Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.5327/fst.00015

Protein nutritional supplement with bacuri almonds (*Attalea phalerata Mart*. Ex Spreng) for vegetarian sports: development, characterization, and shelf life

Flávio da CUNHA[1], Luciana MIAGUSKU[1], Maria Lígia Rodrigues MACEDO[1], Priscila Aiko HIANE[1], Fabiane La Flor Ziegler SANCHES[1*]

Abstract
The aim of the study was to develop and evaluate a protein nutritional supplement formulated with bacuri almonds (Attalea phalerata Mart. Ex Spreng) for vegetarian athletes. The almonds were processed to prepare defatted flour, from which a protein concentrate was obtained through aqueous extraction and lyophilization. The protein concentrate was used to formulate two protein supplement formulations. The nutritional, compositional, and amino acid profiles of the freeze-dried bacuri almond flour and the supplement formulations were characterized. The shelf life of the supplement was evaluated, and its microbiological and sensory composition was analyzed. The results showed that bacuri almonds are a promising source of plant-based protein, and the supplement developed in this study met the nutritional needs of vegetarian athletes. Two final supplement formulations were obtained: one consisting of 70% bacuri almond flour with a protein content of 43%, and the other a protein blend consisting of 40% bacuri almonds and 40% commercial supplement based on rice and peas with a protein content of 47%. Despite some loss of amino acids during the optimization of protein concentration, the supplement formulations had satisfactory microbiological and sensory composition during the 28-day shelf-life test. These findings provide an alternative protein supplement option for athletes and individuals with a vegetarian diet.

Keywords: protein; vegetarian diet; supplementation; native fruits.

Practical Application: Development of an innovative vegetable protein supplement with bacuri almonds for vegetarians: high protein concentration, suitable for sports nutrition, microbiologically stable, and sensorially acceptable.

INTRODUCTION

Vegetarian diets are increasingly popular for various reasons, including health, sustainability, and ethical concerns (Lynch et al., 2016). Studies have shown that vegetarians have a lower body mass index, are overweight, and are obese compared to omnivores, which may be due to higher consumption of vegetables and fiber-rich foods and lower consumption of animal foods (Kahleova & Pelikanova, 2015; Orlich et al., 2015; Yokoyama et al., 2014). However, a balanced vegetarian diet is essential to avoid nutritional deficiencies such as high biological value protein (Slywitch, 2012).

It is possible for vegetarian individuals who practice resistance training to gain muscle mass if they maintain a balanced diet with adequate amino acid consumption (Kleiner & Greenwood-Robinson, 2002). Therefore, vegetarians can replace animal protein sources with plant protein sources to meet their protein needs. This could include meat substitutes and their derivatives (Janssen et al., 2016) or protein-based food supplements (Possebon & Oliveira, 2006).

Protein is essential for athletes and sportsmen to achieve a positive protein balance, ensuring greater protein synthesis and promoting recovery from exercise, adaptation, and anabolism (Van Vliet et al., 2015). The search for alternative sources of nutrients, mainly proteins, is necessary due to the constant increase in consumer demand for protein supplements. Bacuri, a fruit from the Cerrado and Pantanal biomes of Brazil, may be a favorable alternative due to its richness in protein, monounsaturated and polyunsaturated fatty acids, fibers, carotenoids, and minerals (Hiane et al., 2003; Lima e Silva et al., 2014).

The development of a protein nutritional supplement formulated with bacuri almonds for vegetarian athletes was carried out, nutritional characterization was performed, and its shelf life was evaluated. Bacuri is an underutilized food source, and the development of food products that add nutritional and functional value, exploring native fruits, such as the bacuri fruit, as raw materials, can generate innovation and contribute to new research in the area, benefiting the health of the consumer.

Received 23 May, 2023.
Accepted 7 Oct., 2023.
[1]*Universidade Federal of Mato Grosso do Sul, Faculty of Pharmaceutical Sciences, Food and Nutrition, Campo Grande, Mato Grosso do Sul, Brazil.*
*Corresponding author: fabiane.sanches@ufms.br
Conflict of interest: nothing to declare.
Funding: none.

2 MATERIAL AND METHODS

2.1 Material

Bacuri fruits were collected in the city of Campo Grande in the state of Mato Grosso do Sul, Brazil, at the geographic coordinates -20.4600517 and -54.5962478, presenting the registration SisGen (National Management System) number A516D8. After collection, they were transported to the laboratories of the Universidade Federal de Mato Grosso do Sul (UFMS) for the acquisition and processing of almonds. For the protein supplement formulations, the following were used: lyophilized bacuri almond flour; *Natural Vegan protein supplement* from the *Max Titaniun*® brand, which has rice and pea protein sources in its formulation; xylitol from the *Atlhetica*® brand, dextrose from the *Athletica*® brand, *Cargil*® cocoa powder; and Nestlé® *ResourceThickenUp* food thickener and gelling agent, obtained locally.

2.2 Methods

2.2.1 Fruit processing

The fruits were washed and dried in a ventilated oven at 50°C for 3 days. The almonds were removed through a manual breaker and then crushed to obtain the whole flour, using Soxhlet for the extraction of lipids with subsequent elimination of possible solvent residue in a ventilated oven at 40°C, and then lyophilized.

For lyophilization, an aqueous extract was prepared, in which the defatted bacuri almond flour was blended with distilled water in an industrial blender at a concentration of 1:10 and stirred for 3 h. Afterwards, the suspension was centrifuged for 15 min at 2,400 rpm. The supernatant was collected separately, and the residue was washed twice with distilled water in a 1:2 ratio by repeating the centrifugation step between washes and collecting the supernatant in the same container for lyophilization (Vieira et al., 2008).

2.2.2 Nutritional characterization of bacuri almond flour

For the nutritional characterization of the amino acid profile of the freeze-dried flour, the *Spectra System* degasser (Thermo Separation Products) was used; the *Spectra System P4000* quaternary pump module (Thermo Separation Products); the Rheodyne injection valve; the Thermasphere TS-130 HPLC Furnace (Phenomenex—USA Torrance, CA); the Spectra System UV 2000 UV detection module (Thermo Separation Products); and the LUNA C18 100 Å 5μ, 250 × 4.6 mm column (Phenomenex—USA Torrace, CA). About 400 g of sample was homogenized and divided into four equal portions, two symmetrical portions were removed, and the process was repeated until the sample was reduced to approximately 20 g. Then, the sample was crushed in an analytical mill coupled to a cooling bath set at 20°C, at a temperature below 25°C, to prevent loss of moisture and degradation of some amino acids.

For the mineral profile, the content of calcium (Ca), copper (Cu), iron (Fe), phosphorus (P), magnesium (Mg), manganese (Mn), potassium (K), sodium (Na), and zinc (Zn) was determined in the lyophilized flour using an inductively coupled optical emission spectrometer with argon plasma (ICP-OES) brand iCAP 6000® (ThermoScientific, USA). The solutions used for the digestions and calibration curves were prepared with distilled-deionized water (18 MΩ cm, Milli-Q®, Millipore, Bedford, MA, USA) and added nitric acid (65% Sigma-Aldrich). Calibration solutions were prepared from dilutions of a standard solution of 100 mg L^{-1} of Cu, Fe, Mg, Mn, P, and Zn (Specsol, São Paulo, Brazil). Hydrogen peroxide (30% vv^{-1}) (Vetec, Rio de Janeiro, Brazil) and nitric acid were used in digestion.

The materials were decontaminated in a nitric acid solution (10% vv^{-1}) for 24 h. For the digestion of samples, about 0.2500 g were submitted to microwave-assisted digestion using 1.0 mL of concentrated HNO_3, 0.3 mL of H_2O, and 1.0 mL of H_2O_2 in a *Speedwave*® *four* microwave oven (Berghof, Germany).

Then, the quantification procedure was performed by Inductively Coupled Plasma Optical Emission Spectrometry—ICP-OES (Thermo Fisher Scientific, Bremen, Germany), iCAP model 6300 Duo, with axial view mode, using Argon with a high purity of 99.996% (White Martins-Praxair).

2.2.3 Development of supplement formulations

After several pre-tests for the development of protein supplements of vegetable origin from the bacuri almond, considering the nutritional, sensorial, and technological aspects, two final formulations were obtained. The first formulation, called F1, contained 70% Bacuri freeze-dried flour, 15% dextrose, 8% xylitol, 4% cocoa powder, and 3% thickener. The second formulation, described as F2, had 40% Bacuri freeze-dried flour, 40% commercial supplement, 12% dextrose, 5% xylitol, 3% cocoa powder, and 2% thickener.

2.2.4 Nutritional characterization of vegetable protein supplement formulations

The nutritional composition of the bacuri almond protein supplement formulations was determined using methodologies described by Instituto Adolfo Lutz (2005) in triplicate. The moisture of the samples was determined by desiccation (gravimetric method) in an oven at 105°C until constant weight. The ashes were determined gravimetrically by incineration in a muffle at 550°C; crude protein was evaluated by the micro-Kjeldahl method, multiplying the total nitrogen content (percentage) by the conversion factor (6.25); and lipids were determined by continuous extraction in a Soxhlet apparatus using petroleum ether as solvent. The carbohydrates were determined by their differences from the other components.

The total caloric value was determined according to Atwater and Bryant (1906), considering the following values: lipids (9.0 kcal/g), proteins (4.0 kcal/g), and carbohydrates (4.0 kcal/g).

The amino acid profile of the developed supplement formulations was determined by theoretical calculation, according to the results obtained for the determination of the amino acid profile of the freeze-dried flour, detailed in the "Nutritional characterization of bacuri almond flour."

2.2.5 Shelf-life test

The formulations were kept for 28 days, controlling temperature and humidity conditions. At the beginning (T0), 14 days (T1) and 28 days (T2) of controlled temperature conditions in an oven at 25°C were analyzed.

The following parameters were evaluated: analysis of the nutritional composition, microbiological analysis of molds and yeasts (Brazil, 2001), and sensorial analysis with a panel of trained tasters (n = 9) who analyzed the attributes like color, aroma, viscosity, appearance, and global acceptance by affective tests of preference in T0, T1, and T2 and difference in relation to a standard sample at times T0, T1, and T2. For the sensory analysis carried out in T0, the commercial supplement was used as a standard, and for the comparison of the attributes by the tasters in T1 and T2, the samples of the F1 and F2 formulations were vacuum packed, protected from light and humidity, and frozen to serve as a standard in subsequent assessments.

Two 9-point hedonic scales were used, where 9 = extremely better than the standard, 8 = much better than the standard, 7 = regularly better than the standard, 6 = slightly better than the standard, 5 = equal to the standard, 4 = slightly worse than the standard, 3 = regularly worse than the standard, 2 = much worse than the standard, and 1 = extremely worse than the standard (Dutcosky, 2019).

2.2.6 Ethical aspects

The study was submitted to the UFMS Ethics Committee and approved under protocol no. 2.355.448.

2.2.7 Statistical analysis

The data were expressed as the mean and standard deviation. The Kolmogorov-Smirnov test was used to verify data normality. After verification of normality, one-way analysis of variance (one-way ANOVA) was used to compare means using Tukey's *post hoc* test, and $p < 0.05$ was adopted as the significance level. The software used was SPSS (Statistical Package for the Social Sciences).

3 RESULTS

3.1 Nutritional characterization of bacuri almond flour

The amino acid profile of freeze-dried flour is shown in Table 1. The amino acid composition of freeze-dried bacuri almond flour was compared with the values found by Lima e Silva et al. (2014), who used hot defatted bacuri almond flour (DQ) in their study.

There was a considerable reduction in most amino acids after going through the aqueous extraction and lyophilization process when compared to flour defatted by hot extraction.

The mineral profile present in the freeze-dried bacuri almond flour was performed, according to the results shown in Table 2.

Table 1. Amino acid profile of hot defatted (DQ) bacuri almonds and freeze-dried flour.

Amino acids (g.100 g^{-1} of protein)	DQ*	Flour lyophilized
Essential amino acids		
Leucine	8.32	2.72
Valine	7.36	2.31
Isoleucine	3.90	1.40
Histidine	2.18	1.37
Methionine	2.71	0.95
Cysteine	0.68	2.02
Phenylamine	5.09	2.18
Tyrosine	1.92	1.14
Threonine	3.02	1.44
Lysine	5.74	2.65
Tryptophan	NA	NA
Non-essential amino acids		
Asparagine	8.91	2.99
Glutamine	14.12	11.48
Serine	5.95	1.86
Glycine	7.91	2.22
Arginine	11.21	9.61
Alanine	6.77	1.83
Proline	4.20	1.67

*DQ: Hot defatted bacuri flour.
Source: Lima e Silva et al. (2014).

Table 2. The mineral profile present in the freeze-dried bacuri almond flour, obtained by ICP-OES.

Element	Mean ± SD* (mg.100 g^{-1})		
	DQ*	Freeze-dried flour	RDI**
Cu	0.98	10.24 ± 0.18	0.9
Fe	2.2	1.90 ± 0.07	14.0
Mg	3.2	34.54 ± 3.71	260
Mn	190	3.86 ± 0.02	2.3
P	390	1,127.59 ± 7.22	700
Zn	2.1	2.17 ± 0.06	7.0

Mean ± standard deviation (SD) values obtained in triplicate; *DQ: Hot defatted bacuri flour, values obtained from the study by Damasceno Júnior and Souza (2010); **Recommended daily intake (RDI), Instituto Adolfo Lutz (2005).

After the lyophilization process, the values of Cu, Mg, and P had their levels increased when compared to the values of the minerals found in the hot defatted flour (DQ). The Fe and Zn contents remained close to the defatted flour values, showing a considerable reduction only in the Mn values.

3.2 Nutritional characterization of vegetable protein supplement formulations

Table 3 shows the nutritional composition of the commercial supplement (standard) and the formulations of the developed bacuri supplement (F1 and F2).

Comparing the chemical composition of the commercial supplements and formulations, it is observed that the three supplements differed significantly among themselves in all components. All products had a low amount of lipids, and F2 had an

Table 3. Nutritional composition of commercial vegetable protein supplements and formulations (F1 and F2) of protein supplements of bacuri almonds (g.100 g^{-1}).

Components	Nutritional composition Mean ± SD* (g.100 g^{-1})			
	Commercial Supplement	F1	F2	p-value**
Moisture	11.94 ± 0.46^b	15.36 ± 1.80^a	10.61 ± 1.01^b	0.008
Ashes	3.37 ± 0.05^c	6.9 ± 0.53^a	5.76 ± 0.23^b	0.0001
Protein	59.68 ± 0.83^a	43.01 ± 2.49^b	47.23 ± 1.24^b	0.0001
Lipids	2.73 ± 0.26^b	2.48 ± 0.01^b	3.12 ± 0.04^a	< 0.005
Carbohydrates	22.28 ± 0.04	32.24 ± 0.17	33.28 ± 0.63	—
Energetic value	352.41	323.68	350.12	—

*Average values ± standard deviation (SD) obtained by triplicate. F1 = 70% bacuri; F2 = 40% bacuri: 40% commercial. **ANOVA test. Average values followed by equal letters in the same line do not differ from each other by the Tukey's post hoc test at 5% probability.

average significantly higher than the others. Regarding the ash content, higher values were observed for F1, to the detriment of the others. Formulations F1 and F2 showed similar values of carbohydrates.

As for the protein values of the supplements, the focus of the study was that the F1 and F2 formulations had lower levels than the commercial supplement, but in sufficient quantities to be classified as protein supplements.

The protein values found in the commercial supplement are in accordance with the one reported on the product label, which indicates 60% protein for every 100 g; however, the amount of lipids (2.73%) was lower than the label (3.5%) of product.

The amino acid profiles of the F1 and F2 formulations are shown in Table 4.

The values of amino acids present in the commercial supplement showed higher levels when compared to the values of the F1 and F2 formulations, except for cysteine and arginine. This result was expected, considering that the protein content of the commercial supplement was significantly higher than that observed in the formulations.

Regarding the comparison of the values recommended by FAO (2013), both F1 and F2 presented lower values, approaching the contents of essential amino acids valine, isoleucine, histidine, and threonine, especially for F2. On the contrary, the levels of methionine+cysteine and phenylalanine+tyrosine were higher than recommended.

3.3 Shelf-life test

It was determined that the chemical composition, microbiological analysis of molds and yeasts, and sensorial analysis of the formulations were carried out at the beginning of the shelf-life test (T0), after 14 days (T1), and at the end of this test after 28 days (T2). This determination was performed on samples stored at an average temperature of 27.2°C, 27.6°C, and 27.8°C and relative humidity of 65.0%, 55.4%, and 52.9% for periods T0, T1, and T2, respectively.

Table 4. Amino acid composition of commercial vegetable protein supplements, formulations F1 and F2, and values recommended by FAO/WHO, expressed in g 100 g^{-1}.

Components	Supplement Commercial*	F1**	F2**	FAO/WHO standard***
Essential amino acids				
Leucine	5.25	1.9	3.19	6.60
Valine	3.00	1.62	2.12	2.60
Isoleucine	2.75	0.98	1.66	2.00
Histidine	1.25	0.96	1.05	1.00
Methionine	1.25	0.66	0.88	1.50[1]
Cysteine	0.75	1.41	1.11	
Phenylanine	3.25	1.52	2.17	2.50[2]
Tyrosine	2.75	0.8	1.56	
Threonine	2.00	1.01	1.38	1.50
Lysine	3.00	1.85	2.26	3.00
Tryptophan	NA	NA	NA	4.00
Non-essential amino acids				
Asparagine	6.50	2.09	3.8	
Glutamine	11.00	8.03	8.99	
Serine	3.25	1.3	2.04	
Glycine	2.75	1.55	1.99	
Arginine	5.25	6.72	5.94	
Alanine	3.00	1.28	1.93	
Proline	3.00	1.17	1.87	

*Values reported by the manufacturer; **Theoretical calculation obtained through the result expressed in Table 2. F1 = 70% bacuri; F2 = 40% bacuri: 40% commercial ***FAO et al. (2007); NA = not determined; [1]Methionine+cystine; [2]Phenylanine+tyrosine.

The chemical composition of samples F1 and F2 at the three test evaluation times is shown in Table 5.

It is possible to observe that formulation 1 (F1) presented a significantly different variation of protein during the test, where the values were lower in T1 when compared to the other evaluation times, and the moisture content in T0 was higher in relation to T1 and T2 (p = 0.0001), not differing in other nutrients. It was verified for formulation 2 (F2) that there was a difference over time only for humidity, which was significantly higher in T0 in relation to the other evaluated times.

Table 6 contains the results of the microbiological analysis of the shelf-life test.

The microbiological analysis carried out during the shelf-life test showed that the formulations presented stable microbial loads and were compatible with what is expected for powdered products that are innocuous to health. The evaluation of the products did not detect an increase in the microbial count under the evaluated conditions over the stipulated time.

The acceptance test comparing F1 and F2 regarding appearance, aroma, color, viscosity, and overall impression attributes was performed to verify possible sensory changes between the formulations developed over the 28-day shelf-life study. The results are shown in Table 7.

As can be seen in Table 7, there was no significant difference (p > 0.05) in the means of sensory attributes when comparing F1 with F2.

Table 5. Nutritional composition of bacuri protein supplement formulations in periods T0, T1, and T2 of the shelf-life analysis.

	Nutritional composition Mean ± SD*(g.100 g^{-1})				
Sample	**Lipids**	**Protein**	**Moisture**	**Ashes**	**Carbohydrates**
F1 T0	2.48 ± 0.007	43.01 ± 1.84^{a}	15.36 ± 1.34^{a}	6.90 ± 0.37	30.75 ± 0.17
F1 T1	2.63 ± 0.19	36.71 ± 0.23^{b}	6.70 ± 0.63^{b}	7.06 ± 0.30	46.89 ± 0.94
F1 T2	2.61 ± 0.21	41.07 ± 0.99^{a}	7.06 ± 0.51^{b}	7.19 ± 0.57	42.81 ± 0.20
p-value*	0.714	0.007	0.0001	0.701	—
F2 T0	3.12 ± 0.03	47.23 ± 1.24	10.61 ± 0.77^{a}	5.76 ± 0.17	33.28 ± 0.63
F2 T1	3.37 ± 0.32	44.70 ± 0.43	8.11 ± 0.26^{b}	5.94 ± 0.57	37.17 ± 0.45
F2 T2	3.37 ± 0.30	45.77 ± 1.27	7.47 ± 0.30^{b}	5.62 ± 0.17	37.10 ± 0.44
p-value**	0.616	0.115	0.003	0.706	—

Mean ± standard deviation (SD) values obtained in triplicate; F1 = 70% bacuri; F2 = 40% bacuri: 40% commercial *p-value comparing F1 over time using the ANOVA test; **p-value comparing F2 over time using the ANOVA test. Means followed by equal letters in the same column do not differ from each other by Tukey's post hoc test at 5% probability.

Table 6. Microbiological analysis of bacuri protein supplement formulations at times T0, T1, and T2.

	Microbial count of molds and yeasts	
	F1	**F2**
T0	9.0×10^{2} CFU/g	7.0×10^{2} CFU/g
	9.0×10^{2} CFU/g	6.0× 10^{2} CFU/g
	1.0×10^{3} CFU/g	1.0×10^{2} CFU/g
T1	6.0×10^{2} CFU/g	3.0×10^{2} CFU/g
	< 1.0×10^{2} CFU/g	1.0×10^{2} CFU/g
	3.0×10^{2} CFU/g	< 1.0×10^{2} CFU/g
T2	< 1.0×10^{2} CFU/g	1.0×10^{2} CFU/g
	2.0×10^{2} CFU/g	< 1.0×10^{2} CFU/g
	< 1.0×10^{2} CFU/g	1.0×10^{2} CFU/g

F1 = 70% bacuri; F2 = 40% bacuri: 40% commercial.

Table 7. Comparison of the means of the acceptance test of the sensory attributes of the formulations F1 and F2 of the protein supplement of bacuri during the shelf-life test.

Sensory attributes	F1 Mean ± SD	F2 Mean ± SD	p-value*
Time T0			
Appearance	7.78 ± 0.66	7.78 ± 1.56	1.000
Aroma	7.78 ± 1.20	7.22 ± 1.64	0.425
Color	7.89 ± 0.93	7.78 ± 1.64	0.862
Viscosity	7.89 ± 0.60	7.67 ± 0.86	0.536
Global acceptance	7.67 ± 0.86	7.89 ± 1.27	0.670
Time T1			
Appearance	7.78 ± 0.44	7.33 ± 0.86	0.195
Aroma	7.22 ± 1.20	6.89 ± 1.45	0.603
Color	7.67 ± 0.71	7.44 ± 1.01	0.597
Viscosity	7.78 ± 0.83	6.89 ± 1.05	0.065
Global acceptance	7.89 ± 0.78	7.11 ± 0.78	0.051
Time T2			
Appearance	8.33 ± 0.50	7.56 ± 1.67	0.211
Aroma	7.22 ± 0.83	7.22 ± 1.56	1.000
Color	8.11 ± 0.60	7.33 ± 1.12	0.090
Viscosity	8.33 ± 0.50	7.89 ± 1.05	0.270
Global acceptance	8.11 ± 0.33	7.89 ± 1.05	0.560

SD: Standard deviation; F1 = 70% bacuri; F2 = 40% bacuri: 40% commercial; *p-value comparing F1 and F2 at each time point for each sensory attribute using Student's t-test.

When analyzing global acceptance, consumers can be observed to have greater approval for F1 formulation over time, although without significant difference. The comparison was also made over time for each sensory attribute in the F1 formulation.

It was found that there was no significant difference over time for any sensory parameter evaluated for both F1 ($p = 0.214$) and F2 ($p = 0.328$), that is, no sensory change was evaluated in 28 days. However, when analyzing the interaction of time versus sensory attributes, it was observed for F1 that the average of the aroma in T2 (7.22) was significantly lower than the averages of the other attributes in time T2.

In addition to the assessments of the sensory acceptance tests during the shelf-life test, sensory difference tests were also carried out, for which the commercial supplement was used as a standard for comparison at the initial time (T0) and for the other times when formulations 1 and 2 were stored frozen in vacuum-sealed packages and protected from light, thus minimizing any type of degradation.

During the shelf-life test, formulation 1 was sensorially constant and was classified in the three evaluation periods between "slightly better than the standard" and "regularly better than the standard." Likewise, formulation 2, when compared to its standard in T1 and T2, showed the same degree of stability, remaining with means on the hedonic scale between "equal to the standard" and "slightly better than the standard."

Additionally, the difference test was compared over time for each sensory attribute for formulation F1.

For formulation 1, it was found that the sensory attribute variables ($p = 0.245$) and their interaction time versus sensory attribute ($p = 0.526$) had no statistical difference, with a significant influence only on the time variable ($p = 0.036$). Thus, a significant difference ($p = 0.011$) between times T0 and T1 was found only for the attribute viscosity, the other sensory parameters did not differ over the time of the shelf-life test for F1.

For all the attributes evaluated in T0, formulation 2 tended toward a better classification in relation to the other analysis times, with its averages remaining between "regularly better than the standard" and "much better than the standard."

However, there was no significant difference between times T0, T1, and T2 for any evaluated sensory attribute ($p>0.05$), despite the trend toward lower means at times T1 and T2.

4 DISCUSSION

The interest in using plant proteins and their derivatives in the food industry has grown. Research using fruits such as bacuri is necessary due to their nutritional importance, as they are rich in lipids, proteins, fiber, micronutrients, and bioactive compounds (Lima e Silva et al., 2014; Damasceno Jr. & Souza, 2010).

Bacuri almond lyophilized flour is a product that can be consumed in its gross form or added to other ingredients, in accordance with Brazilian legislation (Brazil, 2010), which provides guidelines for food for athletes. The legislation stipulates that protein supplements for athletes should contain at least 10 g of protein and 50% of their total energy value from proteins per serving. The freeze-dried bacuri almond flour, without the addition of ingredients, contains 25.7 g of proteins in 50 g (one portion), providing 102.8 kcal of energy value (Cunha et al., 2021), which meets the requirements of Brazilian law for protein supplements for athletes.

The amino acid profile of bacuri almond lyophilized flour is an important aspect to consider. A study by Lima e Silva et al. (2014) found a reduction in amino acid content in the lyophilized flour compared to the flour that underwent only the hot degreasing process. This reduction can be explained by the results of a study by Porte et al. (2010), which stated that aqueous solutions can accelerate the degradation of some amino acids due to the temperature and pH values to which they are subjected. In the present study, aqueous extracts were used at a concentration of 1:10 with a pH of 7.5 and a temperature of 45°C to obtain the lyophilized flour, which may have contributed to the reduction in amino acid content. It is also possible that some amino acids remained in the residue of the aqueous extractions that were carried out without being solubilized.

Vegetarian individuals consume less protein than omnivores, and thus optimizing protein intake, both in terms of quantity and quality, is important, especially for vegetarian individuals who engage in exercise. Vegetable protein sources are often incomplete, containing lower amounts of essential amino acids and branched chain amino acids, which are important for protein synthesis, compared to animal proteins (Phillips, 2016; Rogerson, 2017). Therefore, future studies should review the processing conditions for optimizing protein content through aqueous extracts and degreased flour obtained from bacuri almonds to preserve the appropriate amino acid profile as much as possible, as found in the study by Lima e Silva et al. (2014).

Micronutrient adequacy is also a matter of concern for athletes. Craig and Mangels (2009) pointed out that attention should be paid to achieving an adequate intake of minerals when designing a vegetarian diet. Poorly designed diets can lead to deficiencies in minerals, regardless of dietary preference, which can have detrimental implications for health and sports performance. Rogerson (2017) described in his study that the Academy of Nutrition and Dietetics recommends paying attention to achieving adequate intake of iron and zinc when designing a vegetarian diet, as female vegetarians tend to have lower iron reserves than omnivores and are more prone to iron deficiency anemia. Male vegetarians have similar iron status to nonvegetarians and are less impacted by iron status, but plant-based foods may pose issues with iron bioavailability, requiring vegetarians to ensure sufficient intake.

In summary, the National Health Surveillance Agency (ANVISA) in Brazil has established recommended daily intake (RDI) values for proteins, vitamins, and minerals with the aim of ensuring adequate nutrition for the population. The inclusion of freeze-dried bacuri almond flour as an ingredient in sports supplements can contribute to meeting these recommendations, as it is a rich source of proteins, fibers, micronutrients, and bioactive compounds. Furthermore, the stability of formulated products during storage, in accordance with ANVISA regulations, suggests that freeze-dried bacuri almond flour may be a viable option in the food industry. However, further studies are needed to optimize processing conditions and ensure the quality and safety of products prior to their incorporation into dietary supplements and other foods intended for human consumption.

5 CONCLUSION

The processing conditions for obtaining aqueous extracts and lyophilization used resulted in a flour that presented high levels of protein, but despite the optimization of the protein amount, there was a decrease in the amino acid profile in the conditions fulfilled.

In the shelf-life test, stability was obtained in the nutritional, microbiological, and sensory composition of the formulations under the evaluated conditions.

Bacuri almonds can therefore be considered an attractive raw material for consumption by the population, in particular, sportsmen, athletes, and vegetarians, and their application in the development of protein supplements of vegetable origin with commercial potential is feasible.

REFERENCES

Atwater, W. O., & Bryant, A. P. (1906). *The chemical composition of American food materials (No. 28)*. US Government Printing Office.

Brazil (2001). Resolution RDC nº 12, of January 2, 2001. Approves the Technical Regulation on microbiological standards for food. *Official Gazette [of the] Federative Republic of Brazil*, *139*(7-E), 45-53.

Brazil (2010). RDC-Resolution No. 18, of April 27, 2010. Provides for Food for Athletes. *Official Gazette [of] the Federative Republic of Brazil*.

Craig, W. J., & Mangels, A. R. (2009). Position of the American Dietetic Association: vegetarian diets. *Journal of the American Dietetic Association*, *109*(7), 1266-1282. https://doi.org/10.1016/j.jada.2009.05.027

Cunha, F. C., Semidei, R., Barbosa, I. P., Barbosa, L., Myagusku, L., Macedo, M. L. R., Hiane, P. A., & Sanches, F. L. F. Z. (2021). Desenvolvimento de suplemento alimentar proteico com amêndoas do bacuri (Attalea phalerata Mart. Ex Spreng.) para esportistas vegetarianos. In F. C. Cunha, R. Semidei, I. P. Barbosa, L. Barbosa, L. Myagusku, M. L. R. Macedo, P. A. Hiane & F. L. F. Z. Sanches (eds.). *Ciência e Tecnologia de Alimentos: pesquisa e práticas contemporâneas* (2ª ed., v. 2, p. 445-456). Científica Digital. Retrieved from https://www.editoracientifica.com.br/articles/code/210805844

Damasceno Jr., G. A., Souza, P. R. (eds.). (2010). *Sabores do Cerrado & Pantanal: receitas e boas práticas de aproveitamento*. Editora UFMS.

Dutcosky, S. D. (2019). *Análise sensorial de alimentos* (5ª ed.). PUCPress / Editora Universitária Champagnat.

Food and Agriculture Organization (FAO) (2013). Report on dietary protein quality evaluation in human nutrition: Recommendations and implications. *Nutrition Bulletin*, *38*(4), 421-428.

Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO) & United Nations University (2007). *Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation*. World Health Organization.

Hiane, P. A., Bogo, D., Ramos, M. I. L., & Ramos Filho, M. M. (2003). Provitamin A carotenoids and fatty acid composition of bacuri fruit and flour (Scheelea phalerata Mart.). *Food Science and Technology*, *23*(2), 206-209.

Instituto Adolfo Lutz (2005). *Métodos físico-químicos para análise de alimentos* (4ª ed.). Brazil: Ministério da Saúde. Retrieved from https://pesquisa.bvsalud.org/portal/resource/pt/sus-19225

Janssen, M., Busch, C., Rödiger, M., & Hamm, U. (2016). Motives of consumers following a vegan diet and their attitudes towards animal agriculture. *Appetite*, *105*, 643-651. https://doi.org/10.1016/j.appet.2016.06.039

Kahleova, H., & Pelikanova, T. (2015). Vegetarian diets in the prevention and treatment of type 2 diabetes. *Journal of the American College of Nutrition*, *34*(5), 448-458. https://doi.org/10.1080/07315724.2014.976890

Kleiner, S. M., & Greenwood-Robinson, M. (2002). Nutrition for strength training. In S. M. Kleiner & M. Greenwood-Robinson (eds.). *Nutrition for Strength Training* (pp. 238-238). Manole.

Lima e Silva, M. C. B., Hiane, P. A., Braga Neto, J. A., & Macedo, M. L. R. (2014). Proteins of Bacuri almonds: nutritional value and in vivo digestibility. *Food Science and Technology*, *34*(1), 55-61. https://doi.org/10.1590/S0101-20612014000100008

Lynch, H. M., Wharton, C. M., & Johnston, C. S. (2016). Cardiorespiratory fitness and peak torque differences between vegetarian and omnivore endurance athletes: a cross-sectional study. *Nutrients*, *8*(11), 726. https://doi.org/10.3390/nu8110726

Orlich, M. J., Singh, P. N., Sabaté, J., Fan, J., Sveen, L., Bennett, H., Knutsen, S. F., Beeson, L., Jaceldo-Siegl, K., Butler, T. L., Herring, R. P., & Fraser, G. E. (2015). Vegetarian dietary patterns and the risk of colorectal cancers. *JAMA Internal Medicine*, *175*(5), 767-776. https://doi.org/10.1001/jamainternmed.2015.59

Phillips, S. M. (2016). The impact of protein quality on the promotion of resistance exercise-induced changes in muscle mass. *Nutrition & Metabolism*, *13*(1), 64. https://doi.org/10.1186/s12986-016-0124-8

Porte, A., Rezende, C. M., Antunes, O. A. C., & Maia, L. H. (2010). Reduction of amino acids in bacuri pulp (*Platonia insignis* Mart), cupuaçu (*Theobroma grandiflorum* Willd ex-Spreng Schum) and murici (*Byrsonima crassifolia* L.) processed (heated and alkalized). *Acta Amazonica*, *40*(3), 573-577. https://doi.org/10.1590/S0044-59672010000300017

Possebon, J., & Oliveira, V. R. (2006). Consumption of supplements in physical activity: a review. *Disciplinarum Scientia | Health*, *7*(1), 71-82. https://doi.org/10.37777/904

Rogerson, D. (2017). Vegan diets: practical advice for athletes and exercisers. *Journal of the International Society of Sports Nutrition*, *14*(1), 36. https://doi.org/10.1186/s12970-017-0192-9

Slywitch, E. (2012). *Vegetarian diet food guide for adults*. Brazilian Vegetarian Society.

van Vliet, S., Burd, N. A., & van Loon, L. J. (2015). The skeletal muscle anabolic response to plant-versus animal-based protein consumption. *Journal of Nutrition*, *145*(9), 1981-1991. https://doi.org/10.3945/jn.114.204305

Vieira, C. R., Lopes Jr., C. D. O., Ramos, C. S., Capobiango, M., & Silvestre, M. P. C. (2008). Extração enzimática das proteínas da farinha de arroz. *Food Science and Technology*, *28*(3), 599-606. https://doi.org/10.1590/S0101-20612008000300015

Yokoyama, Y., Nishimura, K., Barnard, N. D., Takegami, M., Watanabe, M., Sekikawa, A., Okamura, T., & Miyamoto, Y. (2014). Vegetarian diets and blood pressure: a meta-analysis. *JAMA Internal Medicine*, *174*(4), 577-587. https://doi.org/10.1001/jamainternmed.2013.14547