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Physicochemical and microbiological quality of a fermented soybean beverage: effect of modified cassava starches

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Abstract

Intensive heat treatments of fermented soybean beverages affect their quality, which can be prevented by using modified cassava starches. This study aimed to evaluate the influence of modified cassava starch on the quality properties of a fermented soybean beverage (pH, acidity, soluble solids, syneresis, and microbiological quality) by a sensory acceptance test. The soybean beverage fermentation was carried out using a commercial culture of starter microorganisms and probiotics. Three modified cassava starches were added to the samples: octenyl succinic anhydride (OSA), acetylated distarch adipate (ADA), cross-linked-substituted starch (mixed), and a native starch at 1% concentration. They were stored for 3 weeks at 4ºC. The analyses showed a decrease in pH and an increase in acidity due to post-fermentation processes. The syneresis showed significant differences concerning the sample with native starch, but not among the other treatments with modified starches. The microbiological quality was below the regulatory acceptance limit, and the CFU/mL of probiotics was higher than 106. The treatment with OSA and mixed starch had the highest general acceptance in the sensory test.

Keywords: modified starch; physicochemical properties; syneresis; fermenting microorganisms; probiotics.

Practical Application: The application of modified starches improves the rheological and structural characteristics of the fermented soybean beverage. In the same way, this matrix is suitable as a carrier for fermenting and/or probiotic microorganisms by revealing viable cell quantification greater than 106 (CFU/mL) throughout storage, with possible functional properties that help the development of novel foods with nutritional value to meet the diverse food consumption of consumers.

1 INTRODUCTION

Vegetable beverages have been developed as substitutes for dairy beverages. Soybean beverages are important because of their high nutritional value and wide range of health benefits. However, the presence of anti-nutritional factors in soybean, which in traditionally processed products are reduced or eliminated by treatments, requires more intensive heat treatments to reduce or eliminate these components (Aderibigbe et al., 2021). Despite the reduction and degradation of these anti-nutritional factors, the processed beverage shows a reduction in soybean protein solubility kinetics, which also serves as a quality control for the presence of anti-nutritional factors in soy-based products.

The rheological properties of the processed soybean beverage are corrected by adding hydrocolloids to improve its texture, viscosity, consistency, and syneresis (Yuan & Chang, 2010). The effect of hydrocolloids on the gelling behavior of soybean beverage gels has been extensively studied, and their thickening, stabilizing, and/or gelling properties stand out. The function varies according to the molecular weight, structure, compatibility, gelling profile, dispersion, and hydration conditions, which add value to food products (Wei et al., 2023).

Probiotics are live microorganisms that, when applied in adequate amounts, can improve the host's intestinal microbiota and may confer benefits (≥ 10^6 CFU/g) (Hill et al., 2014; Miremadi et al., 2014). Although the health benefits are known, the mechanisms by which probiotics perform their effects are largely unknown (Fazilah et al., 2018). Likewise, certain strains of the *Lactobacillus* and *Bifidobacterium* genus are capable of producing B complex vitamins and bioactive compounds in plant-based milk substitutes as an alternative to bovine milk (Hou et al., 2000; Y. Y. Zhu et al., 2020).

Fermentation processes in soybean beverages have shown improvements in sensory qualities such as the metabolism of indigestible oligosaccharides responsible for flatulence (raffinose and stachyose) (Li et al., 2014; Y. Y. X. Zhu et al., 2020) and the degradation of compounds accountable for bean flavor (n-hexanal and pentanal) by fermenting and probiotic microorganisms of the *Bifidobacterium* genus (Desai et al., 2002; Zhang et al., 2023). In addition, the metabolism and degradation of these oligosaccharides by probiotics of the *Lactobacillus* genus can help metabolize and degrade these oligosaccharides in fermented legume beverages like

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those made of fermented lentil beverages (Verni et al., 2020). Furthermore, modified starches are considered resistant starch (RS) to gastric digestion and behave similarly to dietary fiber with prebiotic properties (Wang et al., 2023; Wen et al., 2022).

Bifidobacterial species are common and often dominant members of the mammalian gut microbiota (Miremadi et al., 2014). *Lactobacillus* is the main and most diverse group of lactic acid bacteria (LAB). *Bifidobacterium animalis* subsp. *lactis* has a great ability to survive technological processes and has a greater tolerance to oxygen and acids. Moreover, it has been shown to produce several powerful bactericides, which can be used as food preservatives (Hyrslova et al., 2021; Seddik et al., 2017). *Lactobacillus acidophilus* stands out for its ability to survive adverse conditions of the gastrointestinal tract (low pH and bile salt toxicity) (Hill et al., 2014), and its viability during refrigerated storage is greater compared to probiotics of the same genus (Mani-López et al., 2014). Likewise, yogurt is traditionally fermented by combining *Streptococcus thermophilus*—due to its high production capacity of exopolysaccharides—and *Lactobacillus delbrueckii* subsp. *bulgaricus*—which contributes to greater water retention, apparent viscosity, better texture, and lower syneresis (Han et al., 2016). Consequently, the aim was to verify the behavior of physicochemical and microbiological properties during storage, and the sensory acceptability of fermented soybean beverages with modified cassava starches (OSA, ADA, cross-linked-substituted starch) and a native starch at 1% concentration.

2 MATERIALS AND METHODS

2.1 Materials

The raw materials used in the experimentation were soybean seeds obtained from a local market in Bogotá. Modified cassava starches were provided by Poltec SAS, La Estrella, Antioquia, Colombia, i.e., starch with octenyl succinic anhydride substitution (OSA, Gel®Lact), acetylated distarch adipate (ADA, Gel®Cream), and cross-linked-substituted starch (mixed starch, Gel®Lact XP). For fermentation, a freeze-dried starter culture was used for direct inoculation with selected strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, and probiotic microorganisms: *Lactobacillus acidophilus* and *Bifidobacterium animalis* (SACCO Lyofast SYAB 1).

2.2 Preparation of fermented soybean beverages

The soybean beverage was prepared based on the methodology proposed by Rodríguez-Ruiz et al. (2023), and each of the starches was added to the beverages at 1% concentration before homogenization at 160 bars and then pasteurized at 85°C for 10 min. For the fermentation of the beverage, commercial culture of starter and probiotic microorganisms (0.003% w/v) (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Bifidobacterium animalis* subsp. *lactis*, and *Lactobacillus acidophilus*) was inoculated into 100 mL (~4.97 ± 0.03 CFU/mL), activated on an orbital shaker (Thermo Scientific, Solaris 4000), poured into flasks (200 mL), and incubated at 42°C until pH 4.5 (~9 h). Then, it was stored at 4°C for further analysis.

2.3 Proximal analysis of the main components of the fermented soybean beverage

The proximal analysis was carried out in triplicate. The moisture content was determined by drying in a convection oven according to AOAC 32.1.03; the ash by the dry method according to AOAC 923.03; the crude fat (or ethereal extract) from the dry matter using the Soxhlet method, according to AOAC 920.39. Protein was determined according to the Kjeldahl titration method (AOAC 984.13); the total dietary fiber, according to the gravimetric–enzymatic method (AOAC 985.29) (AOAC, 2012); finally, carbohydrates by the difference in components.

2.4 Evaluation of physicochemical properties during storage of treatments

The evaluated quality parameters were pH, titratable acidity, soluble solids, and color. Each one was evaluated in triplicate for 21 days at intervals of 7 days (1, 7, 14, and 21). The pH was evaluated by the potentiometric method (AOAC 981.12). The titratable acidity was determined according to AOAC 947.05 using the volumetric titration method with a standardized 0.1 N sodium hydroxide (NaOH) solution. The results are expressed as a percentage of lactic acid. Syneresis was determined by centrifugation and performed in triplicate using a known sample weight for 10 min at 3,500 rpm at 4°C. The supernatant was weighed and then syneresis was expressed as (g supernatant/sample)* 100 g sample (Joon et al., 2017).

The number of soluble solids (Brix degrees) in the commercial beverage was determined according to AOAC 22.019 using a refractometer calibrated at 20°C. The color was determined using a colorimeter (Chroma Meter CR-400, Konica Minolta, Inc., Japan) on the CIELAB scale for L*a*b parameters. The delta E was also determined (Farnand, 2003).

2.5 Microbiological quality assessment

From the sample, 10 g were taken with a sterile pipette and transferred to a dilution bottle with 90 mL of peptone water solution, which constituted the first dilution. Notably, 1 mL of this dilution was taken and transferred to a tube with 9 mL of peptone water, constituting the second dilution. It was further diluted to 10^{-3}. The dilutions were shaken and each one was subsequently inoculated into boxes and tubes (Brunelle, 2016).

For the count of fungi and yeasts, 1 mL of each dilution was inoculated into boxes, and the PDA culture medium was poured, homogenized, and allowed to solidify. It was then incubated invertedly at 25°C for 5 days. Subsequently, the number of fungal and yeast colonies was counted and multiplied by the inverse of the dilution. The results were reported as CFU/mL (Brunelle, 2016).

Total coliforms were determined from the prepared dilutions and seeded in triplicate in 10 mL of 2% brilliant green bile broth with a Durham hood. They were incubated at 37°C for 24–48 h. At 48 h, the fermentation tubes that produced gas and presented turbidity were recorded as positive.

The positive tubes of the presumptive test were inoculated with a microbiological loop in 2% brilliant green bile broth with a Durham hood. They were incubated for 48 h at 44°C and

the readings were taken from the tubes that produced gas and turbidity. The gas and turbidity formation in the broth confirm the presence of total coliforms. Likewise, for the confirmatory test of fecal coliforms in the positive tubes, tubes of tryptone culture broth with Kovacs reagent were inoculated with a loop. If the latter presents a red ring, it is positive for fecal coliforms. For identification and isolation, streak plating was performed in an EMB culture medium, and characteristic colonies of *Escherichia coli* were selected for biochemical tests (Indol, Voges Proskauer, methyl red, and Simmons citrate). The quantification was performed by the most probable number method from the tubes that tested positive for both total and fecal coliforms (MPN) (Brunelle, 2016).

2.6 Viable cell counts of fermenting microorganisms and probiotics

A representative sample of a fermented beverage was suspended by making a dilution of (1:10) in sterile peptone water and then successive dilutions up to 10^{-7}. *S. thermophilus* counts were made using the pour plate technique on deep seeding in duplicate on M17 agar. The boxes were incubated at 37°C for 48 h, and readings were taken during that time. The quantification of *L. delbrueckii* ssp. *bulgaricus* was performed using Man, Rogosa, and Sharpe (MRS) solid culture medium with pH adjusted to 4.5 and incubated at 37°C for 48 h under anaerobic conditions (Cui et al., 2021). For the enumeration of *Bifidobacterium* sp., dilutions were made using MRS agar spiked with dicloxacillin at a concentration of 2 μg/mL and 0.05% (w/v) L-cysteine. According to the methodology developed by Sozzi et al. (1990), the boxes were incubated under anaerobic conditions at 37°C for 48 h. The quantification of *L. acidophilus* was performed using MRS agar with the addition of 0.1 μg/mL clindamycin and 10 μg/mL ciprofloxacin and incubated anaerobically at 37°C for 48 h (Kõll et al., 2008). All the counts were made using the colony-forming unit (CFU) counting methodology on plates. A duplicate count of fermenting and probiotic microorganism colonies in the beverage was carried out at 1, 7, 14, and 21 days. Data were reported in logarithmic units, CFU/mL, and expressed as the mean ± standard deviation.

2.7 Sensory evaluation

Natural blackberry flavoring and blackberry red coloring (betanin E162) (Falvorix Aromáticos S.A., Madrid, Spain) were added to all the treatments for the sensory test. The consumer acceptance sensory evaluation was applied to 100 participants aged between 18 and 55 years: 45 men and 55 women. Treatments were randomly coded with three digits. The participants were given instructions on how to perform the sensory test and a form asking if they were regular consumers of nondairy fermented beverages, allergic to soy-based products, if they had any disease that affected their senses, or were smokers. The sensory test was based on a hedonic scale from one to seven categorized as follows: (1) I dislike extremely, (2) I dislike very much, (3) I dislike it, (4) I neither like nor dislike, (5) I like it, (6) I like it very much, and (7) I like it extremely. The evaluated parameters were general acceptability, texture, taste, smell, and color.

2.8 Statistical analysis of treatments' quality parameters during storage

The analysis was performed using a completely randomized unifactorial design with blocks (days). As a factor, the type of modified cassava starch was examined by substitution with OSA, ADA cross-linked-substituted starch (mixed), and native starch.

The results were statistically analyzed by analysis of variance (ANOVA) followed by Tukey's multiple comparison test with a significance level of 5%. Likewise, the data analysis reported by fermenting microorganisms and probiotics was obtained in duplicate and analyzed by Tukey's test. Statistical analysis was performed with the Rstudio statistical software and results were expressed as the mean ± standard deviation.

The sensory test was analyzed by the nonparametric Kruskal–Wallis test, taking into account that these are noncontinuous data. The comparison between samples was made by the nonparametric multiple tests for each parameter.

3 RESULTS AND DISCUSSION

3.1 Proximal analysis

According to the reported data (Table 1), there are certainly significant differences ($P < 0.05$) among the treatments. The moisture content reported by the treatment with native starch was similar to the ADA and OSA starch, but different from the mixed starch; however, it was similar among modified starches. Dry matter is correlated with moisture; therefore, they have the same significant differences.

Table 1. Proximal analysis of the four treatments*.

Nutritional information	Native starch	ADA	OSA	Mixed starch
Moisture content	90.45 ± 0.08^{b}	90.34 ± 0.07^{ab}	90.27 ± 0.10^{ab}	90.18 ± 0.10^{a}
Dry matter	9.55 ± 0.08^{b}	9.66 ± 0.07^{ab}	9.73 ± 0.10^{ab}	9.82 ± 0.10^{a}
Ashes $_{(wb)}$	0.20 ± 0.01^{a}	0.22 ± 0.02^{a}	0.20 ± 0.01^{a}	0.22 ± 0.02^{a}
Fat $_{(wb)}$	1.87 ± 0.10^{a}	1.77 ± 0.08^{a}	1.63 ± 0.11^{a}	1.81 ± 0.09^{a}
Protein $_{(wb)}$	1.86 ± 0.05^{b}	1.97 ± 0.02^{a}	1.94 ± 0.03^{ab}	2.00 ± 0.01^{a}
Dietary fiber $_{(wb)}$	0.90 ± 0.00^{b}	0.76 ± 0.00^{d}	0.85 ± 0.00^{c}	0.99 ± 0.00^{a}
Carbohydrates $_{(wb)}$	4.72 ± 0.13^{b}	4.95 ± 0.14^{ab}	5.12 ± 0.16^{a}	4.81 ± 0.02^{ab}

*The means of properties with a common letter in a row do not differ significantly at a significance level α = 0.05, according to Tukey's HSD test.

Regarding ash and fat, there was no significant difference among treatments. The protein content was lower in the samples with native starch, and it was higher in the treatments with ADA and mixed starch. The carbohydrate content in the native starch treatment was similar to the ADA and mixed starch but different from the OSA starch. Nevertheless, it was similar between modified starches. In the literature, there are various compositions of both fresh and fermented soybean beverages, with and without extra additives, with higher or lower moisture (Liu et al., 2023). The nutritional importance of soybean beverages is mainly due to being a source of protein; however, in the present study, the protein was lower than that reported in the literature (Barco Coro, 2017; Lee et al., 2015).

The highest dietary fiber value corresponded to the mixed starch sample. It was higher than that reported in the literature (Leon & Joseline, 2017). This is because modified starch behaves like dietary fiber, although starch is usually hydrolyzed by digestive enzymes such as amylase. However, not all starches are digested and absorbed in the intestine. Modified starches resist gastrointestinal digestion and are fermented in the colon in a similar way to dietary fiber.

3.2 Physicochemical properties

The pH is affected throughout the storage mainly by post--fermentative factors. The microorganisms are in a latent phase with a low metabolism at 4ºC, the storage temperature of the product, but over time it is possible to acidify the medium. The titratable acidity is inverse to the pH content, i.e., while the pH decreases, the titratable acidity increases (Vila-Real et al., 2022). This change is due to the anaerobic metabolism of lactic acid microorganisms and to bifidobacteria that transform matrix sugars into lactic acid.

The syneresis behavior of treatments throughout storage is shown in Table 2. Regarding comparisons between treatments, both ADA and OSA starches had similar values and were the ones that presented less syneresis. The treatment with native cassava starch presented the highest syneresis at the end of the product's storage due to the native starch retrogradation. This phenomenon occurs from the decrease in the gelatinized starch temperature, which produces the insolubilization and spontaneous precipitation of the amylose molecules (Altuna et al., 2018; Drunkler et al., 2012).

Modified starches can be more stable over storage because certain chemical modifications interact through different molecular forces between different chemical species in the matrix. For instance, ADA starch can hold water, avoiding retrogradation. It is mainly attributed to the amylose and amylopectin reorganization in a network and the presence of native oligosaccharides in soybeans, as well as bonds arranged by added sugars. ADA starch delays starch retrogradation and provides a longer shelf life, being stable at low-temperature storage and low pH with adverse effects on retrogradation (Zhang et al., 2020).

The physical properties of OSA-modified starch particles affect the ability to effectively stabilize O/W emulsions (Sweedman et al., 2013). Due to the chemical structure with hydrophobic and hydrophilic residues, the molecule has amphiphilic characteristics that weaken the internal hydrogen bonds of the starch particle (Ovando-Martinez et al., 2017). OSA starch gels have also been found to be softer than native starch gels, and OSA-modified starch particles have been shown to aggregate less than native starch (Ovando-Martinez et al., 2017).

Table 2. Changes in pH, titratable acidity, soluble solids, and syneresis of fermented soybean beverage with different types of starch during storage at 4ºC for 21 days*.

Parameter	Treatment	Days			
		1	7	14	21
pH	ADA	$4.62 \pm 0.01^{a\,A}$	$4.57 \pm 0.00^{b\,A}$	$4.43 \pm 0.00^{c\,A}$	$4.15 \pm 0.01^{d\,A}$
	OSA	$4.61 \pm 0.01^{a\,A}$	$4.53 \pm 0.00^{b\,B}$	$4.42 \pm 0.00^{c\,A}$	$4.13 \pm 0.01^{d\,B}$
	Mixed	$4.57 \pm 0.01^{a\,B}$	$4.56 \pm 0.00^{a\,A}$	$4.39 \pm 0.00^{b\,B}$	$4.11 \pm 0.01^{c\,C}$
	Native	$4.56 \pm 0.01^{a\,B}$	$4.50 \pm 0.00^{b\,C}$	$4.33 \pm 0.01^{c\,C}$	$4.20 \pm 0.01^{d\,D}$
Titratable acidity (%)	ADA	$0.33 \pm 0.01^{a\,A}$	$0.37 \pm 0.01^{b\,A}$	$0.40 \pm 0.01^{c\,A}$	$0.62 \pm 0.01^{d\,B}$
	OSA	$0.35 \pm 0.00^{a\,A}$	$0.39 \pm 0.00^{b\,A}$	$0.40 \pm 0.01^{b\,A}$	$0.67 \pm 0.02^{c\,A}$
	Mixed	$0.34 \pm 0.01^{a\,A}$	$0.36 \pm 0.01^{a\,A}$	$0.43 \pm 0.01^{b\,A}$	$0.66 \pm 0.02^{c\,A}$
	Native	$0.34 \pm 0.01^{a\,A}$	$0.38 \pm 0.00^{b\,A}$	$0.42 \pm 0.02^{c\,A}$	$0.56 \pm 0.02^{d\,C}$
Soluble solids (°Brix)	ADA	$10.2 \pm 0.0^{a\,D}$	$10.0 \pm 0.0^{b\,C}$	$10.1 \pm 0.1^{a\,B}$	$10.0 \pm 0.1^{b\,A}$
	OSA	$10.7 \pm 0.0^{a\,A}$	$10.5 \pm 0.0^{b\,A}$	$10.4 \pm 0.0^{b\,A}$	$9.9 \pm 0.1^{c\,B}$
	Mixed	$10.5 \pm 0.1^{a\,B}$	$10.2 \pm 0.0^{b\,B}$	$10.1 \pm 0.0^{b\,B}$	$9.5 \pm 0.1^{c\,C}$
	Native	$10.6 \pm 0.0^{a\,C}$	$10.3 \pm 0.0^{b\,B}$	$10.1 \pm 0.0^{c\,B}$	$10.2 \pm 0.1^{b\,D}$
Syneresis (%)	ADA	$34.13 \pm 1.83^{a\,AB}$	$35.89 \pm 1.42^{a\,A}$	$39.37 \pm 0.97^{ab\,A}$	$42.52 \pm 1.15^{b\,A}$
	OSA	$33.61 \pm 1.88^{a\,B}$	$37.73 \pm 1.86^{ab\,A}$	$41.34 \pm 0.78^{bc\,AB}$	$42.73 \pm 0.55^{c\,A}$
	Mixed	$36.56 \pm 1.80^{a\,AB}$	$42.08 \pm 2.17^{b\,B}$	$45.06 \pm 2.08^{b\,BC}$	$46.55 \pm 0.74^{b\,A}$
	Native	$38.11 \pm 1.74^{a\,A}$	$43.08 \pm 1.39^{b\,B}$	$49.43 \pm 0.16^{c\,C}$	$56.40 \pm 1.02^{d\,B}$

*a–d superscripts of means by days (row) with significant differences ($P < 0.05$). A–D superscripts of means by treatment (column) with significant differences ($P < 0.05$).

3.3 Color

Changes in CIELab color values are shown in Table 3. None of the treatments had significant changes in lightness (L*) throughout storage. The a* values tended to be on the scale of slightly green tones as they had a negative value. No change was observed in the a* value during storage for each treatment, but significant differences were observed between the treatments with ADA and OSA starch compared to the native starch evaluated on the first day. Regarding the b* value, it tended toward yellow tones due to the natural pigment in soybeans and presented significant differences during storage among the treatments with ADA, mixed, and native starch; but there were no changes in the one with OSA starch.

The delta E value—which is the difference in color perception—was compared between treatments throughout the storage (Table 3). Delta E > 3 is differentiable to the untrained human eye; however, the difference in color perception depends on the observer and it can be more or less. There were no color differences between the ADA and OSA treatments during storage, while the comparison between the other treatments with at least one day of storage shows differences in color perception.

3.4 Microbiological quality

The sanitary regulation that best adjusts to the product obtained is Resolution 2310 of 1986, which is aimed at dairy products. A regulation on fermented plant-based products is needed in Colombia. For fermented milk, the microbiological acceptability limit for total coliforms is ≤ 20 (Table 4). According to the information provided by the Resolution, all the treatments comply with quantification of < 20 CFU/mL inclusive of the end of the storage time (21 days), and no fecal coliforms were reported. Moreover, the acceptability limit for fungi and yeasts (Table 4) must be ≤ 500 CFU/mL. Despite all the treatments meeting this limit in the first week, at the end of storage, the treatments with ADA starch reported values above 1,000 CFU/mL; therefore, the product would be withdrawn.

3.5 Count of LAB and probiotics during storage

The general count of LAB includes the quantification of fermenting and/or probiotic microorganisms; the general count of viable cells was above 8 (log CFU/mL). The count of *Lactobacillus delbrueckii* ssp. *bulgaricus* was relatively similar between treatments except for the treatment with mixed starch, which shows a much lower viable cell count than the other treatments (Figure 1A). Regarding the count of *L. delbrueckii* ssp. bulgaricus during the first week, it kept the microbial population constant, but there was a reduction of viable cells at the end of the third week in all treatments. The *Streptococcus thermophilus* viable cell count varied in each treatment and there was a reduction in the general count in treatments with modified starches during storage, whereas an increase in the number of viable cells was observed in the native starch (Figure 1B).

Table 3. Color change in the CIELab system of the fermented soybean beverage with the different types of starch during storage at 4ºC for 21 days**.

CIELab	Days				
	Treatment	1	7	14	21
L*	ADA	76.40 ± 1.21$^{a\ A}$	74.69 ± 2.20$^{a\ A}$	75.38 ± 2.68$^{a\ A}$	77.36 ± 2.97$^{a\ A}$
	OSA	75.63 ± 2.69$^{a\ A}$	76.84 ± 2.58$^{a\ A}$	76.69 ± 2.39$^{a\ A}$	76.28 ± 0.53$^{a\ A}$
	Mixed	73.05 ± 2.21$^{a\ A}$	70.65 ± 2.90$^{a\ A}$	72.19 ± 2.67$^{a\ A}$	73.99 ± 1.59$^{a\ A}$
	A. native	75.96 ± 2.21$^{a\ A}$	75.65 ± 2.41$^{a\ A}$	74.11 ± 0.37$^{a\ A}$	72.28 ± 0.06$^{a\ A}$
a*	ADA	-0.47 ± 1.35$^{a\ A}$	-0.50 ± 0.17$^{a\ A}$	-0.36 ± 0.06$^{a\ A}$	-1.64 ± 0.11$^{a\ A}$
	OSA	-0.11 ± 0.79$^{a\ A}$	-0.27 ± 0.46$^{a\ A}$	-1.31 ± 0.09$^{a\ A}$	-1.19 ± 0.47$^{a\ A}$
	Mixed	-0.86 ± 1.14$^{a\ AB}$	-1.76 ± 0.01$^{a\ A}$	-1.55 ± 0.10$^{a\ A}$	-1.81 ± 0.12$^{a\ A}$
	Native	-2.28 ± 0.72$^{a\ B}$	-1.38 ± 0.37$^{a\ A}$	-0.94 ± 0.09$^{a\ A}$	-1.17 ± 0.07$^{a\ A}$
b*	ADA	14.88 ± 0.80$^{a\ AB}$	11.39 ± 0.46$^{b\ B}$	9.95 ± 1.68$^{b\ A}$	11.71 ± 0.47$^{b\ A}$
	OSA	13.15 ± 1.25$^{a\ B}$	10.79 ± 0.82$^{a\ B}$	12.20 ± 0.03$^{a\ A}$	11.59 ± 0.10$^{a\ A}$
	Mixed	16.31 ± 1.53$^{a\ A}$	9.73 ± 1.43$^{b\ B}$	11.20 ± 0.32$^{b\ A}$	12.62 ± 0.52$^{b\ A}$
	Native	14.90 ± 1.94$^{a\ AB}$	15.11 ± 1.51$^{a\ A}$	10.79 ± 0.38$^{b\ A}$	11.64 ± 0.06$^{b\ A}$
Delta E*	ADA–OSA	1.93	2.24	2.77	1.18
	ADA–mixed	3.66	4.55	3.63	3.49
	ADA–native	1.86	3.94	1.63	5.10
	OSA–mixed	4.15	6.45	4.62	2.59
	OSA–native	2.81	4.62	2.96	4.00
	Mixed–native	3.53	7.35	2.06	2.07

**a–b superscripts of means per row (days) with significant differences ($p < 0.05$). A–B superscripts of means per column (treatments) with significant differences ($p < 0.05$).

According to Resolution 11961 of 1989 on fermented milk with *Bifidobacterium* sp., the count must be higher than 105 CFU/mL to be considered a product with functional effects. In contrast, the probiotic strains (*Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis)* showed viable cell counts higher than 6 (log CFU/mL), i.e., higher than what the regulatory framework indicates to consider it a product with probiotics. These are the minimum count requirements to achieve a probiotic effect. For bifidobacteria, the treatment with the highest viable cell count during storage was the one with mixed starch, with an increasing behavior. The other treatments did not show predictable trends, but it stands out that, in general, the bifidobacteria in the food matrix grew above 8 (log CFU/mL) (Figure 1C). Regarding *Lactobacillus acidophilus*, the viable cell count was much lower compared to bifidobacterial: less than 7.4 (log CFU/mL). The treatment with the highest number of viable cells was the one with OSA starch, while the treatment with the lowest number of viable cells was the mixed one (Figure 1D).

According to the results, the bifidobacterial count was much higher than 8 (log CFU/mL) for the probiotic *L. acidophilus*. This result was possibly due to the metabolic capacity of most bifidobacterial; they contain the enzyme α-galactosidase, which is responsible for hydrolyzing oligosaccharides present in soybeans, such as raffinose and stachyose, as a carbohydrate source (Desai et al., 2002; Tabasco et al., 2007; X. Zhu et al., 2020). Raffinose and stachyose can be used as a culture medium for selective quantification of viable *Bifidobacterium* cells (Desai et al., 2002). Likewise, probiotics can make simple sugars bioavailable to lactobacilli, which in turn provide peptides for probiotics via proteolytic enzymes to promote a symbiotic relationship between strains (Cui et al., 2021; X. Zhu et al., 2020). Therefore, native soybean oligosaccharides act as prebiotic fiber that helps the gastrointestinal system stimulate the growth of probiotic bacteria and gives functional properties for the protection, care, and well-being of the digestive system (Fazilah et al., 2018).

Table 4. Total coliform count for each treatment by most probable number (MPN) method. Count of fungi and yeasts for each treatment by plate count method.

Día	Treatment	10^{-1}	10^{-2}	10^{-3}	NMP	(Fungi and yeast) CFU/mL
2	ADA	1	1	0	7.4	450
	OSA	0	1	2	9,2	270
	Mixed	2	0	0	9.2	163
	Native	0	0	0	0	88
21	ADA	1	1	1	11	1050
	OSA	2	1	1	20	157
	Mixed	1	1	1	11	280
	Native	1	0	1	7.2	210

Verni et al. (2020) demonstrated that the fermentation of a lentil beverage from different species of the genus *Lactobacillus*, including *L. acidophilus*, resulted in the degradation of sucrose, fructose, and glucose, as well as oligosaccharides typical of the lentil (raffinose, stachyose, and verbascose). However, the *L. acidophilus* bacterium in this study had a lower growth rate, below 7.2 (log CFU/mL). This result may be because *L. acidophilus* possibly requires a specific consortium of LAB for the degradation and optimal growth of this bacterium.

From another point of view, the quantification of probiotics of both *B. animalis* and *L. acidophilus* may be underestimated due to the use of antibiotics for the selective growth of these bacteria. Even though, Sozzi et al. (1990) guarantee a quantification of bacteria of the genus *Bifidobacterium* with the antibiotic dicloxacillin and a method for the quantification of *L. acidophilus* with clindamycin (Cui et al., 2021), there is always a risk that the quantification is lower than the real one, so it would be recommended to use other specific culture media as a comparison method.

3.6 Sensory analysis

A sensory test of general acceptability, texture, smell, taste, and color was carried out using a nonparametric Kruskal–Wallis analysis (Table 5). The native starch had a low acceptability score. Regarding texture, both the treatment with OSA starch and the mixed one had a higher texture score than that of the native starch. The consumers expressed that the samples with native starch had a very fluid and not a creamy mouthfeel, while the samples with OSA and mixed starch were very creamy, with body and texture. Additionally, most of the consumers expressed that the samples with ADA starch were very fluid. Regarding the smell and color attributes, there were no significant differences among treatments. Regarding the flavor attribute, the native starch sample was judged for a rather acidic and fermented taste, related to unpleasant flavors, while the OSA and mixed starches were rated as pleasant with a dairy flavor or similar to dairy beverages. ADA starch had a neutral dairy connotation, with a lack of flavor or increased sweetness, although the same sweetener concentration and the same blackberry flavoring were used in all treatments to mask the characteristic soybean flavor.

Consumers who performed the sensory evaluation were familiar with fruit-flavored yogurt, but not with plant-based fermented lactic acid beverages. These data agree with Santos et al. (2019), who reported that due to demand, they are commercially dominated by colorful and sweet yogurts or fermented

Table 5. Acceptability averages according to the score expressed by consumers*.

Parameters	Native	ADA	OSA	Mixed
Acceptance	4.5 ^{a}	5.1 ^{b}	5.5 ^{b}	5.5 ^{b}
Texture	4.6 ^{a}	5.0^{ab}	5.2 ^{b}	5.3 ^{b}
Smell	4.7 ^{a}	5.1 ^{a}	5.2 ^{a}	5.1 ^{a}
Flavor	4.5 ^{a}	5.0^{a}	5.4 ^{b}	5.3 ^{b}
Color	5.4 ^{a}	5.5 ^{a}	5.4 ^{a}	5.6 ^{a}

*The means of treatments with a common letter in a column do not differ significantly at a significance level $\alpha = 0.05$, according to the Kruskal–Wallis test.

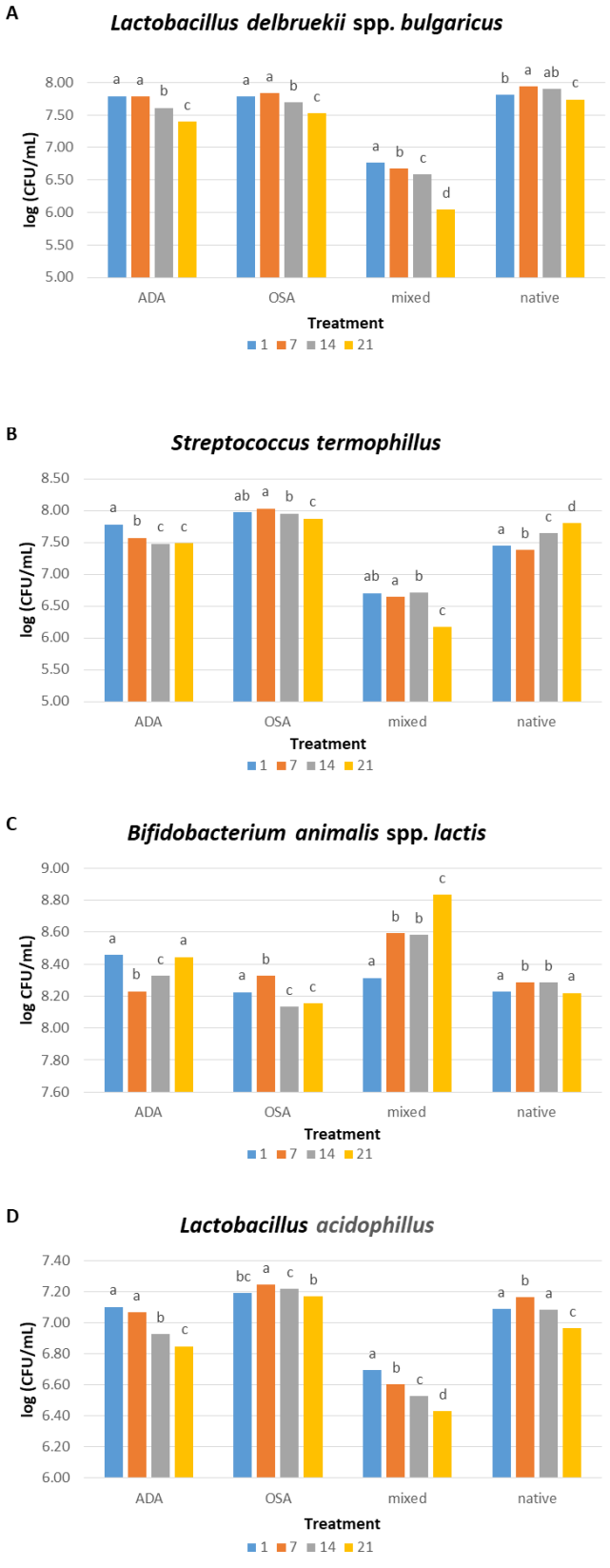

Figure 1. The viable cell concentrations of (A) *Lactobacillus delbrueckii* ssp. *bulgaricus,* (B) *Streptococcus thermophilus*, (C) *Bifidobacterium animalis* subsp. *lactis,* and (D) *Lactobacillus acidophilus* for each treatment during storage.

milk drinks, which are preferred by consumers. Therefore, some fermented soybean beverage sensory properties, such as the appearance, color, smell, and even the intensity of the blackberry flavor, may have been underestimated or confused by consumers not used to its sensorial characteristics.

4 CONCLUSIONS

The pH decreased and the titratable acidity increased during the storage of all treatments due to post-fermentative processes. Soluble solids were relatively stable for the ADA and native starch treatments. The treatments with ADA, OSA, and mixed starch had no differences in syneresis at the end of storage, while the native starch had an increase in syneresis due to the starch retrogradation, thus forming lumps. The color (delta E) did not differ between ADA and OSA starch during storage. The LAB viable cell counts are significantly high for all treatments during storage, as well as for probiotics (*B. animalis* subsp. *lactis* and *L. acidophilus*), higher than 6 log CFU/mL; thus, it is considered a product with probiotic properties. Sensory analysis showed high acceptance of the OSA treatment; however, the treatment with mixed starch had a similar acceptance score among consumers.

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