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FOOD TECHNOLOGY

Elaboration, sensorial acceptance and characterization of fermented flavored drink based on water-soluble extract of baru almond

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ABSTRACT: *The impact of alimentation on life's quality have encouraged the search for alternative foods with better quality and use of native fruits with technological and nutritional potentials contributes to the development of new products. Thus, the aim of this study was to develop a fermented flavored drink, potentially probiotic, based on the water-soluble extract of baru almond, perform sensory evaluation, determine its chemical composition and monitor the shelf life. The extract was fermented by a culture containing Streptococcus thermophilus, Lactobacillus acidophilus and Bifidobacterium. After fermentation, thickeners, sucrose and plum pulp were added, these latter two in accordance to the proposed factorial design that resulted seven assays. These formulations were subjected to sensorial analysis, which included the acceptance test and purchase intention. The optimized formulation had its physical-chemical and nutritional characteristics evaluated trough the determination of chemical and mineral composition, viability of the probiotic bacteria and the acidity and pH parameters were evaluated during the twenty-eight storage days. Formulations obtained high rates of acceptability and purchase intention and the optimized formulation achieved good nutritional characteristics, being a food with high manganese content and a source of magnesium and phosphorus. During the storage, the pH and acidity values varied and probiotic microorganism count increased. Probiotic potential of fermented drink can be justified by presence of both microorganisms and by the verified counting obtained since the seventh storage day of the product.*
Key words: *probiotics, sensorial analysis, water-soluble extract, Dipteryx alata* Vog.

Elaboração, aceitação sensorial e caracterização de bebida fermentada saborizada à base de extrato hidrossolúvel da amêndoa de baru

RESUMO: *Os impactos da alimentação na qualidade de vida têm incentivado a busca de alternativas alimentares de melhor qualidade e a utilização de frutos nativos com potencial tecnológico e nutricional contribui para o desenvolvimento de novos produtos. Assim, o objetivo deste trabalho foi elaborar uma bebida fermentada saborizada, potencialmente probiótica, à base de extrato hidrossolúvel da amêndoa de Baru, realizar a avaliação sensorial, determinar sua composição centesimal e acompanhar a vida de prateleira. O extrato foi fermentado por uma cultura contendo Streptococcus thermophilus, Lactobacillus acidophilus e Bifidobacterium. Após a fermentação foram incorporados espessantes, sacarose e polpa de ameixa, estes dois últimos segundo o delineamento fatorial proposto que totalizou sete ensaios. As formulações foram submetidas a análise sensorial, que compreendeu o teste de aceitação e de intenção de compra. A formulação otimizada teve suas características físico-químicas e nutricionais avaliadas pela determinação da composição centesimal e de minerais. Avaliou-se a viabilidade das bactérias probióticas e os parâmetros de pH e acidez durante vinte e oito dias de armazenamento. As formulações obtiveram altos índices de aceitabilidade e intenção de compra e a bebida otimizada apresentou boas características nutricionais, sendo um alimento com alto conteúdo de manganês e fonte de magnésio e fósforo. Durante o armazenamento os valores de pH e acidez variaram e a contagem de microrganismos probióticos aumentou. Justifica-se a potencialidade probiótica dessa bebida pela presença de ambos micro-organismos e pela contagem verificada a partir do sétimo dia de armazenamento do produto.*
Palavras-chave: *probióticos, análise sensorial, extrato hidrossolúvel, Dipteryx alata Vog.*

INTRODUCTION

Among many factors, the characteristics of the consumed diet are directly related to the life quality and this consensus has driven a growing interest in functional foods (PINTO & PAIVA, 2010), which are as foods made up of nutrients that confer physiological or medical benefit to the consumers (OLAIYA et al., 2016). Because of the awareness of the impact of food on health, the demand for probiotic functional foods is growing and, given the high prevalence of lactose intolerance, various non-dairy probiotic products obtained by the fermentation of grains, fruits and vegetables, have been developed and received the attention of the scientific and consumers community (TRIPATHI & GIRI, 2014).

Almonds are rich in mono and polyunsaturated fatty acids, vegetable protein, fiber,

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vitamins and minerals (YADA et al., 2011) and its extracts have compounds that act on some current chronic diseases, obesity and some cancers (KAMIL & CHEN, 2012). The Baru almond (*Dipteryx alata* Vog.), native fruit of the Cerrado, has a high content of lipids, proteins, several amino acids; fatty acids and minerals (FREITAS & NAVES, 2010) and its extract can be used to fermentation.

Thus, the aim of this study was to develop a fermented flavored drink, potentially probiotic, based on the water-soluble extract of baru almond, perform the sensorial evaluation, determine its chemical composition and monitor shelf life.

MATERIAL AND METHODS

Obtention of water-soluble extract

Obtention of water-soluble extract followed the methodology proposed by D'OLIVEIRA (2015). Baru almonds, from São Manoel Settlement at Anastácio MS - Brazil, located at the coordinates 20°42'31"S and 55°41'35"O (LIMA & ALVES, 2010), acquired in Campo Grande/MS, were sanitized with sodium hypochlorite solution (100ppm) for 15 minutes, heat treated in boiling water (1:3, w:v) for five minutes, manually dehulled and milled in a cutter for 3 minutes. They were then homogenized in boiling water (295g of ground almonds/1 liter of extract) in a blender for five minutes and the resulting extract was filtered through a 60 sieve mesh. It was added to the extract 5% of commercial sucrose (Estrela®) and performed the pasteurization process (65°C/30 minutes) in sterile glass bottles, being after this, cooled and stored under refrigeration (8°C) until fermentation time.

Elaboration of the fermented drink

The extract was heated, controlled by thermometer until it reaches the temperature of 45°C, then the culture (BioRich®, Chr. Hansen A/S Denmark) containing *Streptococcus thermophilus*, *Bifidobacterium* BB-12 and *Lactobacillus acidophilus* LA -5 (400mg L^{-1}) was inoculated, under aseptic conditions and slow agitation and the material was incubated at 45°C until pH 4.7 (5h). After fermentation, the fermented drink was transferred to a refrigerator (8°C) where it remained until the next day, when these ingredients were added during agitation: 0.15% xanthan gum (Sabor Alternativo®) and 0.15% carboxymethylcellulose (Arcólor®) in addition to the plum pulp (PP) (JEB®) and sucrose (S), these latter two in levels determined in the experimental design, which was based on a 2^2 factorial, with two equidistant levels of variation and a central point with two replications, resulting in 7 formulations (F) (F1 - 20% of PP and 5% of S; F2 - 20% of PP and 0% of S; F3 - 10% of PP and 5% of S; F4 - 10% of PP and 0% of S; F5 (central point), F6 and F7 (replications) - 15% of PP and 2.5% of S). To ensure the microbiological safety of the formulations, before sensorial evaluation, analysis of coliform at 45°C g^{-1} was performed accordingly to SILVA et al. (2010), looking forward the requirements in the microbiological regulation food standards (BRAZIL, 2001), using the fermented milk as a reference.

Sensorial analysis

The analyses were performed on two consecutive days, in the first day the analysts tasted four samples and in the second day three. Around 50ml of the fermented drink formulations were offered in plastic cups, in a balanced and monadic way, for a panel of 21 untrained tasters, chosen according to the availability. Sensorial analysis was performed in single cabins and the sensory record had parameters of evaluation for appearance, color, aroma, flavor, texture, sweetness and overall acceptance, and was adopted a hedonic scale of nine points (1-dislike extremely and 9-like extremely) (DUTCOSKY, 2013). The analysts were also asked in the same record about the product purchase intention, which had a hedonic scale of 5 points (1-definitely would buy and 5-definitely would not buy). With the data of the acceptance test were calculated the acceptability indices by the formula: AI (%) = Ax100/B (where A = average score obtained for the product and B = top marks gave to the product) and AI values ≥70% were considered good acceptance (DUTCOSKY, 2013).

Physicochemical evaluation

Physicochemical characterization of the optimized formulation was performed in triplicate. Moisture content was obtained by drying at 105°C to constant weight; the total mineral content was obtained after incineration in a muffle at 550°C; protein was obtained by Micro-Kjehdahl method; the lipid content was determined by the hot solvent extraction method, in a Soxhlet extractor device; the pH determination was performed with digital pH meter and titratable acidity was obtained by titration with 0.1N NaOH following the Adolfo Lutz Institute methods (ZENEBON et al., 2008). Total caloric value was determined by Atwater conversion values of 4.07kcal g^{-1} of protein, 3.47kcal g^{-1} of carbohydrates and 8.37kcal g^{-1} of lipids (MERRIL & WATT, 1973).

Mineral analysis

The determination of the minerals content calcium (Ca), copper (Cu), iron (Fe), phosphorus (P), magnesium (Mg), manganese (Mn), potassium (K), sodium (Na) and zinc (Zn) in the optimized formulation, was performed in duplicate using an inductively coupled argon plasma optical emission spectrometer (ICP-OES), based on the methodology described by CÔNSOLO (2014).

Shelf life

The optimized drink was evaluated on the 1^{st}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of storage at 8°C, in relation to the values of pH, acidity and count of viable probiotic cells of *Lactobacillus acidophilus* and *Bifidobacterium* according to ALVES et al. (2009).

Statistical analysis

Physicochemical analysis results were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was performed in the data of the sensory test and in the mean comparison it was used the Tukey test, at 1% significance level (COSTA NETO, 2002; DUTCOSKY, 2013).

RESULTS AND DISCUSSION

Microbiological and Sensorial analysis of the formulations

All analyzed formulations obtained counts <3MPN g^{-1} of Coliforms at 45°C, which shows that they were within the law recommended limits (10MPN g^{-1}) (BRASIL, 2001). In the test with hedonic scale the average scores for appearance (7.06), color (7.02), aroma (6.86), flavor (6.87), texture (6.88), sweetness (6.65) and overall acceptance (7.03) were among the terms "like slightly" and "like moderately", for all formulations. It wasn't detected significant differences between them neither with ANOVA nor with the Tukey test.

The highest percentages of purchase intent of the formulations were the options "maybe/maybe not" (F1 and F7), "probably would buy" (F2, F3, F4, F5 and F6) and "definitely would buy" (F4). In the acceptability indices, only the sweetness attribute of F1 had a rate of 68.78% and for the other attributes of formulations, the indexes ranged from 71.96% to 80.95%, which indicates good acceptance, according to DUTCOSKI (2013), because they exceed the minimum of 70%. MARTINS et al. (2013) and KOLLING et al. (2014) reported lower indexes 65.70%-75.70% and 63%-70%, respectively, in soy based yogurts with prebiotics.

Physicochemical evaluation

The physicochemical evaluation was performed in the optimized formulation, which was the one that had the highest average for the "overall acceptance" (F5). The physicochemical evaluation data are shown in table 1.

The moisture content of the optimized drink was lower when compared to the levels observed by KOLLING et al. (2014) of 85.4% in soy yogurt with addition of prebiotic and BICUDO et al. (2012), of 90.9% in fermented drink based on water soluble extract of quinoa with fruit pulp, but the levels of carbohydrates, lipids and total caloric value where higher compared to the same authors, who had values of 9.8% and 4.39% of carbohydrates; 1.2% and 1.2% of lipids and 61.2 and 40.96kcal $100g^{-1}$ of total caloric value, respectively. These variations between the results of these studies are due to the use of different raw materials.

Mineral analysis

Values of RDI (Recommended Daily Intake) for calcium, iron, magnesium, zinc, phosphorus and manganese are: 1000mg day^{-1}; 14mg day^{-1};

Table 1 - Physical and chemical composition of the optimized formulation of fermented flavored drink based on water soluble extract of baru almond.

Characteristics*	Optimized formulation
pH	4.67±0.03
Acidity (g lactic acid $100g^{-1}$)	0.51±0.00
Moisture (g $100g^{-1}$)	76.25±0.15
Total minerals (g $100g^{-1}$)	0.46±0.06
Proteins (g $100g^{-1}$) **	2.94±0.09
Lipids (g $100g^{-1}$)	6.50±1.64
Glucose (g $100g^{-1}$)	3.96±0.26
Sucrose (g $100g^{-1}$)	8.52±0.18
Starch (g $100g^{-1}$)	ND***
Total carbohydrates (g $100g^{-1}$)****	12.49±0.17
Total caloric value (kcal $100g^{-1}$)	109.69±14.69

*Values are mean±standard deviation of triplicates.
**Nitrogen to protein conversion factor - 6.25.
***ND - Not detectable.
****Constitute the sum of values obtained for glucose and sucrose.

160mg day^{-1}; 7mg day^{-1}; 700mg day^{-1} and 2.3mg day^{-1}, respectively (BRASIL, 2005). Optimized formulation has 14.86±4.46mg $100g^{-1}$ of Ca; 0.11±0.01mg $100g^{-1}$ of Cu; 0.64±0.05mg $100g^{-1}$ of Fe; 90.83±3.35mg $100g^{-1}$ of P; 22.98±0.46mg $100g^{-1}$ of Mg; 0.44±0.02mg $100g^{-1}$ of Mn; 162.91±10.48mg $100g^{-1}$ of K; 11.79±7.39mg $100g^{-1}$ of Na and 0.42±0.01mg $100g^{-1}$ of Zn. The 54th RDC of November 12, 2012 (BRASIL, 2012) determines the minimum of 15% of the RDI reference for food be considered as a source, and a minimum of 30% of the RDI to be ranked as high minerals content, represented both in 100g or ml as in portions. Considering a portion of 200ml of the optimized drink, this may provide 28.72% of the RDI for magnesium, 25.95% for phosphorus and 38.26% for manganese. Therefore, this drink can be considered a food with high content of manganese and a source of magnesium and phosphorus (BRASIL, 2012). Phosphorous and magnesium are nutritionally important minerals and the deficiency of such elements usually proves fatal unless intervened properly and manganese acts as an activator of enzyme and as a component of metalloenzymes (PRASHANTH et al., 2015).

Shelf life

Table 2 shows the parameters of pH and acidity and *Lactobacillus acidophilus* and *Bifidobacterium* count during 28 days of storage of the optimized formulation. There was an increase in acidity and a decrease of pH during the refrigerated storage. According to VAHEDI et al. (2008) it should be considered the continuation of the activity of lactic acid bacteria during the product storage, a fact described as post-acidification. Current legislation recommends a minimum number of *Bifidobacterium* only to fermented milk (at least 10^6CFU g^{-1}) (BRASIL, 2007), for GALLINA et al. (2011), the minimum viable amount for the probiotics should be in the range of 10^8 to 10^9CFU in daily recommendation of the product, which corresponds to a consumption of 100g of product containing 10^6 to 10^7CFU ml^{-1} or g^{-1}. Although the number of viable cells of *Bifidobacterium* does not reach this minimum since the beginning of storage, the probiotic appeal is justified by the concomitant presence of *L. acidophilus*, endowed with the same probiotic properties. The sum of these two meets the minimum considered ideal to promote beneficial effects starting on the 7th day of the storage period.

Increase in counts of both probiotic microorganisms was certainly influenced by the presence of plum in the formulation. Addition of this pulp promoted the development and maintenance of these bacteria during storage, because it has a high content of soluble fibers (SILVA & UENO, 2013), which selectively stimulate bacteria growth present in the colon, serving as a substrate and contributing to the increase of these microorganisms (SAAD, 2006).

CONCLUSION

Results shown the feasibility of flavored fermented drink production based on the water-soluble extract of baru almond with probiotics, given the physical-chemical, mineral and microbiological properties, its sensorial acceptance and the viability of probiotics bacterias during storage. Thus, there arises an alternative for the industry to provide a vegetable-based product with probiotic appeal, which constitutes an alternative, with high nutritional value, for dairy drinks and soy-based drinks.

Table 2 - Parameters of pH and acidity and count of *Lactobacillus acidophilus* and *Bifidobacterium* during the storage period (28 days) of the optimized formulation.

Parameters	------Time (days)------				
	1	7	14	21	28
L. acidophilus (CFU g^{-1})	$5.6x10^3$	$4.5x10^5$	$1.37x10^7$	$1.61x10^8$	$3.83x10^8$
Bifidobacterium (CFU g^{-1})	$2.5x10^3$	$9.0x10^5$	$1.06x10^7$	$3.71x10^7$	$6.0x10^8$
Total count	$8.1x10^3$	$1.35x10^6$	$2.43x10^7$	$1.98x10^8$	$9.83x10^8$
pH*	4.75±0.01[a]	4.54±0.01[b]	4.39±0.00[c]	4.20±0.02[d]	3.98±0.02[e]
Acidity (g lactic acid $100g^{-1}$)*	0.47±0.01[a]	0.55±0.00[b]	0.59±0.01[c]	0.68±0.01[d]	0.89±0.02[e]

*Values are mean±standard deviation of triplicates, averages followed by different letters in the same line, differ significantly by Tukey test (P<0.01).

BIOETHICS AND BIOSSECURITY COMMITTE APPROVAL

This study has been approved by the Ethics Committee (CEP) of the Universidade Federal do Mato Grosso do Sul (UFMS) (protocol number 6751.98/2015 and CAAE 31229114.4.0000.0021).

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