	49.1 ± 0.9 ^{aA}	47.2 ± 0.7 ^{ab}
	BJ10	49.4 ± 3.4 ^{aA}	49.3 ± 3.4 ^{aA}	42.4 ± 0.2 ^{bA}	48.6 ± 1.7 ^{aA}	49.4 ± 0.3 ^{aA}
pH	BJ5	4.18 ± 0.02 ^{bA}	4.07 ± 0.01 ^{cb}	4.19 ± 0.01 ^{bA}	4.17 ± 0.01 ^{bb}	4.27 ± 0.01 ^{aA}
	BJ10	4.14 ± 0.07 ^{aA}	4.17 ± 0.01 ^{aA}	4.17 ± 0.01 ^{aA}	4.24 ± 0.01 ^{aA}	4.27 ± 0.01 ^{aA}
Titratable acidity (%)	BJ5	0.259 ± 0.031 ^{aA}	0.254 ± 0.001 ^{aA}	0.247 ± 0.001 ^{aA}	0.242 ± 0.002 ^{aA}	0.252 ± 0.001 ^{aA}
	BJ10	0.260 ± 0.030 ^{aA}	0.280 ± 0.001 ^{ab}	0.246 ± 0.002 ^{aA}	0.250 ± 0.000 ^{aA}	0.251 ± 0.001 ^{aA}
TPC (mg GAE 100 g ⁻¹ d.m.)	BJ5	172.8 ± 4.4 ^{ab}	170.0 ± 2.2 ^{ab}	147.8 ± 0.3 ^{bb}	149.0 ± 7.8 ^{bb}	134.0 ± 3.7 ^{cA}
	BJ10	186.8 ± 1.8 ^{abA}	195.7 ± 5.1 ^{aA}	160.1 ± 0.0 ^{cA}	175.9 ± 7.5 ^{bA}	136.2 ± 7.8 ^{dA}
Ascorbic acid (mg100 g ⁻¹ d.m.)	BJ5	27.9 ± 2.5 ^{aA}	23.5 ± 1.3 ^{abA}	21.1 ± 0.9 ^{bA}	22.9 ± 0.9 ^{abA}	22.0 ± 1.5 ^{abA}
	BJ10	26.7 ± 3.6 ^{aA}	22.2 ± 1.2 ^{aA}	23.1 ± 1.5 ^{aA}	23.2 ± 0.6 ^{aA}	21.4 ± 0.3 ^{aA}
Microbiology						
AMB (log CFU mL ⁻¹)	BJ5	Absent	Absent	Absent	Absent	Absent
	BJ10	Absent	Absent	Absent	Absent	Absent
YM (log CFU mL ⁻¹)	BJ5	Absent	Absent	Absent	Absent	Absent
	BJ10	Absent	Absent	Absent	Absent	Absent

BJ5 and BJ10: blend juice after pasteurization for 5 and 10 min at 95°C, respectively; w.b.: wet basis; d.m.: dry matter; AMB: aerobic mesophilic bacteria; YM: yeast and mold; CFU: colony forming unit. For one parameter, in each column, different capital letters indicate significant differences determined by the t-Student test at $p < 0.05$, while different lowercase letters in each row indicate significant differences based on Tukey's test at $p < 0.05$. Data are presented as mean \pm standard deviation.

Moisture content (Table 5) of blend juice pasteurized (5 and 10 min) decreased significantly ($p < 0.05$) during storage at 25 °C, however, for each storage day, it was similar between the two thermal treatments of blend juice. According to Choo et al. [31] for noni juice and Mandha et al. [44] for watermelon, pineapple and mango juice, high moisture content (81.7-80.7%) of the blend juice allows microbial growth and chemical reactions during storage, therefore, pasteurization at 80 °C for 1 to 15 min prevents microbial growth and preserves the juices.

The ΔE , determined by comparing blend juice immediately after pasteurization (5 and 10 min) and after each day of storage, showed values from 4.4 to 1.8, which is considered very and medium difference in color, according to Adekunle et al. [26]. The a^* decreased significantly ($p < 0.05$) for both pasteurization times after two weeks of storage, which corresponds to the measure of red ($+a^*$) hue.

Soluble solids increase significantly ($p < 0.05$) for blend juice pasteurized at 5 and 10 min during storage, which agrees with the evolution of total soluble solids in watermelon juice pasteurized at 80 \pm 2 °C for 10 and 15 min, and stored at 4 °C for 14 days [44]. These authors mentioned a similar behavior for roselle-mango juices attributed to the hydrolysis of polysaccharides into monosaccharides during storage.

pH increased significantly ($p < 0.05$) for the blend juice pasteurized at 5 min during storage. In contrast, for BJ10, it remained stable, possibly due to the combined evolution of solid soluble and titratable acidity, which remained constant during storage.

The total polyphenol content was affected during storage, being significantly lower ($p < 0.05$) at the eighth week compared to the blend juice immediately after pasteurization at 95 °C by 5 and 5 min treatments. Phenol degradation was reported during storage of strawberry pulp and pasteurized watermelon juice for Gonçalves et al. [15] and Mandha et al. [44], respectively.

4. Conclusion

The blend juice formulated with a mixture of 70% BSG liquid fraction and 30% strawberry pulp, was pasteurized at 95 °C for 5 and 10 min and immediately cooled. It exhibited stable physicochemical, microbiological, and sensory properties. No significant differences ($p > 0.05$) were observed in moisture content, color, chemical composition, microbiological quality, or sensory attributes after thermal treatment. Furthermore, the sensory characteristics, overall acceptability, and purchase intent of BJ5 and BJ10 were comparable to those of two commercial Chilean blend juices (FS and AFE).

During storage at 25 °C for 8 weeks, BJ5 and BJ10 maintained their color, chemical, and microbiological stability; however, significant changes ($p < 0.05$) were observed in moisture content, soluble solids, pH, and total polyphenol content. Both formulations remained free from aerobic mesophilic bacteria, yeasts, and molds throughout storage, confirming the microbiological stability achieved under both pasteurization conditions.

Overall, the use of the brewer's spent grain liquid fraction, obtained by pressing, as a dilution medium in combination with strawberry pulp enabled the development of a novel blend juice with an extended shelf life of up to 8 weeks at 25 °C. This product represents a promising innovation for the food and gastronomic industries, contributing to the valorization of brewery by-products and supporting the principles of a circular economy.

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