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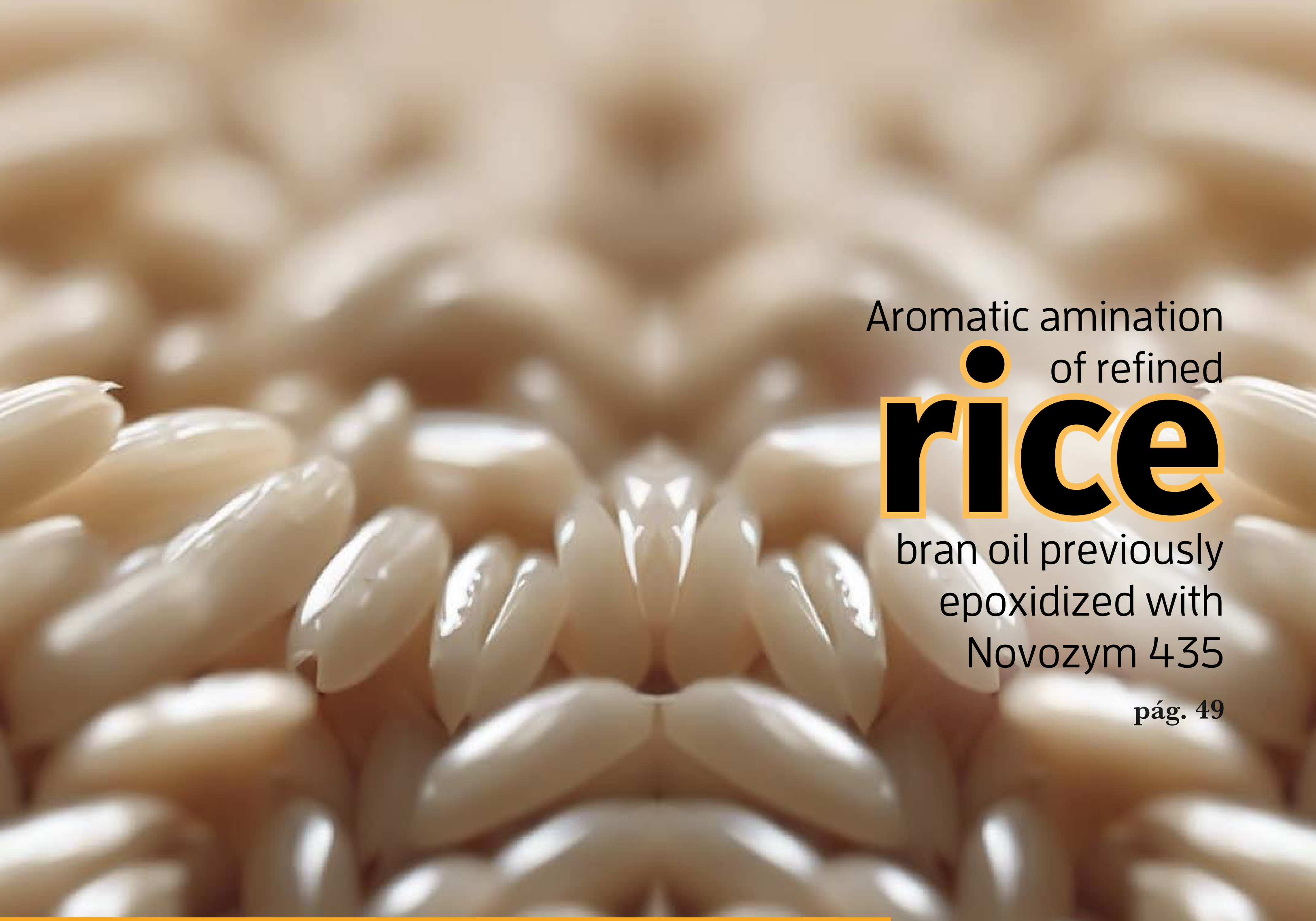

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Colegio de Postgraduados

Antioxidant properties of soy-dairy milk blends fermented with probiotics

De Jesús-José, Edgar[1†]; Hidalgo-Fuentes, Belén[1†]; Rosas-Espejel, Monzerrat[2]; Rayas-Amor, Adolfo A.[2]; Jiménez-Guzmán, Judith[2]; Zambrano-Zaragoza, María L.[3]; González-Reza, Ricardo M.[3]; Liceaga, Andrea M.[4]; Aguilar-Toalá, José E.[2*]

[1] Licenciatura en Ciencia y Tecnología de Alimentos, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana, Unidad Lerma. Av. de las Garzas 10. Col. El Panteón, Lerma de Villada 52005, Estado de México, Mexico.
[2] Departamento de Ciencias de la Alimentación, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana, Unidad Lerma. Av. de las Garzas 10. Col. El Panteón, Lerma de Villada 52005, Estado de México, Mexico.
[3] Laboratorio de Procesos de Transformación y Tecnologías Emergentes de Alimentos-UIM, FES-Cuautitlán, Universidad Nacional Autónoma de México, Cuautitlán Izcalli, Estado de México 54714, México.
[4] Protein Chemistry and Bioactive Peptides Laboratory, Purdue University, 745 Agriculture Mall, West Lafayette, IN, 47907, USA.
*- Correspondence: j.aguilar@correo.ler.uam.mx
† These authors contributed equally to this work.

ABSTRACT
Objective: Evaluate the effect of the substitution of cow milk with soy beverage on the antioxidant properties, physicochemical parameters, and sensory quality of the probiotic and conventional fermented beverages.
Design/methodology/approach: Different combinations of soy beverage (T1=80%, T2=60%, T3=40%, and T4=20%) with cow milk (20%, 40%, 60%, and 80%, respectively) were fermented with either conventional or probiotic cultures. The antioxidant activity of fermented beverages was evaluated by DPPH method and the samples were also characterized for protein, fat, solids non-fat, density, and acidity. Sensory evaluation was done in order to determine the acceptability of the fermented beverages.
Results: Overall, most treatments fermented with the probiotic culture showed higher ($P<0.05$) antioxidant capacity compared to those fermented with the conventional culture. In contrast, for both starter cultures, it was observed that the T1 treatment displayed the highest ($P<0.05$) antioxidant activity compared with the other treatments (T2, T3, and T4). Similarly, the treatment T1-probiotic culture was the most preferred, being the aroma and appearance, the sensory properties scored with the highest degree of liking.
Study Limitations: Follow-up research is needed to identify the bioactive compounds responsible for antioxidant properties exhibited by fermented soy-dairy milk beverages.
Findings/conclusions: Probiotic cultures can be used to generate soy-dairy milk fermented beverages with noticeable antioxidant and sensory properties.

Keywords: probiotic; fermentation; antioxidant; sensory acceptability; plant-based beverage.

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INTRODUCTION

In recent years, plant-based beverages have gained more attention from the food industry and consumers because they are a good alternative to cow milk, especially for people with lactose intolerance, milk allergies, and prevalence of hypercholesterolemia

(Sethi, Tyagi, & Anurag, 2016). Apart from the foregoing, many consumers are looking for a more sustainable diet with a plant-based lifestyle (*i.e.*, vegetarianism), growing ethical concerns about animal welfare, and the negative environmental impacts associated with dairy production (Mongi & Gomezulu, 2022; Penha, Santos, Speranza, & Kurozawa, 2021).

Among the plant-based beverages available in the market, soy-based beverages are the most consumed because of their nutritive value, especially their higher protein content, and lower price (Sethi *et al.*, 2016; Siddiqui *et al.*, 2022). Despite these advantages, soy-based beverages are characterized for presenting an unpleasant beany off-flavor and grassy aroma, which are generated during their production (B. Wang *et al.*, 2021). Some strategies have been used in order to improve the sensory profile of soy-based beverages including their fermentation and mixing with two or more materials (e.g., plant-based or non-plant-based) (Montemurro, Pontonio, Coda, & Rizzello, 2021; Silva, Silva, & Ribeiro, 2020). These strategies can improve the sensory properties and nutritional composition of the resulting beverage, and also offer the opportunity to develop functional foods with health benefits because the generation of bioactive compounds and the inclusion of probiotics (Marsh, Hill, Ross, & Cotter, 2014). To the best of our knowledge, there are no previous studies reported on the antioxidant activity of fermented soy-dairy milk blends. Thus, the aim of this work was to evaluate the effect of the substitution of cow milk with soy beverage on the antioxidant properties, physicochemical parameters, and sensory quality of the probiotic fermented beverages.

MATERIAL AND METHODS

Materials

Soy-based commercial beverage (Ades, Cola-Cola®) and comercial whole cow milk (Lala®) were obtained at local store in Toluca, state of Mexico, Mexico. Commercial starter cultures (as freeze-dried powders) were obtained from Vivolac Culture Corporation (Greenfield, IN, USA). Chemicals used for the analysis were of analytical grade obtained from Sigma-Aldrich.

Preparation of soy-dairy milk fermented blends

The conventional mixed starter culture for yogurt (Yogurt Dri-Set 442, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus*) and probiotic yogurt culture (Bioflora Dri-Set ABY 438, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium* ssp.) were activated (0.2% w/v) in commercial pasteurized cow milk under sterile conditions and incubated at 42 °C for 6 h to obtain stock cultures. Different blends of soy-based beverage with cow milk were fermented, which are shown in Table 1. The stock cultures were added (5% v/v) aseptically to 100 mL of pasteurized soy-dairy milk blends in 120-mL sterile bottles. Fermentation was complete when the pH value reached at least 4.2 (5 h), then samples were stored at 4 °C, and all the analyses were performed within 24 h.

Table 1. Experimental combinations of the substitution of cow milk with soy beverage for the production of fermented beverages.

Treatment codes	Soy-based beverage (%)	Dairy (cow) milk (%)
T1	80	20
T2	60	40
T3	40	60
T4	20	80

Physicochemical analysis

The fermented soy-dairy milk blends were analyzed for protein, fat, solids non-fat, and density through milk analyzer device Milkotester Master Eco (Milkotester Ltd, Belovo, Bulgaria). A calibrated digital pH meter was used to determine the pH values of the samples and titratable acidity was measured by titrating the samples with 0.1 N NaOH solution according to the NOM-243-SSA1-2010. All analyses were carried out in triplicate.

Determination of antioxidant activity

Assessment of the antioxidant activity of fermented soy-dairy milk blends was carried out using the DPPH method (Centenaro *et al.*, 2014). A working solution of DPPH (0.1 mM) in 80% ethanol was prepared. A volume of 1000 μL of DPPH in ethanol was added to 2000 μL of diluted soy-dairy milk fermented blends samples (2% v/v), well vortexed and incubated for 30 min in the dark at room temperature. Trolox (6-hydroxy-2,3,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich, St. Louis, MO, USA) was used as a standard to prepare a reference curve (20 to 250 μM). The results were expressed as micromoles of Trolox equivalents.

Sensory analysis

Two sensory analysis tests were performed on soy-dairy milk blends fermented with conventional and probiotic cultures, 2 days after the production of the samples. Regular consumers of fermented dairy products were recruited at the Metropolitan Autonomous University - Campus Lerma. Panelist pool consisted of university students, lecturers, and employees, aged between 18 and 50 years, 54% female and 46% male. For the sensory tests, first, a preference ranking test with the participation of 22 panelists was applied in order to select the best beverage. Next, the most preferred beverage was evaluated by panelists (N=71) for overall acceptance using a 9-point hedonic scale (from 1="disliked extremely" to 9="liked extremely") evaluating aroma, appearance, flavor, sweetness, and overall liking. Purchase intent, using a 5-point scale (from 5="definitely would buy" to 1="definitely would not buy") was also asked. Panelists were given water and unsalted crackers to cleanse their palate in between samples.

Statistical analysis

The statistical analysis of experimental data was made using ANOVA followed by Tukey's test to compare the results among treatments or Student's.

unpaired t-test to compare the results between the type of starter culture used. Differences were considered to be significant when P<0.05. Ranking data of the sensory analysis was analyzed using the Friedman test. All analyses were performed using the Minitab software version 19.1 (Minitab Inc., Pennsylvania, USA). Each experiment was repeated three times.

RESULTS AND DISCUSSION

Physicochemical characterization

The fat, solids non-fat, density, and protein content of the samples were not significantly different (P>0.05) among treatments for each culture. However, particularly the acidity showed statistical difference (P<0.05) among treatments for each culture, being higher with the increase of the proportion of dairy milk in the treatments. This indicates that lactose, which is converted into organic acids, mainly lactic acid, influences the gel formation (consistency) in fermented milks (Moreno-Montoro *et al.*, 2018). In contrast, in most parameters, there was no difference (P>0.05) between the type of starter culture for each treatment, except for the treatment T4, in which the content of solids non-fat,

Table 2. Physicochemical characterization of fermented soy-dairy milk blends.

Parameter	Treatments	Type of starter culture	
		Conventional	Probiotic
Fat	T1	2.40±0.1 a, A	2.53±0.92 a, A
	T2	2.40±0.1 a, A	2.90±1.45 a, A
	T3	2.63±0.66 a, A	2.06±0.75 a, A
	T4	2.80±0.87 a, A	2.46±0.11 a, A
Solids non-fat	T1	7.83±1.80 a, A	6.83±2.15 a, A
	T2	7.70±1.70 a, A	6.80±1.74 a, A
	T3	7.50±1.12 a, A	7.33±1.97 a, A
	T4	7.43±1.05 a, A	8.9±0.81 a, B
Density	T1	27.63±2.83 a, A	30.37±2.80 a, A
	T2	33.70±6.70 a, A	33.03±7.10 a, A
	T3	31.90±7.75 a, A	31.47±6.21 a, A
	T4	26.10±3.75 a, A	36.63±4.51 a, B
Protein	T1	3.96±1.15 a, A	3.20±0.40 a, A
	T2	3.76±0.56 a, A	3.33±0.83 a, A
	T3	3.50±0.43 a, A	3.6±0.40 a, A
	T4	3.16±0.75 a, A	3.8±0.60 a, B
Acidity	T1	0.23±0.004 a, A	0.21±0.012 a, A
	T2	0.31±0.011 b, A	0.30±0.012 b, A
	T3	0.33±0.005 c, A	0.32±0.004 c, A
	T4	0.35±0.009 d, A	0.33±0.005 c, A

Values are mean±standard deviation of three replicates. Different lowercase letters (a-d) indicate statistical difference (P<0.05) among treatments for each culture (per column). Different uppercase letters (A,B) indicate statistical difference between type of starter culture for each treatment (per row). Treatments codes are defined in Table 1.

density, and protein were higher ($P<0.05$) in the probiotic culture sample compared to the conventional culture. This could be explained by the microbial activity of the probiotic culture, which is more active than conventional cultures; thus, more production/release of metabolites is expected during their growth in the fermented food matrix (Conway, 1996; Salminen *et al.*, 1998). However, further studies using more sensitive and robust methods (*e.g.*, official and reference methods) such as Kjeldahl (protein content) and Soxhlet (fat content) for physicochemical characterization of soy-dairy milk blends should be done.

Antioxidant activity

Dietary antioxidants are crucial in the prevention of the production of reactive oxygen species and help to reinforce the organism protection mechanism against oxidative stress (Ponnampalam *et al.*, 2022). Our Results (Table 3) show that all fermented soy-dairy milk blends were able to exhibited antioxidant activity ranging from 102.55 to 192.75 μM of Trolox for those fermented with the conventional culture and from 107.45 to 270.20 μM of Trolox for those fermented with the probiotic culture. Overall, most treatments fermented with the probiotic culture showed higher ($P<0.05$) antioxidant capacity compared to those fermented with conventional culture. In contrast, for both starter cultures it was observed that the treatments T1 was those that displayed the highest ($P<0.05$) antioxidant activity followed by T2 compared with the other treatments (T3 and T4). For example, in probiotic fermented soy-dairy milk blends, treatment T1 and T2 showed up to 2.5 times more antioxidant activity than the other two treatments; where the greater the substitution of cow's milk for soy, the greater the antioxidant capacity.

It has been reported that different bioactive components are responsible for the antioxidant activity of both fermented dairy milk and fermented soy-based beverages. For example, fermented dairy milk has bioactive compounds occurring naturally or as a result of microbial activities during the fermentation such as bioactive peptides, exopolysaccharides, fatty acids, organic acids, vitamins, and γ-aminobutyric acid (GABA), which have demonstrated to exhibit antioxidant properties (Fardet & Rock, 2018; Stobiecka, Król, & Brodziak, 2022). In contrast, some studies have shown that fermented soy-based beverages had bioactive compounds with antioxidant properties, mainly phytochemicals such as

Table 3. Antioxidant activity (μM of Trolox) of soy-dairy milk blends fermented with conventional and probiotic cultures.

Treatments	Type of starter culture	
	Conventional	Probiotic
T1	192.75±4.49 a, A	270.20±3.40 a, B
T2	153.53±16.38 b, A	210.39±8.49 b, B
T3	151.57±26.36 b, A	114.31±28.87 c, A
T4	102.55±16.98 c, A	107.45±16.98 c, A

Values are mean±standard deviation of triplicate determinations. Different lowercase letters (a-d) indicate statistical difference ($P<0.05$) among treatments for each culture (per column). Different uppercase letters (A,B) indicate statistical difference between type of starter culture for each treatment (per row). Treatments codes are defined in Table 1.

polyphenols, isoflavone aglycones (*e.g.*, daidzein, genistein, and glycitein), and flavonoids. Moreover, others studies have found that during fermentation, other compounds are present and/or produced including bioactive peptides, and GABA, that exhibit antioxidant properties (de Queirós *et al.*, 2020; Sanjukta, Rai, Muhammed, Jeyaram, & Talukdar, 2015). In addition, it has been found that whole cells of lactic acid bacteria and probiotics possess antioxidant properties (Feng & Wang, 2020; Y. Wang *et al.*, 2017). Thus, with this in mind it is plausible that some of these compounds are present in the fermented soy-dairy milk blends used in this study. According to our results, the antioxidant activity of the samples was higher with the increase of the proportion of soy-based beverage in the fermented blends, which suggested that mainly the compounds produced during the fermentation are derived from soy and are the main ones responsible for the antioxidant properties. However, further studies aimed at identifying of these bioactive compounds responsible for the antioxidant activity exhibited by the samples are necessary.

In similar studies, it has been reported the antioxidant activity of either soy-based beverage or fermented milks, but not on fermented beverages consisting of soy-dairy blends. For example, Tonolo *et al.* (2019) and Marazza, Nazareno, de Giori, and Garro (2012) reported DPPH radical scavenging around 30% of fermented soy-based beverages. On the other hand, Csatlos *et al.* (2023) found values of antioxidant activity of fermented soy-based beverage added with *Chlorella vulgaris* ranging from 301 to 497 μM of Trolox, which are higher that our results. However, these could be due to the addition of Chlorella vulgaris powder.

The comparison of our results from different studies is difficult as the different methods were used or the way the results are presented; our results indicate that fermented soy-dairy milk blends are source of dietary antioxidants with potential health benefits related with this bioactivity. Nevertheless, further work is necessary to carry out *in vivo* studies to test its antioxidant effect in order to identify novel bioactive compounds responsible for such benefit.

Sensory analysis

Overall, it was observed that with increased proportion of soy-based beverage in the treatments, the preference was also increased (Figure 1). The results of total rank sum obtained by Friedman's test show that the treatment T1 (80% soy, 20% dairy milk), for both types of starter cultures, was the most preferred ($P<0.05$) by consumers according to preference-ranking test. Thus, T1-probiotic culture was selected for further sensory evaluation in the acceptance test.

The acceptance test (degree of liking, DOL) indicated that the aroma and appearance were the sensory properties scored the highest (DOL>6.4), whereas flavor and sweetness were the sensory properties scored the lowest (DOL<5.0). In contrast, the overall liking was >5.0. However, it could be noted that the fermented samples were produced without the addition of additives (*e.g.*, sweeteners, flavoring agents, etc.), which could improve their sensory characteristics and improve their overall acceptability. In a similar study, Otolowo, Omosebi, Araoye, Ernest, and Osundahunsi (2022) reported that yoghurt samples prepared with soy-dairy milk blends showed scores ranging from 6 to 8 for color, consistency, aroma,

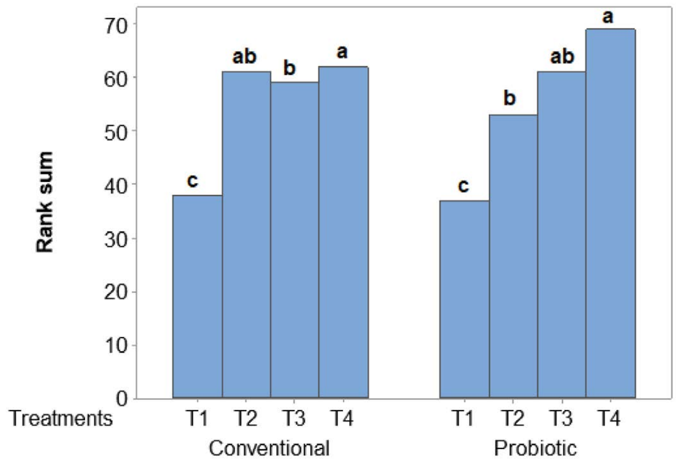

Figure 1. The ranking score of soy-based beverages (N=22). Lower rank sum indicated higher overall acceptance and higher rank sum indicated lower acceptance. Different letters on top of the bars mean significant differences according to Friedman's test. Treatments codes are defined in Table 1.

taste, and overall acceptability indicating 'like slightly' to 'like very much" on the 9-point hedonic scale. These sensory attributes were rated higher in comparison with our study, possibly as a result of flavoring agents being added to the fermented products in that study. However, Jimoh (2020) indicates that the addition of flavoring agents (*i.e.*, banana

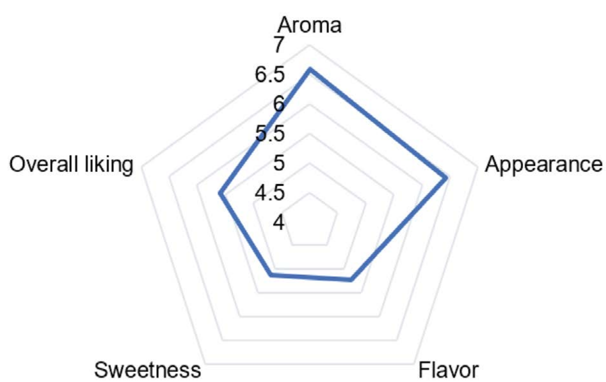

Figure 2. Sensory acceptance results for the fermented soy-dairy milk blend T1 (80% soy and 20% dairy milk blend) obtained from 9-point hedonic scales. N=71.

Table 4. Purchase intention of selected fermented soy-based beverage T1.

Scale	Score	%
Definitely would buy	9	13
Probably would buy	17	24
May or may not buy	28	39
Probably would not buy	6	8
Definitely would not buy	11	15
Total	71	100

puree) to dairy yoghurt or soy-based yoghurt decreased significantly their scores on color, appearance, taste, aroma, mouthfeel, and overall acceptability compared with those samples without the banana puree.

Regarding purchase intent, 37% of the panelists indicated that they would buy, whereas 39% said that they may or may not buy the selected soy-based beverage T1. Only 23% of the panelists indicated they would not buy the fermented beverage.

These results indicate that the development of soy-dairy milk blend fermented with probiotics may be an interesting product for consumers because it has some desirable sensory characteristics, but it is necessary to improve the formulation of this beverage.

CONCLUSIONS

To the best of our knowledge, this is the first study that reports the antioxidant activity of fermented soy-dairy milk blends. Overall, fermentation with the probiotic culture improved the antioxidant capacity of the soy-dairy milk blends compared to those fermented with the conventional yogurt culture, which is interesting and important because of the additional health benefits offered by the consumption of probiotics. Besides, it was observed that the antioxidant activity of samples was related with the proportion of soy-based beverage in the fermented blends, which represents an opportunity to develop plant-based functional foods. However, further studies are needed to determine the bioactive compounds present in soy-dairy milk blends responsible for the antioxidant properties.

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