DEVELOPMENT AND CHARACTERIZATION OF A PROBIOTIC FERMENTED BEVERAGE BASED ON PLANT EXTRACT FLAVORED WITH AÇAÍ PULP

DESENVOLVIMENTO E CARACTERIZAÇÃO DE BEBIDA FERMENTADA PROBIÓTICA A BASE DE EXTRATO VEGETAL SABORIZADA COM POLPA DE AÇAÍ

DESARROLLO Y CARACTERIZACIÓN DE UNA BEBIDA FERMENTADA PROBIÓTICA A BASE DE EXTRACTO VEGETAL SABORIZADA CON PULPA DE AÇAÍ

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Joanna Tamashiro Ujlaki[1], Dirlei Diedrich Kieling[2]

ABSTRACT

Functional foods are traditionally developed and produced in such a way as to provide health benefits to the consumer. Considering the importance of probiotic foods due to their functional health properties, as well as the need for research aimed at finding alternative, non-dairy probiotic sources, the objective of the present study was to develop and evaluate the characteristics of a probiotic fermented beverage based on oat extract and pea protein concentrate, flavored with açaí pulp. A total of 6 formulations were developed with oat extract (10 or 15% oat flakes w/v), which were inoculated with the probiotic culture (*Lactobacacillus casei* and/or *Lactobacillus acidophilus*), and the oat extract and probiotic used varied according to the formulation: F1 (10% and *L. casei*), F2 (15% and *L. casei*), F3 (10% and *L. acidophilus*), F4 (15% and *L. acidophilus*), F5 (10% and *L. casei* and *L. acidophilus*), and finally, F6 (15% and *L. casei* and *L. acidophilus*). The beverages were developed in such a way that it was possible to evaluate the formulation that would be most favorable for the development of a probiotic beverage. The formulations were subjected to physicochemical analyses such as pH, acidity, ash, total solids, soluble solids, lipids, proteins, and Lactic Acid Bacteria (LAB) count. Among the 6 formulations, the one that showed the best performance in LAB count (F4), with 9.56 log CFU/mL, was selected, for which anthocyanin analysis was carried out in the final beverage. The viability of the probiotic in F4 was also evaluated after 14 days of refrigerated storage. The beverages were acidic, with pH < 4.5, and protein content close to that of milk (approximately 2.9%). The anthocyanin content was 158.64 ± 9.45 mg cyanidin-3-glucoside/L, demonstrating the antioxidant potential of the açaí beverage. The beverage maintained its probiotic potential over 14 days under refrigeration, presenting a satisfactory count (1.22×10^7 CFU/mL) during this period. The developed formulation constitutes a beverage rich in nutritional and functional components, reaching consumers with lactose intolerance or milk protein allergy, or those who choose not to consume products of animal origin, such as vegetarians and vegans, in addition to being an alternative, non-dairy source of probiotic consumption.

[1] Graduated in Food Engineering. Universidade Federal do Paraná (UFPR).
E-mail: ujlakijoanna306@gmail.com
[2] Dr. in Food Science. Universidade Federal do Paraná (UFPR).
E-mail: dirlei.kieling@ufpr.br Orcid: https://orcid.org/0000-0002-0917-6120

Keywords: Probiotics. Functional Food. *Lactobacillus acidophilus*. *Lactobacillus casei*.

RESUMO
Os alimentos funcionais são tradicionalmente desenvolvidos e produzidos de tal forma a trazer benefícios à saúde do consumidor. Considerando a importância dos alimentos probióticos devido às suas propriedades funcionais de saúde, bem como a necessidade de pesquisas voltadas a encontrar fontes probióticas alternativas, não lácteas, o objetivo do presente estudo foi desenvolver e avaliar as características de uma bebida fermentada probiótica a base de extrato de aveia e concentrado proteico de ervilha, saborizada com polpa de açaí. Ao todo foram desenvolvidas 6 formulações com extrato de aveia (10 ou 15% de flocos de aveia m/v), as quais foram inoculadas com a cultura probiótica (Lactobacillus casei e/ou Lactobacillus acidophilus), sendo que o extrato de aveia e o probiótico utilizado variou conforme a formulação: F1 (10% e L. casei), F2 = (15% e L. casei), F3 (10% e L. acidophilus), F4 (15% e L. acidophilus), F5 (10% e L.casei e L. acidophilus), e por fim, F6 (15% e L. casei e L. acidophilus). As bebidas foram desenvolvidas de modo que fosse possível avaliar a formulação que seria mais favorável ao desenvolvimento de uma bebida probiótica. As formulações foram submetidas a análises físico-químicas como pH, acidez, cinzas, sólidos totais, sólidos solúveis, lipídios, proteínas e contagem de Bactérias Ácido Lácticas (BAL). Dentre as 6 formulações, foi escolhida a que apresentou melhor desempenho na contagem de BAL (F4), com 9,56 log UFC/mL, para qual realizou-se a análise de antocianinas na bebida pronta. Avaliou-se também a viabilidade do probiótico na F4 após 14 dias de armazenamento refrigerado. As bebidas apresentaram-se ácidas, com pH < 4,5, e teor de proteína próximo ao do leite (aproximadamente 2,9%). O teor de antocianinas foi de 158,64 ± 9,45 mg cianidina-3- glucosídeo/L, evidenciando o potencial antioxidante da bebida com açaí. A bebida manteve seu potencial probiótico ao longo de 14 dias em refrigeração, apresentando contagem satisfatória (1,22x107 UFC/mL) neste período. A formulação desenvolvida configura uma bebida rica em componentes nutricionais e funcionais, que alcança o público com intolerância à lactose ou alergia à proteína do leite, ou que opta por não consumir produtos de origem animal, como vegetarianos e veganos, além de ser uma fonte alternativa, não láctea, de consumo de probióticos.

Palavras-chave: Probióticos. Alimento Funcional. *Lactobacillus acidophilus*. *Lactobacillus casei*.

RESUMEN
Los alimentos funcionales son tradicionalmente desarrollados y producidos de tal manera que aportan beneficios a la salud del consumidor. Considerando la importancia de los alimentos probióticos debido a sus propiedades funcionales para la salud, así como la necesidad de investigaciones orientadas a encontrar fuentes probióticas alternativas, no lácteas, el objetivo del presente estudio fue desarrollar y evaluar las características de una bebida fermentada probiótica a base de extracto de avena y concentrado proteico de guisante, saborizada con pulpa de açaí. En total, se desarrollaron 6 formulaciones con extracto de avena (10 o 15% de hojuelas de avena m/v), las cuales fueron inoculadas con el cultivo probiótico (*Lactobacillus casei* y/o *Lactobacillus acidophilus*), siendo que el extracto de avena y el probiótico utilizado variaron según la formulación: F1 (10% y *L. casei*), F2 (15% y *L. casei*), F3 (10% y *L. acidophilus*), F4 (15% y *L. acidophilus*), F5 (10% y *L. casei* y *L. acidophilus*), y finalmente, F6 (15% y *L. casei* y *L. acidophilus*). Las bebidas fueron desarrolladas de manera que fuese posible evaluar la formulación más favorable para el desarrollo de una bebida probiótica. Las formulaciones fueron sometidas a análisis fisicoquímicos como pH, acidez, cenizas, sólidos totales, sólidos solubles, lípidos, proteínas y recuento de Bacterias Ácido Lácticas (BAL). Entre las 6 formulaciones, se eligió aquella que presentó mejor desempeño en el recuento de BAL (F4), con 9,56 log UFC/mL, para la cual se realizó el análisis de antocianinas en la bebida final. También se evaluó la viabilidad

del probiótico en F4 después de 14 días de almacenamiento refrigerado. Las bebidas se presentaron ácidas, con pH < 4,5, y contenido de proteínas cercano al de la leche (aproximadamente 2,9%). El contenido de antocianinas fue de 158,64 ± 9,45 mg de cianidina-3-glucósido/L, evidenciando el potencial antioxidante de la bebida con açaí. La bebida mantuvo su potencial probiótico durante 14 días en refrigeración, presentando un recuento satisfactorio (1,22x10^7 UFC/mL) en este período. La formulación desarrollada constituye una bebida rica en componentes nutricionales y funcionales, dirigida a consumidores con intolerancia a la lactosa o alergia a la proteína de la leche, o que optan por no consumir productos de origen animal, como vegetarianos y veganos, además de ser una fuente alternativa, no láctea, de consumo de probióticos.

Palabras clave: Probióticos. Alimento funcional. *Lactobacillus acidophilus*. *Lactobacillus casei*.

1 INTRODUCTION

The search for functional products and the search for a healthier lifestyle has required researchers to develop new types of products in a way that appeals to consumers and meets the claim of functional food. According to Salgado (2017), functional foods are composed of natural substances that have positive effects, which can increase well-being and health or reduce the risk of diseases, promoting health benefits and increasing quality of life, adding to physical, psychological and behavioral performance. Its effects do not cure, however, they can help prevent certain pathologies, such as cancer, diabetes, hypertension, Alzheimer's disease, bone, cardiovascular, inflammatory and intestinal diseases.

Resolution No. 2 of 01/07/2002, of the National Health Surveillance Agency (ANVISA), describes functional food as any food or ingredient that, in addition to basic nutritional functions, when consumed in the usual diet, produces metabolic and/or physiological effects beneficial to health, and must be safe for consumption without medical supervision (Brasil, 2002). On the other hand, the Functional Food Science in Europe (Fufose, 2001) defined functional foods as those that have been satisfactorily shown to beneficially affect one or more target functions of the body, in addition to their own nutritional effects, so that they are relevant for a better state of health and well-being or for the reduction of disease risks.

About 60% of functional foods bring benefits and improvements to digestive health, in which they have probiotics and prebiotics that perform their functions in the intestinal tract of the human being, through different mechanisms of action (Binns, 2014). The term probiotic means "for life" and is attributed to microorganisms that provide health benefits to humans and animals (FAO/WHO, 2001). According to Moreira (2019), the consumption of probiotic products helps regulate the intestinal flora, as well as provides benefits to the immune system, gastrointestinal resistance to colonization by pathogens, in addition to increasing the absorption of minerals and vitamins.

Generally, probiotics are related to dairy products, such as yogurts and fermented milk drinks, which include in their composition, among the main microbial genera, *Lactobacillus* and *Bifidobacterium* (Gallina et al., 2012; Komatsu; Buriti; Saad, 2008). In these products, probiotics contribute to the production process itself, conferring sensory characteristics through the fermentation of lactose. In addition to functional dairy-based products, cereals have proven to be an alternative for the production of foods of this type. They can be used as a fermentable substrate for the growth of probiotic microorganisms and may also present oligosaccharides with potential prebiotic properties. According to Ranadheera et al. (2017), soy products, cereal-based products, fruit and vegetable juices, as well as fermented meat and fish products can be considered the main non-dairy probiotic foods available on the market today.

The term prebiotic refers to selectively fermented ingredients, which result in specific changes in the composition and/or activity of the gastrointestinal microbiota, providing benefits to the health of the host (Gibson et al., 2010). According to Ramirez (2017), some types of prebiotics are galactooligosaccharides, xylooligosaccharides, fructooligosaccharides, inulin, phosphooligosaccharides, isomaltooligosaccharides, isomaltooligosaccharides, lactulose, and pectin.

The chemical and structural composition of the oat grain is unique among cereals, which gives it its suitability for use in human food (Castro et al., 2012). According to Mendonça et al., (2009), with regard to cereals, oats and barley are the ones with the highest content of β-glucans, having an average of 3-7% and 3-11% respectively, being these important dietary fibers. According to Angelov et al., (2018) fermented oat-based beverages are considered functional due to the symbiotic effect of probiotic starter cultures and β-glucan prebiotic fibers. This fiber has been proven to reduce blood cholesterol and glucose absorption in the gut. In addition to fiber, oats are rich in protein, polysaccharides, minerals such as magnesium, iron, manganese, copper and zinc, and vitamins B1 and B5. By reducing blood cholesterol, oats prevent heart disease and are considered a functional food, with their chemical composition and nutritional quality being high and superior to that of other cereals.

Açaí is also considered a food rich in fiber, helping in intestinal transit, since fiber promotes a healthy digestive system (Rocha, 2015). Souza and Bahia (2010) state that in addition to the historical and socioeconomic factor, açaí proved to be a product on the rise and of great nutritional prominence because it is a source of vitamins, such as E and B1, as well as rich in lipids. According to Nascimento et.al, (2008), açaí oil presented high levels of unsaturated fatty acids, ranging from 68% to 71%. Among the unsaturated fatty acids, oleic acid and palmitic acid are the most abundant, highlighting this fruit in an attractive condition for the food market, as well as a source of minerals such as iron and phosphorus and antioxidants.

Bernaud and Funchal (2011), verified the important antioxidant activity of açaí, which is almost entirely attributed to anthocyanins, and state that this class of flavonoids stands out for its protective effects against many diseases, especially cardiovascular diseases and cancer. Anthocyanins are water-soluble compounds that, in addition to conferring antioxidant capacity, are responsible for the dark red color characteristic of açaí pulp. According to Portinho et al. (2012) the main anthocyanins present in açaí pulp are cyanidin-3-glucoside, cyanidin-3-rutinoside, perlagonidin-3-glucoside, cyanidin-3-sambioside, peonidin-3-glucoside, and peonidin-3-rutinoside.

According to Batista et al. (2018), lactose intolerance affects around 65% to 75% of the world's population, thus presenting a distinct predominance among populations, being 80 to 100% in Indigenous Americans and Asians, 60 to 80% of blacks and Latinos, and between 2 and 15% of Northern European descent. According to Mattar and Mazo (2010), lactose malabsorption or indigestion consists of a reduction in the body's ability to hydrolyze lactose, which is a result of hypolactasia. Hypolactasia means decreased activity of the enzyme lactase in the mucosa of the small intestine, also known as non-persistent lactase. Typical symptoms include abdominal pain, a feeling of bloating in the abdomen, flatulence, diarrhea, borborygmus, and, particularly in young people, vomiting (Silva et al. 2020).

In addition, the Brazilian Vegetarian Society (SVB, 2018) estimates that 14% of Brazilians declare themselves openly vegetarian and/or vegan and 55% of Brazilians would consume more vegan products if it were indicated on the packaging. Thus, due to the high number of people with lactose intolerance, allergy to milk protein and even those who have opted for vegetarianism or veganism, the demand for plant-based drinks has increased considerably. Thus, the objective of the present study was to develop and evaluate the characteristics of a probiotic fermented beverage based on oat extract and pea protein concentrate, flavored with açaí pulp.

2 MATERIAL AND METHODS

2.1 RAW MATERIALS AND INGREDIENTS

Whole rolled oats and refined sugar were purchased at a local market in Jandaia do Sul - PR. Pea protein concentrate (85% protein) was obtained from a health food store. The 100% natural açaí pulp was purchased in a market in São Bernardo do Campo - SP. The probiotics *Lactobacillus casei* and *Lactobacillus acidophilus* (109 CFU in each capsule) were obtained in a compounding pharmacy.

2.2 OBTAINING OAT EXTRACT

The water-soluble oat extract was obtained following the methodology of Dezideiro (2019) with some modifications regarding the proportion of oats. Thus, it was defined to evaluate the proportions of 15 g oats/100 mL water and 10 g oats/100 mL water. After weighing, the whole rolled oats were crushed with filtered drinking water at room temperature (25°C) for 2 minutes in a domestic blender to obtain a homogeneous mixture. Subsequently, filtration was carried out in organza mesh to separate the extract and solid waste.

2.3 PREPARATION OF DRINK

For the preparation of the beverage, an experimental design was developed with six formulations of water-soluble oat extract and probiotic, as shown in Table 1. The formulations were prepared in duplicate.

Initially, 10% and 15% oat extracts were prepared for the beverage formulations, as shown in Table 1. The amount of açaí was defined through preliminary tests. The amount of pea concentrate was estimated so that the beverages had around 3% protein (average protein content of bovine milk). The calculation was based on the protein content informed on the label of pea concentrate (85%) and the average protein content of oat extracts (1.05 to 2.04%) found in the literature (Dezideiro 2019; Almeida et al. 2020). Sucrose (10 g/100 mL) was also added to each of the formulations, as indicated by Costa et al. (2017).

All ingredients were mixed in a home blender on high speed for 2 minutes. After obtaining a homogeneous mixture, a sample of each beverage was separated to determine the initial pH (before fermentation). Then, all formulations were pasteurized at 85°C for 30 min and then cooled in cold water until reaching a temperature of 45°C. Next, inoculation was carried out, using one capsule of each probiotic according to the formulation of the beverage (Table 1). The capsules of each probiotic culture were opened aseptically and inserted into the bottle of each formulation. The flasks were stored in an incubation oven to carry out the fermentation process for 48 h at a temperature of 42°C ± 5°C (Costa et al., 2017).

Table 1

Formulations of fermented vegetable drink based on oat extract and pea protein concentrate

FORMULATION	OAT EXTRACT	PEA PROTEIN CONCENTRATE	AÇAÍ PULP	PROBIÓTICO1
F1	10% (m/v) 100 mL	3 g	20 g	*L. I married*
F2	15% (m/v) 100 mL	3 g	20 g	*L. I married*
F3	10% (m/v) 100 mL	3 g	20 g	*L. acidophilus*
F4	15% (m/v) 100 mL	3 g	20 g	*L. acidophilus*
F5	10% (m/v) 200 mL	6 g	40 g	*L. casei + L. acidophilus*
F6	15% (m/v) 200 mL	6 g	40 g	*L. casei + L. acidophilus*

1One capsule of each probiotic has been added to each formulation. Source: The authors (2024).

Each of the six formulations (Table 1) was evaluated in terms of lactic acid bacteria (LAB) count to choose the best formulation for the development of the probiotic drink. The same formulations were also analyzed for composition (moisture, total solids, soluble solids, total minerals, protein and lipids), acidity and pH. The fermented beverage obtained, prepared from the chosen formulation, was also analyzed in relation to the anthocyanin content. The probiotic potential of the ready-to-drink was also evaluated by means of the lactic acid bacteria count (LAB) after 14 days of cold storage (5 ± 1°C).

2.4 PHYSICOCHEMICAL ANALYSIS

For the production and elaboration of food, it is of paramount importance to carry out physicochemical analyses to ascertain the quality of the product. To carry out these analyses, the methodologies proposed by the Adolfo Lutz Institute (IAL, 2008) were used. With this, the moisture content, total solids (TS), soluble solids (SS), total ash, total nitrogen and protein, lipids, pH and titratable acidity were determined.

2.4.1 Moisture content, total solids and soluble solids

To determine moisture and total solids, drying in an oven at 105°C for 24 hours was used. To determine the soluble solids content, a refractometer was used and the reading was performed as a percentage of soluble solids (°Brix).

2.4.2 Total ash

The ashes were obtained through incineration in the muffle furnace, at a temperature of 550°C, around 3 to 4 hours. For this, 3 g of sample was used in previously prepared crucibles.

2.4.3 Protein

The determination of protein was based on the determination of total nitrogen, performed through the micro Kjeldahl process. This method is based on three steps: digestion, distillation, and titration. Organic matter is decomposed and existing nitrogen is transformed into ammonia. A factor of 6.25 was taken into account to transform the number of g of nitrogen found into the number of g of proteins, considering that the nitrogen content of the different proteins is approximately 16%.

2.4.4 Total lipids

To obtain the desired lipid fraction and to ensure a better efficiency of total lipid extraction, samples resulting from the moisture removal process were used. The method used was hot solvent extraction in a Goldfish extractor (Lucadema®). The method is based on three steps: extraction of the fat from each sample with petroleum ether solvent (90%), elimination of the solvent by evaporation and quantification by weighing.

2.4.5 Determination of pH

The pH was measured using a benchtop pH meter and, for this, 10 mL of each sample was used. For the calibration of the equipment, buffer solutions of pH 4.0 and 7.0 were used.

2.4.6 Titratable acidity

This step was based on titration of 10 mL of sample of each formulation with a 0.1 N sodium hydroxide (NaOH) solution and three drops of phenolftalein as an indicator. The result was expressed as % lactic acid.

2.4.7 Anthocyanins

For the determination of anthocyanins, the method proposed by Ribeiro (2016) was used, where the total anthocyanin content of the extracts was determined by the differential pH method, where buffer solutions of potassium chloride (0.025 mol/L, pH 1.0) and sodium acetate buffer (0.4 mol/L, pH 4.5) were used. Thus, about 10 mL of each sample was separated, which were centrifuged at 5000 rpm for 15 minutes. After centrifugation, 200 µL of the supernatant was taken, which was mixed with 3800 µL of each buffer solution. Finally, the absorbance was measured using a spectrophotometer with wavelengths of 510 nm and 700 nm. From this, the calculation was performed to determine total anthocyanins, according to Equation (1).

$$AT = \left[\frac{Abs_{510} - Abs_{700\ pH_{1,0}}\ x\ 10^{3}\ x\ PM_{cianidina\text{-}3\text{-}glicosideo}\ x\ fd}{\varepsilon} \right] \times 100 \qquad (1)$$

2.5 MICROBIOLOGICAL ANALYSIS

To find out which formulation performed better, lactic acid bacteria (LAB) was counted. After choosing the best formulation, the LAB count was performed again after 14 days of storage at 5 ± 1 oC, thus allowing to analyze the performance of the probiotic strain during cold storage. The BAL count (CFU/mL) was determined on Man Rogosa & Sharpe agar (MRS), through the depth plating technique, using an overlayer of the same medium, to ensure microaerophilic conditions, completed by subsequent incubation of the samples at 35 °C for 72 h (Silva et al., 2017).

2.6 STATISTICAL ANALYSIS

The results obtained for soluble solids and total solids, ash, protein, lipids, pH, titratable acidity in lactic acid and BAB count, were submitted to Analysis of Variance (ANOVA) and Tukey's test ($p \leq 0.05$), with the aid of the Statistica 7® program. The results are presented as mean and standard deviation of three replications.

3 RESULTS AND DISCUSSION

3.1 PHYSICOCHEMICAL CHARACTERIZATION OF FERMENTED BEVERAGE FORMULATIONS

The results achieved in the physicochemical analyses (g/100mL) of the six formulations developed with oat extract, pea protein concentrate and açaí, are shown in Table 2.

Table 2

Physicochemical analyses of fermented vegetable beverage formulations

Parameters	F1	F2	F3	F4	F5	F6
Humidity (%)	84.37 ± 0.24a	81.84 ± 0.20b	84.55 ± 0.39a	82.54 ± 0.09b	84.88 ± 0.07a	82.10 ± 0.01b
ST (%)	15.63 ± 0.24b	18.16 ± 0.21a	15.45 ± 0.39b	17.46 ± 0.09a	15.12 ± 0.07b	17.90 ± 0.01a
Ash(%)	0.30 ± 0.13a	0.33 ± 0.03a	0.29 ± 0.03a	0.35 ± 0.03a	0.29 ± 0.01a	0.34 ± 0.03a
Proteins (%)	2.83 ± 0.33a	3.06 ± 0.12a	2.61 ± 0.04a	2.96 ± 0.05A	2.49 ± 0.00A	2.99 ± 0.08a
Lipids (%)	0.75 ± 0.24a	0.58 ± 0.04a	0.59 ± 0.20a	0.85 ± 0.09a	0.85 ± 0.11a	0.75 ± 0.12a
Acidity (% lactic acid)	0.64 ± 0.01b	0.78 ± 0.02b	0.88 ± 0.13b	1.17 ± 0.01a	0.79 ± 0.10b	0.85 ± 0.02b
pH1	4.55 ± 0.01a	4.25 ± 0.28A	4.05 ± 0.49A	3.62 ± 0.25A	3.67 ± 0.15A	3.72 ± 0.04a
SS (°BRIX)	12.63 ± 0.53ab	13.75 ± 0.71ab	12.00 ± 0.71b	14.88 ± 0.88a	11.75 ± 0.00b	11.38 ± 0.53b

Different letters in the same line correspond to a significant difference according to Tukey's test ($p \leq 0.05$). 1Initial pH of formulations = 6.39 ± 0.02. Source: The authors (2024).

It is noted that in relation to moisture content and total solids, there was a significant difference between the formulations (Table 2). These differences in moisture and ST results are explained by the different concentrations of oat extract used in beverages, while for F2, F4 and F6 the extract has 15% rolled oats (w/v), for F1, F3 and F5 it has 10% (w/v).

The high moisture content of the formulations is due to the fact that açaí pulp has an average of 88.7% moisture, according to the Brazilian Table of Food Composition (TACO, 2011). According to Furtado and Ferraz (2007), moisture content is one of the main analytical determinations made in order to verify identity and quality standards in food. This parameter is directly linked to the lifespan of this product, considering that high moisture levels accelerate the deterioration of the food. In the case of a beverage, a high moisture content was already expected, which is responsible for providing fluidity to the product, favoring consumption.

In the centesimal composition analyses carried out by Almeida et al. (2020), a content of 10.09% of total solids was found in the oat extract. The fermented beverage in question was formulated with more ingredients than in the study addressed, so the amount of total solids tends to increase and, therefore, this parameter was between 15.12% and 18.16%.

Regarding the ash analysis, there was no significant difference between the samples. As it is an oat extract, there is a significant loss of solid waste, so the amount of ashes varied from 0.29% to 0.34%. According to Heiden et al. (2014) the ash content found in the oat flake was 1.7%, while the value found by Bayer (2019) was 0.095% in the oat extract. From TACO (2011) it was possible to verify that açaí pulp has an ash content of 0.3%. Therefore, it was expected that the ash content of the drink would not be very high.

Regarding the amount of proteins present, it was found that there was no significant difference between the samples. In the study conducted by Bayer (2019), oat extract had a protein content of 0.927%. The low protein content found in oat extract may be related to the low solubility of its proteins in water or the interference of lipid concentration with the solubility of proteins. Thus, due to the low protein content of oat extract, pea protein concentrate has been added to bring the protein content of the plant-based drink closer to the protein content of bovine milk (minimum of 2.9%). This goal was achieved.

For the lipid content, it is possible to observe that there was no significant difference between the samples, ranging from 0.58 to 0.85%. According to data obtained by Minighin et al. (2019), the average percentage of lipids obtained from purple açaí pulp was 6.57%. In turn, Almeida et al. (2020) obtained a value of 1.87% in oat extract. The low percentages found in this study are explained by the fact that a small fraction of açaí pulp (20g/100mL) was added to the formulation of the beverage, in addition to other ingredients, thus reducing the lipid content in the ready-made beverage. The low lipid content obtained points to a low-calorie drink.

Regarding the titratable acidity, a significant difference was observed between the formulations. According to Silva (2019) this parameter indicates the amount of organic acids contained in the sample. Among the samples analyzed, the lowest titratable acidity was found for F1 (0.64%) and the highest was for F4 (1.17%). In a study conducted by Thamer and Penna (2006), it was possible to observe that the higher the percentage of soluble solids in functional milk beverages fermented by probiotics and added with prebiotics, the higher the titratable acidity. It can be observed that the F4 formulation, with a higher content of soluble solids, presented the highest titratable acidity, which must be due to the fermentation process itself (lactic fermentation), perhaps more intense in this formulation, in which there is conversion of fermentable sugars into organic acids, primarily lactic acid.

pH is a parameter that deserves attention, because, according to Anvisa's Ordinance No. 27, of January 13, 1998, the pH of beverages must be lower than 4.5 to prevent the germination of spores and the growth of *Clostridium botulinum* during storage in the absence of oxygen (Brasil, 1998). In the formulations developed, it can be observed through the pH that the beverages remained acidic, ranging from 3.62 to 4.55, with no significant difference between them. Thus, through the results obtained in this study, the pH levels are in accordance with the Brazilian legislation and guarantee microbiological safety to the product.

According to Silva et al. (2022), the soluble solids content (°BRIX) is used in the food industry to determine the approximate content of sugars in fruits, fruit juices, wines, beverages in general, as well as in other solutions. The content of soluble solids is an important characteristic to be analyzed in fruits and their derivatives, being used, for standardization, maturity index, sweetness and quantity of substances that are dissolved, and this measurement is important for a better evaluation of the quality of fresh fruits, and derived products. According to Normative Instruction No. 01, of January 2000 (Brasil, 2000), which sets the standards of identity and quality for fruit juices, açaí must present, in relation to °Brix, from 8% to 14%. The main ingredient contributing to the drink's SS values was sugar (10 g) and, in second place, açaí pulp (20 g). The variations found are due to the soluble solids from the oat extract, which was prepared with different proportions of rolled oats (w/v), and the pea concentrate.

3.2 MICROBIOLOGICAL ANALYSIS OF THE FERMENTED BEVERAGE

Table 3 shows the counts of lactic acid bacteria obtained through microbiological analyses of the 6 formulations of fermented beverage previously prepared with oat extract, pea protein concentrate and açaí pulp.

Table 3

BAL count for fermented vegetable beverage formulations

Formulations	BAL1 (Log CFU/mL)
F1	6.39 ± 0.23c
F2	5.84 ± 0.51c
F3	7.87 ± 0.04b
F4	9.56 ± 0.03a
F5	8.82 ± 0.27b
F6	8.87 ± 0.05b

1BAL = Lactic acid bacteria. Different letters in the same column correspond to a significant difference according to Tukey's test (p ≤ 0.05). Source: The authors (2024).

From the microbiological analyses, a significant difference was observed in the BAL count for the 6 beverage formulations produced. In order for foods to be classified as functional, it is necessary that the probiotic microorganisms reach the minimum necessary count of 106 CFU/mL (Baú, Garcia and Ida, 2014). In this way, all formulations can be considered viable, but the shelf life may be short, making commercial production unfeasible. On the other hand, Terhaag et al. (2020) point to a minimum daily consumption of 106 -109 CFU/mL of the probiotic microorganism to obtain the beneficial effect and cite that factors such as the composition of the food matrix, form of processing, temperature, among others, affect the viability of the probiotic culture during storage.

Regarding the choice of the best formulation for the development of the probiotic drink, F4 was considered, which presented the highest count value for BAL (9.56 ± 0.03 log CFU/mL), highlighting that in this formulation 15% (w/v) oat extract was used and only *L. acidophillus* as a probiotic, which showed better performance compared to *L. casei*. The association of *L. acidophillus* with *L. casei*, in turn (F5 and F6), did not show a favorable result, on the contrary, there is evidence that there may have been competition between both since *L. acidophillus* alone reached higher counts (F4).

In general, when probiotic cultures are used in certain foods, they require a significant fermentation period until low pH values (pH < 4.5) are reached. According to Smid and Kleerebezem (2014), the main applicability of LAB in industries is due to the fact that the fermentation of food is related to the short acidification time of the food matrix, caused by the rapid conversion of fermentable carbohydrates into lactic acid. Thus, the rapid acidification and rapid consumption of fermentable sugars are two factors that result in the competitive advantage of these bacteria in nutrient-rich environments, whether the raw materials are of animal or vegetable origin. The fermentation time of the developed beverages was 48 hours. Thus, F4 was the one that obtained the highest titratable acidity, showing a more effective fermentation, in view of the greater development of the probiotic, in the same period of time.

According to Leroy and Vuyst (2004), the rapid acidification of a beverage and the production of lactic acid, ethanol, aromatic compounds, bacteriocins, exopolysaccharides and enzymes increase the shelf life and safety of the product, in addition to favoring the development of a pleasant sensory profile. For Smid and Kleerebezem (2014), the low pH and a certain concentration of organic acids suggest a greater durability of food products fermented with lactic acid bacteria, in addition, LABs not only influence the shelf life of the product, but the food matrix leads to the production of other functionalities such as texture, nutritional value and aroma.

According to Rhee (2011), the fermentation process in foods makes them resistant to microbial spoilage and the development of food toxins, less likely to transfer pathogenic microorganisms, preserves food between the time of harvest and consumption, modifies the flavor of the original ingredients and often improves the nutritional value.

Table 4 shows the BAB count in order to verify the viability of the probiotic lactic culture after 1 and 14 days of cold storage (5°C ± 1 °C).

Table 4

BAL count after 1 and 14 days of cold storage

	F4 (1 day at 5oC)	F4 (14 days at 5oC)
BAL1 (Log CFU/mL)	(3.70 ± 0.28) x 109	(1.22 ± 0.25) x107

1BAL = Lactic acid bacteria. Source: The authors (2024).

During the monitoring of the fermented beverage during storage, it was found that the beverage maintained its probiotic potential during the 14 days in refrigeration, still presenting satisfactory counts after this period, in view of the minimum recommended count of 106 CFU/mL for probiotic foods and beverages (Baú, Garcia and Ida, 2014). According to Garcia et al. (2020) LABs are capable of metabolizing different substrates, resulting in biochemical changes in the composition of the medium, in addition, lactic acid fermentation is linked to improved nutritional properties, flavor, and health-related aspects of food products. It is important to note that the survival capacity of probiotics depends on the genus, species and strain, and the composition of the other ingredients in the preparation also interferes with the viability of the product to be developed. Because of this, changes in the number of viable probiotic bacteria during functional food storage should be studied more deeply (Kang et al., 2012).

Kang et al. (2012) point out that factors such as low temperature and the presence of sugars favor the survival of LABs in an acidic environment, which makes it necessary to store the beverage in environments with reduced temperatures. In a study conducted by Yoon et al. (2004), it was possible to notice that there was a 1000-fold decrease in the BAL population after 28 days of storage in tomato juice fermented with *L. plantarum*. Storage in non-refrigerated conditions with high relative humidity and exposure to an oxygen-containing atmosphere may contribute to decreased viability of probiotics over time. This is because long-term storage can cause damage to the cell membrane. Thus, cold storage aims to ensure safety and maintain product quality (Kang et al., 2012). In the fermented beverage of the present study, there was a 303-fold reduction in the BAL count after 14 days of refrigerated storage, and an evaluation for a longer period of time is recommended in order to estimate the final viability of the probiotic strain used.

According to Poppi et al. (2008), color is a sensory attribute of paramount importance to determine the quality of a food, exerting an enormous influence on appearance and helping consumers accept a variety of food products. In natural products, most of the substances responsible for coloring belong to the class of flavonoids. Two classes can be considered as the most important for these compounds, namely flavonols and anthocyanins. In the analysis carried out, a total anthocyanin content of 158.64 ± 9.45 mg cyanidin-3-glucoside/L was obtained in the fermented beverage. According to Cohen et al. (2006), anthocyanins, in addition to dyes, are natural antioxidants. In açaí beverages developed by Albarici et al. (2006), these compounds were identified as cyanidin-3-arabinoside and cyanidin-3-arabinosil-arabinoside, with a total anthocyanin content of 263 mg/100g. In the research carried out by Carbonell et al. (2015), which consisted of the elaboration of a drink with açaí pulp, total anthocyanin levels of 40.4 to 49.3 mg cyanidin-3-glucoside/L were found, therefore lower than the levels determined in the present work. According to Ribeiro and Seravalli (2004), anthocyanins are unstable pigments and have greater stability under acidic conditions.

The differences observed in the anthocyanin content in different beverages are related to the composition of the açaí pulp used in its preparation. According to a study carried out by Rufino et al. (2010) the value obtained of total anthocyanins in açaí pulp was 73.54 mg/100g, and this fruit is considered a source of this pigment. Santos et al. (2008), in turn, found levels ranging from 13.93 to 54.18 mg/100g of total anthocyanins when analyzing açaí pulps from 12 different commercial points in Fortaleza/CE. Tonon et al. (2009) obtained 32.81 mg/100g of anthocyanins in açaí pulp from the city of Pará. It is worth noting that the difference between the values of anthocyanins analyzed may be related to the processing and the time of packaging of the pulp, considering that in the places where the studies by Santos et al. (2008) and Tonon et al. (2009) were carried out, the açaí is not a typical fruit, being acquired in processed form (pasteurized and frozen).

4 FINAL CONSIDERATIONS

The formulation chosen for the production of the fermented beverage based on oat extract and pea protein concentrate, flavored with açaí pulp, was F4, which is composed of 15% oat extract and was fermented by the probiotic *L. acidophilus*, which proved to be viable during 14 days of refrigerated storage. However, all six formulations produced in this study are considered viable for the production of the probiotic drink. The main advantage of the innovative formulation developed is the fact that it is a drink rich in nutritional and functional components, reaching certain audiences with some type of intolerance or allergy, or even by those who choose not to consume animal products, in addition to being an alternative, non-dairy source of probiotic consumption.

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