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Quality of high-protein diet bar plus chia (*Salvia hispanica* L.) grain evaluated sensorially by untrained tasters

Natalie VEGGI[1]*, Fabrício Azevedo VOLTARELLI[2], Jeann Marcos Nascimento PEREIRA[3], Wanessa Costa SILVA[4], James Wilfred NAVALTA[5], Daniela Fernanda Lima de Carvalho CAVENAGHI[3], Wander Miguel de BARROS[1]

Abstract

The objective of this study was to develop, analyze composition and evaluate the microbiological and sensory characteristics of high-protein diet bars (PB) with the addition of chia grain (*Salvia hispanica* L.), partially replacing isolated soy protein and concentrated whey protein, in proportions of 0, 10, 15 and 20%. The proximate composition was analyzed of PB, for microbiological quality of *Bacillus cereus*, Filamentous fungi and yeast count, total fecal coliforms, and *Salmonella* ssp. search. Sensory analysis was performed utilizing acceptance testing of characteristics on a nine-point hedonic scale for various attributes, including purchasing intention of the tested PB. Bars showed 20% moisture, 2.3% ash, 20-23% protein and 19% lipids. The effect of increasing of chia was to increase crude fiber content and decrease total carbohydrate and total energy value. All samples were within the microbiological food standards established by current legislation. All PB formulations obtained a good overall impression index and all characteristics were above mean grades, with the exception of taste (63%) in the PB containing 0% chia. Chia grain has a positive influence on sensory aspects and appears to be an alternative way to increase the nutritional quality of high-protein diet bars.

Keywords: high-protein diet bars; functional food; oleaginous.

Practical Application: High-protein bar with chia (low sugar, rich in proteins and fibers) had good acceptability between the tasters.

1 Introduction

With the displacement of rural society to urban centers, there has been a change in eating habits and, due to accelerated routines that do not accommodate traditional dining practices, individuals have sought to feed on quick, easily obtainable products, known as "fast-foods" which are rich in saturated fatty acids and substitute natural elements with processed and refined components. When added to the sedentary lifestyle that is prevalent in modern society, the result is increased physiological stress, which increases the chances of developing chronic diseases, such as cardiovascular complications, cancer, cerebrovascular accident, atherosclerosis, liver diseases, and type 2 diabetes *mellitus*.

Food bars are convenient and practical for nutrient intake, representing an alternative food supplement based on carbohydrates, proteins and fibers and, in addition, can be supplemented by some health-promoting substances, such as antioxidants (Peuckert et al., 2010), which is a current trend in the food sector.

Chia (*Salvia hispanica* L.) is a grain that can be considered a functional food, which is nutritionally rich in fiber and contains beneficial substances to health. It is composed of protein (15-25%), oils (30-33%), carbohydrates (26-41%), dietary fiber (18-30%), ashes (4-5%), minerals, vitamins and antioxidants, such as tocopherols, phenolic compounds and polyphenols. Chia has high levels of polyunsaturated fatty acids, and is considered the highest plant source of alpha-linolenic acid (Omega-3), which provides an exceptionally high content of linoleic acid (Omega-6) (Ayerza & Coates, 2011; Jiménez et al., 2013). The main benefits of Chia to health include the reduction of issues related to constipation, reduced risk of cardiovascular diseases, and protection against some types of cancer (Sierra et al., 2015; Oliva et al., 2013).

In the year 2000, the U.S. Dietary Guidelines recommend the ingestion of Chia as a primary food source, not exceeding the amount of 48 g/day (Mohd Ali et al., 2012) and the European Commission approved the use of this grain for baking purposes (Borneo et al., 2010). In an interesting study which investigated factors that influence the consumption of "diet/light" products it was observed that individuals are aware of healthy eating practices and have higher purchasing power, and also have a higher chance of avoiding the emergence of chronic non-communicable diseases, such as hypertension, diabetes mellitus, and cancer (Hall, 2006).

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[1] *Programa de Pós-graduação em Ciência e Tecnologia de Alimentos, Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso – IFMT, Cuiabá, MT, Brasil*
[2] *Programa de Pós-graduação em Ciências da Saúde, Faculdade de Medicina, Universidade Federal de Mato Grosso – UFMT, Campus Cuiabá, Cuiabá, MT, Brasil*
[3] *Curso de Engenharia de Alimentos, Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso – IFMT, Campus Bela Vista, Cuiabá, MT, Brasil*
[4] *Programa de Pós-graduação em Engenharia de Alimentos, Faculdade de Engenharia de Alimentos, Universidade de Campinas – UNICAMP, Campinas, SP, Brasil*
[5] *Department of Kinesiology and Nutrition Sciences, University of Nevada, Las Vegas, NV, United States of America*
*Corresponding author: veggi.natalie@gmail.com

Therefore, the aim of this study was to develop a functional product in the form of a high-protein diet bar based on soy protein and whey, with the addition of chia, in order to obtain an easily consumed food with low levels of sugars, protein and rich in polyunsaturated fatty acids, especially Omega-3 and Omega-6, as well as greater acceptability, given the growing demand for functional foods aiming healthy eating habits.

2 Materials and methods

2.1 Material

The ingredients used for the production of protein bars (PB) were: Isolated soy Protein 90% (ISP) (Chá e Cia®), Concentrated Whey Protein (CWP) 80% (Elmar®), food hydrolyzed collagen (Estação do Grão®), oat bran and Chia (Estação do Grão®), sucralose sweetener (Linea®), soy lecithin emulsion (Grings®), Sorbitol 70% solution (Native®), oily acetate vitamin E (Native®), glycerin (Native®), palm fat (Tauá®), flavoring orange (Mix®) and diet milk chocolate (Harald®). The other ingredients, such as sodium chloride and anhydrous citric acid, were purchased in markets located in the city of Cuiabá, Mato Grosso, Brazil.

2.2 Formulations of PB

A formula for PB (PB1) and three other formulations were developed, with partial replacement of ISP and CWP for whole grain chia. The total weight of each PB formulation was 100 g, with ingredients as described in Table 1; with the four formulations containing a chia content ranging from zero (standard) to 10% (PB2), 15% (PB3) and 20% (PB4).

Table 1. Ingredients used in the PB formulations.

Ingredients	PB1	PB2	PB3	PB4
Dry ingredients				
ISP 90% (g)	14.5	13.05	12.325	11.6
CWP 80% (g)	14.5	13.05	12.325	11.6
Oat bran (g)	12.0	12.0	12.0	12.0
Collagen (g)	3.3	3.3	3.3	3.3
Sucralose* (g)	1.5	1.5	1.5	1.5
Citric acid (g)	0.3	0.3	0.3	0.3
Salt (g)	0.3	0.3	0.3	0.3
Chia (g)	0	2.9	4.35	5.8
Wet and semi-solid ingredients				
Soy lecithin (g)	7.0	7.0	7.0	7.0
Sorbitol (g)	6.7	6.7	6.7	6.7
Flavoring (g)	2.0	2.0	2.0	2.0
Palm fat (g)	4.0	4.0	4.0	4.0
Vitamin E (g)	0.4	0.4	0.4	0.4
Glycerin (g)	0.2	0.2	0.2	0.2
Diet chocolate (g)	33.3	33.3	33.3	33.3
Water (mL)	15.0	15.0	15.0	15.0
Total (g)	100.0	100.0	100.0	100.0

*Diluted 1:50 g concentrated whey protein; ISP 90% = Isolated soy Protein 90%; CWP 80% = Concentrated Whey Protein 80%; PB1 = Protein Bar 0% chia; PB2 = Protein Bair with 10% chia grain; PB3 = Protein Bar with 15% grain chia; PB4 = Protein Bar with 20% grain chia.

The dry ingredients were homogenized, except for calcium chloride and citric acid, which were dissolved in 15 ml of water in a separate container and, after mixing the wet and semi-solid ingredients, portions of approximately 14 grams were formed, which were molded into a PVC (polyvinyl chloride) baking tin and covered with diet milk chocolate, totaling 20 grams of final mass, becoming similar to currently marketed protein bars. Finally, the PB were packed and wrapped and refrigerated (Electrolux, Air Flow System, model DC40,Brasil) (10 to 14 °C).

2.3 Centesimal analysis

Centesimal analysis was carried out according to the methods described by the Association of Official Analytical Chemists (2012). Moisture was determined by the gravimetric method in heater (Nova Ética, model 400/2ND-300. Brazil) oven at 105 °C by the 925.09 method, ashes by incineration of residue obtained in a furnace (Quimis-SP, model D21, Brazil) at 550 °C through the 923.03 method, lipids by Soxhlet (Tecnal, model TE 044, Brazil), according to the method 920.39, and protein by modified kjeldahl (Tecnal, model TE 0363, Brazil) (method 991.20 and 6.25 conversion factor). Crude fibers were determined according to Cecchi (2003) and Bobbio & Bobbio (2001) and carbohydrate content by difference, as resolution No. 360, December 23, 2003 (Brasil, 2003). The caloric value (kcal/100.g^{-1}) was determined by the sum of the values of conversion, which considers 4 kcal.g^{-1} for proteins and carbohydrates and 9 kcal.g^{-1} for lipids.

2.4 Microbiological analysis

Microbiological analysis was carried out according to RDC nº 12, 02 January 2001, of the Anvisa (Brasil, 2001), which regulates Microbiological Standards for Foods. The procedures described by Silva et al. (2010) were performed by indicative samples. *Salmonella* ssp. by International Organization for Standardization (2007); *Bacillus cereus* and filamentous fungi and yeasts by direct count method on plates (Bennett & Belay, 2001). Total and thermotolerant coliforms were determined by the Most Probable Number (MPN) technique.

2.5 Sensory analysis

Sensory evaluation was performed through the Affective Method using the Preference/Acceptability test (Dutcosky, 1996), which was applied to Team Nogueira® and AFC®, located in the city of Cuiabá, Mato Grosso, Brazil. A total of 50 male and female untrained tasters (i.e., had no previous contact with the PB used in this study) were employed, between the ages of 18 and 50 years old.

All subjects were informed about the experimental protocol and provided written consent to participate in the study. The project was approved by the Committee of Ethics in Research Involving Humans of the Mato Grosso State University (UNEMAT), as it regulates the resolution 196/96 (Brasil, 2012) of the National Council of Health (Process number: 48601615.3.0000.5166).

Affective testing was employed to determine perceived acceptability according to a hedonic likert-like scale that included nine points, ranging from "really like" (9 points) to "extremely

dislike" (1 point). The attributes that were evaluated were aroma, flavor, texture, color and overall impression. The Acceptability Index (AI) percentage was determined by the values obtained from the hedonic scale test, which multiplies the mean points for 100 and divides the resulting value by the maximum score, according to Equation 1 below:

$$\%AI = \left(\frac{\text{mean of acceptability}}{9} \right) \times 100 \quad (1)$$

where: AI = Acceptability Index.

The purchasing intention test was evaluated by Affective Test, using a five-point scale, ranging from "certainly would buy" (5) "certainly wouldn't buy" (1). The results were evaluated by the frequencies allocated in that scale of intent.

2.6 Statistical analysis

With regard to the centesimal analysis, the statistical design was performed with four repetitions, all in triplicate, with normality determined by the Shapiro-Wilk method. Parametric data (via analysis of variance [ANOVA]) and nonparametric data (Scott-Knott test) were subsequently evaluated, followed by post-hoc Tukey test to identify differences as warranted (ASSISTAT 7.7 version). The sensory analysis of PB acceptance and the frequency histogram, applied to describe the purchasing intention of PB, were calculated with the aid of Microsoft Excel® version 2010.

3 Results

No significant differences were observed with respect to humidity, ashes, proteins and lipids when considering the centesimal analysis of these components (Table 2). Significant differences for crude fiber were evident among the samples, demonstrating that the amount of chia is directly related to the percentage of fiber. It is important to note that the increasing replacement of protein by chia decreased the carbohydrate content and, consequently the total energy value (TEV).

The results of the microbiological analysis of the formulated bars are presented in the Table 3. The data show that all samples are in compliance with the microbiological standards set forth by RDC n° 12, 02 January 2001, ANVISA (Brasil, 2001). Therefore, it can be affirmed that the hygiene, handling and storage procedures of the bars ensured microbiological safety of these products.

Sensory analysis of PB by unfamiliar testers demonstrated that most attributes presented values higher than 70% on the Acceptability Index, with the exception of the flavor in BP1, which was rated at 63.5% (Table 4).

Forty-six percent of untrained tasters preferred the BP4 formulation (Figure 1), indicating that the PB with the highest percentage of chia positively affected the characteristics of the product.

The purchasing intention, shown in Figure 2 (according to sample taster's favorite), shows that 26%, who chose the BP4, attributed score 5, 10% score 4, 6% score 3, and 2% score 1; the formulation B3 was the second most selected (obtained score 5 of 8% of the tasters).

The tasters who consumed diet/light products represented 68% of the sample population, and 32% did not make a conscious effort to consume these products. With regard to the frequency of use of PB, only 26% of the tasters consume PB twice or more per week, while 20% did consume PB at all (Table 5).

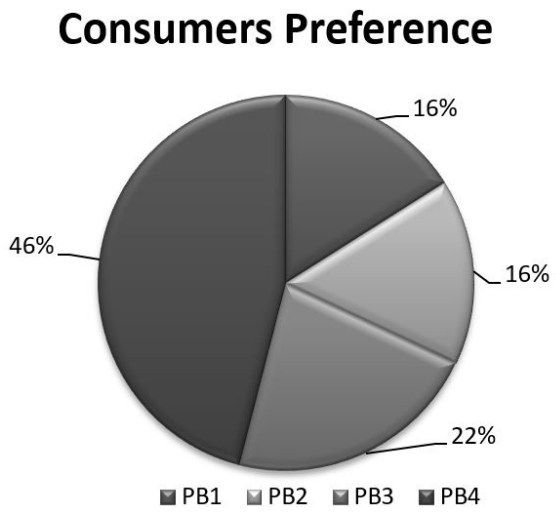

Figure 1. Preference of PB. PB1 = Protein Bar 0% chia; PB2 = Protein Bair with 10% chia grain; PB3 = Protein Bar with 15% grain chia; PB4 = Protein Bar with 20% grain chia.

Table 2. Results of centesimal analysis in reference to the 4-protein bar (PB) formulations.

Constituents	Formulations				CV(%)
	PB1	PB2	PB3	PB4	
Moisture[1] **(%)**	20.39 ± 1.63[a]	19.78 ± 0.40[a]	20.34 ± 1.03[a]	19.53 ± 0.56[a]	8.13
Ashes[1] **(%)**	2.30 ± 0.04[a]	2.36 ± 0.04[a]	2.39 ± 0.12[a]	2.40 ± 0.05[a]	6.28
Proteins[2] **(%)**	20.28 ± 1.26[a]	23.42 ± 2.57[a]	22.65 ± 1.06[a]	21.44 ± 1.65[a]	7.92
Lipids[2] **(%)**	19.09 ± 1.27[a]	19.69 ± 0.61[a]	19.25 ± 1.22[a]	19.72 ± 0.71[a]	5.15
Crude Fibres[2] **(%)**	12.36 ± 0.47[c]	15.52 ± 1.17[b]	20.41 ± 0.57[a]	22.16 ± 0.98[a]	4.84
Carbohydrates[2]*** (%)**	25.65 ± 1.37[a]	19.21 ± 3.02[b]	14.94 ± 1.23[c]	14.10 ± 2.34[c]	7.53
TEV[2]**** (Kcal/100g)**	355.53[a]	347.77[a]	323.68[b]	319.70[b]	2.56

The results are expressed as Mean ± Standard Deviation. Means with equal letters, in the same line, do not differ in significance level of 5% by [1]Scott-Knott test and [2]Tukey; *Carbohydrate content calculated by difference; **TEV = Total Energy Value; CV = coefficient of variation; PB1 = Protein Bar 0% chia; PB2 = Protein Bair with 10% chia grain; PB3 = Protein Bar with 15% grain chia; PB4 = Protein Bar with 20% grain chia.

Table 3. Results of microbiological analyses.

Microbiological analyses (CFU/g)	Protein Bars			
	PB1	PB2	PB3	PB4
Coliforms count at 45 °C	< 50	< 50	< 50	< 50
Total coliforms count	< 50	< 50	< 50	< 50
Bacillus cereus count	< 500	< 500	< 500	< 500
Search for *Salmonella ssp*.	absent	absent	absent	absent
Filamentous fungi and mould count	< 50	< 50	< 50	< 50

CFU/g = colony-forming unit per gram of sample. Microbiological standards for cereal bar (Brasil, 2001): Coliforms count at 45 °C: 5 x 10^2 CFU/g; *Bacillus cereus* count: 5 x 10^3 CFU/g; Search for *Salmonella* ssp. in 25g: absent; PB1 = Protein Bar 0% chia; PB2 = Protein Bair with 10% chia grain; PB3 = Protein Bar with 15% grain chia; PB4 = Protein Bar with 20% grain chia.

Table 4. Acceptability Index (%) of PB in relation to aroma, flavor, texture, color and overall impression.

Formulations	Attributes				
	Aroma	Flavor	Texture	Color	Overall Impression
PB1	77.77%	63.55%	77.77%	86.44%	74.66%
PB2	82.66%	74.44%	81.77%	87.55%	81.55%
PB3	81.77%	75.55%	83.55%	88.88%	83.77%
PB4	79.11%	79.77%	83.33%	88.66%	83.55%

PB1 = Protein Bar 0% chia; PB2 = Protein Bair with 10% chia grain; PB3 = Protein Bar with 15% grain chia; PB4 = Protein Bar with 20% grain chia.

Table 5. Frequency of consume of PB of the tasters.

Frequency of consume	%
Twice or more a week	26
Once a week	18
Each 15 days	16
Once a month	20
Never	20

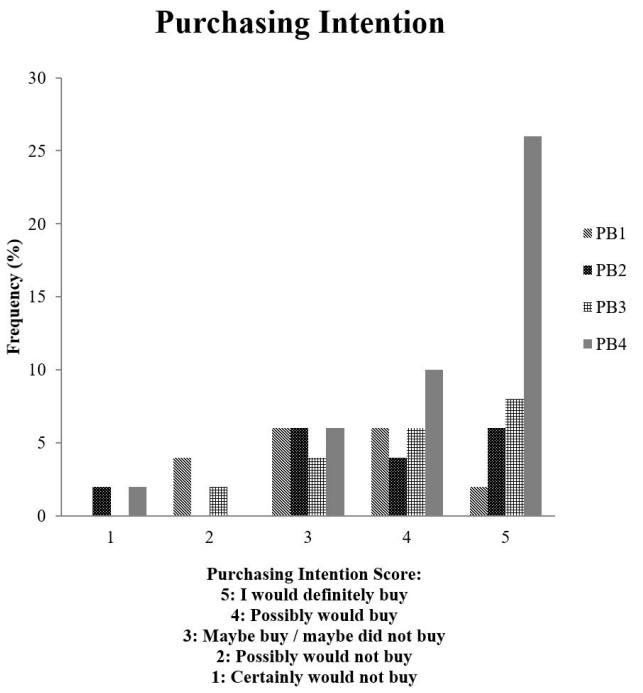

Figure 2. Frequency histogram of scores of purchasing intention of BP. PB1 = Protein Bar 0% chia; PB2 = Protein Bair with 10% chia grain; PB3 = Protein Bar with 15% grain chia; PB4 = Protein Bar with 20% grain chia.

4 Discussion

Our results show that the moisture of PB ranged between 19 and 20.4%, which are similar to values reported by Nadeem et al. (2012); on the other hand, they found lower values for protein (14.96%), lipid (8.37%) and fibre (3.88%). The quantification of ashes represents the amount of non-volatile minerals present in the food. Our results showed no significant difference with respect to ash composition; however, we observed a slight increase in relation to the proportion of chia. Atala (2015) and Coelho & Salas-Mellado (2015) also reported that there is a relationship between the amount of minerals and the percentage of chia in their products. Nadeem et al. (2012) and Farias et al. (2018) reported a growing trend in the amount of ashes with the addition of CWP, with calcium being the most quantified salt. Moreover, Parreiras et al. (2014) observed that 100 g of CWP contains 600 mg of calcium, which is an essential mineral for maintaining health and homeostasis of several organic functions.

The PB pioneer was Morgan, who patented his formula in 1974. The main protein sources reported were casein and lactalbumin; the centesimal composition was 35-40% protein, 30-40% fat and 20%-35% carbohydrate and approximately 250 Kcal/bar, which was developed to supply the military and individuals engaging in physical exercises (Morgan, 1974). Since that time, PB formulations have evolved in order to satisfy a wide range of consumers that have varied health aims. One of the main changes is the protein source employed, however a relatively few number of PB on the market utilize soy and milk proteins as exclusive sources (Loveday et al., 2009).

The protein content of bars in the present study were greater than the values of high-protein food bars designed by Freitas & Moretti (2006) and Baú et al. (2010) (PB1 = 4.05%; PB2 = 4.68%; PB3 = 4.53%; PB4 = 4.28%). On the other hand, PB in the current investigation were lower in protein content compared with commercial products, since these formulations include isolated soy, concentrated whey protein, textured soy protein, and albumin.

The values of lipids in our study were higher compared to those reported by Nadeem et al. (2012) because our PB contained a chocolate covering. Furthermore, the PB developed by Farias et al. (2018) showed higher levels of proteins and crude fibre, can be explained by the use of roasted soybeans as a topping. Atala (2015) verified that there is a significant increase in lipid content when Chia is added into the formulations, and Brito & Moreira (2016) and Coelho & Salas-Mellado (2015) have conformed these findings. These results can be explained, at least in part, by the fact that chia in flour form, has a greater contact surface, thus facilitating the extraction of lipids which may become more available than whole grain.

In the present study, the replacement of proteins by Chia grain significantly increased the levels of fibre in the PB1 (12.36%) and PB4 (22.16%) formulations, which can be very advantageous. Similar findings have been reported by Atala (2015) (bars) and Coelho & Salas-Mellado (2015) (elaborated breads). It is important to note that the fibre content of Chia can be as high as 37% (Marineli et al., 2014). Foods with higher fiber content have slower absorption due to delayed gastric emptying and decreased gastrointestinal transit time, both phenomena that are known to prevent the occurrence of glycemic peaks (Giuntini & Menezes, 2011). The results obtained by Salmerón et al. (1997) and Schulze et al. (2004) provide evidence that foods with a high glycemic load, associated with a low amount of cereal fibers, increases the chances of developing type 2 diabetes *mellitus*. In addition, these authors suggest that Chia should be eaten in a minimally refined way to reduce and/or prevent the occurrence of this pathophysiology.

Regarding the percentage of carbohydrates, there were no significant differences between the PB3 and PB4 formulations, but differences between PB1 and PB2 were observed. Additionally, there was a decrease in values in relation to the percentage of Chia as CWP contains carbohydrates from raw material, so the total energetic value was also reduced. Consequently, each of the four PB formulations in the current investigation was lower compared to commercial PB. These results corroborate the findings of Costantini et al. (2014), which compared the PB with added Chia to the control formulation (containing no Chia).

Physico-chemical, nutritional, sensory and microbiological aspects are essential to ensuring the quality of a product. These characteristics help to determine a favorable choice by the consumer. Therefore, characteristics such as aroma, flavor, texture, color and overall appearance are essential in the development of new products (Dutcosky, 2013). Furthermore, the A.I. must be at least 70% in order for a product to be considered acceptable (Teixeira et al., 1987).

In the present study, the attribute of flavor in PB1 obtained mean A.I. score of 63.5%, which is less than recommended by Teixeira et al. (1987). Alternatively, the BP4 formulation obtained highest mean percentage (79.77%) with respect to the A.I score for flavor. Although the formulations looked dark in color (evaluated with the naked eye, only), the A.I. for the color attribute obtained higher percentages for BP3 (88.88%) and BP4 (88.66%), being more attractive than BP1 in this regard. The remaining attributes (texture, flavor and overall impression) were sensorially well accepted by tasters, regardless of the concentrations of chia used. In another study (Chiareli et al., 2017), cakes made with chia seed, chia and oat flour were well accepted by consumers and 86% would buy.

The PB4, followed by PB3 formulation, was a favorite of tasters, a fact that demonstrates the versatility of Chia, because this grain can be added into food and/or in the formulation of food products (Brito & Moreira, 2016; Coelho & Salas-Mellado, 2015; Atala, 2015). Dutra et al. (2015) reported that, independent of the concentration of chia seed flour used as a partial replacement of wheat flour in the bread produced, consumers are very receptive to this new product.

This work presents some limitations, such as the absence of any specific analysis of dietary fiber. Further studies are needed in order to evaluate and identify the sensory profile of consumers and contribute to the best choice of food components, mainly with regard to physical exercise practitioners, which seek for foods that are able to be consumed quickly, are economically viable, tasty and, above all, nutritious and healthy.

5 Final considerations

Incorporating chia's whole grain in the formulations provided an increased nutritional result for PB4, which showed higher crude fiber content as well as a reduced value of total carbohydrates and TEV. Different chia concentrations affected the acceptance of the constituted formulas, with the flavor of PB1 lower than recommended, whereas the other formulations were well accepted. Importantly, we found that PB4 obtained both a higher frequency of preference as well as a higher score on the purchasing intention test.

These results demonstrate that it is possible to use alternative protein-based formulations without added sugar, that are well accepted sensorially. Because of this, it is possible such a product would appeal to both practitioners of physical exercise (healthy population) as well as patients with diabetes *mellitus* (special population). As this product responded to the expectations of consumers not only with regard to organoleptic requirements but also in terms of practicality for consumption, we recommend the addition of 20% of chia to the PB formulation. Further studies are indispensable in order to assess potential subchronic effects of PB with added chia grain on blood biochemical profile (health biomarkers). Finally, due to the scarcity of research incorporating PB, an analysis of physical- chemical interactions, such as color, water activity, texture, pH and acidity are necessary.

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