

Research Article

Effect of Marolo (*Annona crassiflora* Mart.) Pulp Flour Addition in Food Bars

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Due to increasing consumer demand for healthy foods, attempts are being made to improve their nutritional value. Thus, there is a necessity to develop food bars enriched with dietary fiber and palatable nutritional components. The aim of this study was to evaluate the shelf life of food bars, prepared with different proportions of marolo pulp flour (20, 30, 40, and 50% replacement of oatmeal) for six-month storage, through nutritional and physical analysis, as well as sensory acceptance. The total dietary fiber content showed an average of $7.1 \text{ g} \cdot 100 \text{ g}^{-1}$. There was a significant increase in antioxidant activity, vitamin C, and total carotenoids content according to increasing concentration of marolo pulp flour in the food bars. In sensory evaluation, the food bar with addition of 50% marolo pulp flour showed higher averages for all evaluated attributes and was the favorite by the evaluators.

1. Introduction

Brazil is a country with enormous biodiversity, and Cerrado is its second largest biome, with many native species that produce fruits with excellent nutritional, sensorial, and functional potential. However, most of these fruits remain poorly explored scientifically [1].

The marolo (*Annona crassiflora* Mart.) is a typical fruit of the Brazilian Cerrado and is among the 20 most common species in regional food [2]. da Silva et al. [3] determined the physical and chemical characteristics of the marolo flour and found $29.2 \text{ g} \cdot \text{kg}^{-1}$ of protein, $48.3 \text{ g} \cdot \text{kg}^{-1}$ lipid and $174.3 \text{ g} \cdot \text{kg}^{-1}$ of dietary fiber, and $1830.6 \text{ mg} \cdot \text{kg}^{-1}$ vitamin C.

The fiber constituting the edible portion of plants is resistant to digestion and absorption in the small intestine, playing an important role in gastric emptying and control of peristalsis. It fulfills an important role in nutrition, promoting

beneficial effects for health and may delay the onset of chronic diseases and improving the quality of life [4].

Due to the increasing consumer demand for healthy food, attempts are being made to improve the nutritional value of foods such as cereal bars [5]. The consumption of such food has grown due to its convenience, with the change in people's lifestyle.

The expanding market for cereal bars and foods recognized as healthy products is leading the industry to diversify the variety of flavors and attributes, such as products enriched with nutrients; products developed for a specific group of people or with new features that benefit health [6, 7].

Given the above, we see the need to develop food bars enriched with dietary fiber, nutritional components, and palatable taste. Therefore, the aim of this study was to evaluate the shelf life of food bars, made with marolo pulp flour in different concentrations, during the period of 6 months,

TABLE 1: Formulation of the food bars produced with marolo pulp flour coming from the southwest of the state of Goiás, Brazil.

Ingredients	Formulation (g·100 g ⁻¹)				
	F1	F2	F3	F4	F5
Rice flakes	15.9	15.9	15.9	15.9	15.9
Oatmeal	24.1	19.28	16.87	14.46	12.05
Flour marolo	0	4.82	7.23	9.64	12.05
Sorbitol	5	5	5	5	5
Salt	0.3	0.3	0.3	0.3	0.3
Brown sugar	4.6	4.6	4.6	4.6	4.6
Soy lecithin	0.4	0.4	0.4	0.4	0.4
Glucose syrup	28.79	28.79	28.79	28.79	28.79
Vegetable fat	3.58	3.58	3.58	3.58	3.58
Gum acacia	2.99	2.99	2.99	2.99	2.99
Water	12.34	12.34	12.34	12.34	12.34
Maltodextrin	2	2	2	2	2
<i>Total</i>	100	100	100	100	100

F1: Control Food Bar; F2, F3, F4, and F5: food bars prepared with, respectively, 20, 30, 40, and 50% of marolo flour in substitution of oatmeal.

through physical, chemical, and antioxidant activity and nutritional analysis as well as sensory acceptance.

2. Material and Methods

To conduct the study, marolo (*Annona crassiflora* Mart.) was acquired by local vendors in native Cerrado area of the southwestern state of Goiás, Brazil. The other ingredients for the development of food bars were received as a donation by the Food Technology Institute (ITAL).

2.1. Preparation of Marolo Pulp Flour. The fruits were washed and sanitized with sodium hypochlorite solution at 300 ppm for 30 min. The pulp was separated from the seed using a pulper (Hauber Macanuda JEM-05). The pulp was dehydrated at 65°C for 48 hours in an oven with forced air circulation until it reached the final moisture content of 7%, and then the pulp was milled in an industrial mixer to obtain the flour, which was then stored in a freezer at the temperature of -4°C.

2.2. Processing of Food Bars. The development of the food bars was performed at the Food Technology Institute (ITAL), in Campinas, Brazil. The bars had their base formulation comprising dry ingredients (marolo pulp flour, rice flakes, and oatmeal) and binders (soybean lecithin, glucose syrup, vegetable oil, sorbitol, maltodextrin, acacia gum, salt, sugar, and water) as presented in Table 1. The dry ingredients and binders were sanitized separately, and the latter were heated in the mixing pot, when then the dry ingredients were added. The dough was placed on a laminating table and, after cooling, the bars were cut into uniform pieces approximately 30 g, packed in flexible laminated packaging, labeled, and stored at room temperature until the performed of the nutritional, physical, microbiological, and sensory analysis.

2.3. Physical, Nutritional, and Microbiological Analyses. The analyses were performed at the Physics and Chemistry Laboratory and LABMULTI, at the Sector of Food Engineering (School of Agronomy); at the Food Chemistry and Biochemistry laboratory (School of Pharmacy); at the Laboratory of Food Hygiene and Health Control (School of Nutrition), all located at the Federal University of Goiás (UFG), Brazil; and at the Food Chemistry and Biochemistry Laboratory, Federal University of Lavras (UFLA), Brazil. All analyses were performed in triplicate.

The analyses of proximate composition of the food bars (moisture, ash, total lipids, protein, dietary fiber, and carbohydrates) were performed only at months 0 and 6.

2.3.1. Proximate Composition. Proximate composition was performed according to the methods proposed by the AOAC [8], except for the total lipid content that followed the method of Bligh and Dyer [9]. The moisture content was determined by drying at 105°C until weight was constant. The determination of ash was performed by the gravimetric incineration method in muffle furnace at 550°C. The protein content was determined by the Kjeldahl method, considering 6.25 as the conversion factor for protein. The method for determining the total lipid content was based on the mixture of three solvents: water, methanol, and chloroform. The soluble and insoluble fibers were determined by the enzymatic-gravimetric method with use of enzymes (α -amylase, protease, and amyloglucosidase). The total carbohydrate content was calculated by difference. The results of proximate composition analyzes were expressed in g·100 g⁻¹.

2.3.2. Total Energetic Value. The total energetic value was calculated by using the Atwater factors (carbohydrates = 4.0 Kcal/g, lipids = 9.0 Kcal/g, and proteins = 4.0 Kcal/g) [10].

2.3.3. Soluble Solids. The soluble solids content was determined in a digital refractometer (AR 200), according to the

method proposed by AOAC [8]. The results were expressed in %.

2.3.4. Hydrogenionic Potential (pH). The pH determination was performed using a digital potentiometer (TEC-3MPP). The device was calibrated with a buffer solution at 4.0 and 7.0 pH, and then a pH reading was taken according to the methodology proposed by AOAC [8].

2.3.5. Titratable Acidity. The titratable acidity was determined by titrating with sodium hydroxide (NaOH) solution 0.1 N, according to AOAC [8].

2.3.6. Water Activity. The water activity was determined using an Aqualab device (Aqualab CX-2) at 25°C.

2.3.7. Minerals. The determination of minerals, calcium, magnesium, phosphorus, copper, iron, manganese, and zinc, was performed by the method of nitroperchloric digestion according to the methodology proposed by Malavolta et al. [11]. Mineral analyses were performed with 1 month of storage (T1).

2.3.8. Vitamin C. The total vitamin C was determined by the method of dinitrophenylhydrazine (2,4-DNPH) according to Strohecker and Henning [12]. Vitamin C was extracted with 0.5% oxalic acid, under stirring, and after filtration the determination in the extract was carried out, using 2,4-dinitrophenylhydrazine and using ascorbic acid as the standard. Quantification was performed at 520 nm and results were expressed in mg of ascorbic acid per 100 g of sample.

2.3.9. Total Carotenoids. The extraction was performed according to Higby [13], using an extracting solution of isopropyl alcohol:hexane (3:1). Readings were taken at 450 nm and the results were expressed in $\text{mg}\cdot 100\text{ g}^{-1}$.

2.3.10. Antioxidant Activity

(1) Collection and Preparation of Extracts. The ethereal, alcoholic, and aqueous extracts were consecutively prepared, in which 2.5 g of sample was weighed and stirred with 50 mL of ethyl ether at room temperature for 1 hour. The solution was filtered with filter paper and the volume was completed to 50 mL with ethyl ether. The residue was subjected to drying at 45°C for 1 hour in order to be used in the alcoholic extraction. Absolute ethyl alcohol was added to the residue in the ratio of 1:20 (p/v), followed by stirring and filtration with the same procedures conducted for the ethereal extract. The volume was completed as to the initial volume of absolute ethyl alcohol. For the aqueous extract, distilled water was added in the ratio of 1:20 (p/v) to the residue dried at 45°C for 1 hour, followed by stirring and filtration with the same procedures done for the ethereal and ethanolic extracts. The volume was completed to the initial volume of distilled water. The ethereal, ethanolic, and aqueous extracts were stored in a freezer at -18°C and used for analysis of antioxidant activity by ABTS, FRAP, and DPPH methods.

(2) ABTS. The antioxidant activity by ABTS⁺ method [2,2-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid)] was performed according to the methodology described by Rufino et al. [14]. The reading was taken at 734 nm, and ethanol was used as a blank. As reference Trolox, a synthetic antioxidant analogous to the vitamin E, was used at concentrations of 100–2000 μM . Results were expressed in μM Trolox per g of sample (Trolox equivalent antioxidant activity).

(3) FRAP. A method of reducing power of iron ions was carried out as described by Rufino et al. [15]. The standard curve was constructed with ferrous sulphate solution at concentrations from 500.0 to 2000.0 μM . The absorbance of the samples was read at 595 nm and the results were expressed as μM of ferrous sulfate per g of sample.

(4) DPPH. The antioxidant activity was based on the absorption extinction of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), according to Rufino et al. [16] at a wavelength of 517 nm in a spectrophotometer (Rayleigh UV-1800), in triplicate. The results were expressed in the amount of antioxidant required to decrease the initial concentration of DPPH by 50% (IC 50).

2.3.11. Texture Profile Analysis. In analysis of the Texture Profile, modified TPA [17] was performed in a TA-XT Plus texturometer (Stable Micro Systems) using a cylindrical aluminum tube of 20 mm in diameter, pretesting, testing, and posttesting speed of 1 mm/s and 50% compression of the sample. With the data generated by “Texture Expert” program, cohesiveness, springiness, and chewiness parameters were calculated. The analysis of shear strength was determined using HDP BSK/Set blade. The equipment operated under the following conditions: pretesting, testing, and posttesting speed of 1 mm/s with deformation of 150%. Each sample was analyzed separately, with 3 repetitions.

2.3.12. Microbiological Analysis. The microbiological analysis follows guidelines of the Brazilian legislation (Brasil, 2001), which include coliforms at 45°C, *Bacillus cereus* and *Salmonella* sp. The methods used are recommended by the American Public Health Association [18].

2.3.13. Sensory Analysis. The sensory evaluation of the food bars was conducted with 120 volunteers who habitually consumed the product. The food bars were evaluated in appearance, aroma, flavor, and texture in a hedonic scale of nine points [19]. Attributes were scored on a scale that ranged from 9 = “like extremely” up to 1 = “dislike extremely,” labeling all points. The samples were coded with random three-digit numbers and served in random order, using water between samples.

2.4. Statistical Analysis. The experimental design was completely randomized in a factorial 5×6 (5 formulations and 6 months of storage) with three repetitions. Statistical analyses were performed with the aid of the SISVAR program [20]. After analysis of variance, Tukey’s test was used to find significant differences at 5% probability between means. The analysis of proximate composition was submitted to Tukey

TABLE 2: Proximate composition (wet base) of marolo flour food bars originated from the southwest of the State of Goiás, Brazil.

Parameter	Time	Formulation				
		F1	F2	F3	F4	F5
Moisture (g·100 g ⁻¹)	0	8.81 ^{Aa} ± 0.54	8.31 ^{Aa} ± 0.04	7.94 ^{Aa} ± 0.37	7.26 ^{Aa} ± 0.33	8.44 ^{Aa} ± 0.03
	6	9.65 ^{Aa} ± 0.68	9.45 ^{Aa} ± 0.33	9.14 ^{Aa} ± 0.38	9.47 ^{Ab} ± 2.3	9.04 ^{Aa} ± 0.18
Ashes (g·100 g ⁻¹)	0	1.22 ^{Aa} ± 0.05	1.39 ^{Aa} ± 0.15	1.34 ^{Aa} ± 0.04	1.21 ^{Aa} ± 0.18	1.44 ^{Aa} ± 0.13
	6	1.19 ^{Aa} ± 0.13	1.37 ^{Aa} ± 0.26	1.28 ^{Aa} ± 0.24	1.29 ^{Aa} ± 0.26	1.36 ^{Aa} ± 0.23
Total lipids (g·100 g ⁻¹)	0	7.15 ^{Aa} ± 0.20	7.36 ^{Aa} ± 0.11	7.37 ^{Aa} ± 0.33	7.40 ^{Aa} ± 1.14	7.93 ^{Aa} ± 0.42
	6	6.71 ^{Aa} ± 0.03	7.55 ^{Aa} ± 1.54	7.34 ^{Aa} ± 0.97	7.18 ^{Aa} ± 0.62	7.26 ^{Aa} ± 0.40
Proteins (g·100 g ⁻¹)	0	7.06 ^{Aa} ± 0.09	6.49 ^{Ba} ± 0.13	5.68 ^{Ca} ± 0.10	6.04 ^{Ca} ± 0.54	5.73 ^{Ca} ± 0.11
	6	6.44 ^{Ab} ± 0.06	5.42 ^{Bb} ± 0.03	5.25 ^{Bb} ± 0.12	5.01 ^{Bb} ± 0.10	4.25 ^{Cb} ± 0.10
Insoluble dietary fiber (g·100 g ⁻¹)	0	3.78 ^{Aa} ± 2.36	4.25 ^{Aa} ± 2.18	4.64 ^{Aa} ± 1.69	4.80 ^{Aa} ± 1.80	4.70 ^{Aa} ± 3.19
	6	2.45 ^{Aa} ± 3.54	3.28 ^{Aa} ± 1.60	3.79 ^{Aa} ± 3.19	5.15 ^{Aa} ± 2.81	4.53 ^{Aa} ± 1.84
Soluble dietary fiber (g·100 g ⁻¹)	0	3.04 ^{Aa} ± 0.71	3.02 ^{Aa} ± 0.82	3.41 ^{Aa} ± 1.50	3.17 ^{Aa} ± 0.00	3.78 ^{Aa} ± 0.85
	6	2.78 ^{Aa} ± 0.25	2.79 ^{Aa} ± 0.95	2.73 ^{Aa} ± 0.14	3.08 ^{Aa} ± 0.76	2.33 ^{Aa} ± 0.19
Total dietary fiber (g·100 g ⁻¹)	0	6.83 ^{Aa} ± 3.08	7.28 ^{Aa} ± 2.99	8.05 ^{Aa} ± 3.20	7.97 ^{Aa} ± 1.80	8.49 ^{Aa} ± 4.04
	6	5.24 ^{Aa} ± 3.79	6.08 ^{Aa} ± 2.55	6.51 ^{Aa} ± 3.05	8.23 ^{Aa} ± 3.57	6.87 ^{Aa} ± 1.65
Total carbohydrates (g·100 g ⁻¹)	0	75.76 ^{Aa} ± 0.27	76.45 ^{Aa} ± 0.15	77.67 ^{Aa} ± 0.28	78.09 ^{Aa} ± 0.57	76.47 ^{Aa} ± 0.25
	6	76.01 ^{Aa} ± 0.14	76.22 ^{Aa} ± 1.72	76.99 ^{Aa} ± 1.19	77.05 ^{Aa} ± 0.36	78.09 ^{Aa} ± 0.25
Total energetic value (Kcal·100 g ⁻¹)	0	395.64 ^{Aa} ± 0.85	398.00 ^{Aa} ± 0.81	399.73 ^{Aa} ± 1.76	403.12 ^{Aa} ± 5.73	400.12 ^{Aa} ± 2.50
	6	390.21 ^{Aa} ± 0.77	394.47 ^{Aa} ± 7.50	395.02 ^{Aa} ± 4.56	392.86 ^{Ab} ± 3.10	394.71 ^{Aa} ± 2.82

Mean values ± standard deviation of three repetitions. Means followed by the same capital letter in the line and lowercases in the column do not differ statistically from each other by Tukey test at 5% probability; F1: Control Food Bar; F2, F3, F4, and F5: food bars prepared with, respectively, 20, 30, 40, and 50% of marolo flour in substitution of oatmeal.

test at 5% probability for the variable formulation and *t*-test at 5% probability for the variable time.

3. Results and Discussion

The data of the marolo pulp flour food bars proximate composition are shown in Table 2.

The moisture content of food bars averaged 8.7 g·100 g⁻¹ and was not significantly influenced between the different formulations. During storage of 6 months, there was significant increase in the moisture only for F4 formulation. According to Coultate [21], when two or more ingredients are brought together in an airtight environment, as are food bars, moisture exchange occurs between them, changing the biological, chemical, and physical properties of the product. Changes in moisture content can be explained mainly by the heterogeneity of the ingredients used in the preparation of food bars. Brazilian legislation [22] limits moisture for cereal-based products to 15%. All formulations evaluated in this study showed moisture values lower than that required by the legislation. High moisture levels favor undesirable reactions, such as nonenzymatic browning and microbial growth. In addition, high moisture reduces the crispy sensory attribute of cereal bars. For cereals, the crispness indicates freshness and quality of the product, and its loss, characterized by softening, is one of the causes for consumer rejection [23–25].

The ash content averaged 1.3 g·100 g⁻¹ and showed no significant difference between the different formulations and during storage of 6 months. The ash contents were similar to those found in the literature for cereal bars, whose values in g·100 g⁻¹ were 1.13 [26], 1.40 to 1.61 [27], and 1.15 to 1.38 [28].

The total lipid content averaged 7.3 g·100 g⁻¹ and did not present significant difference between the different formulations and during storage. Munhoz et al. [29] studied cereal bars with bocaiuva and found higher lipid content, which ranged from 12.31 to 13.55 g·100 g⁻¹. da Silva et al. [3] also studied marolo pulp flour cereal bars and found lower values for the lipid content, ranging from 2.12 to 2.6 g·100 g⁻¹. This difference can be explained mainly by the composition and quantity of ingredients used in the preparation of the food bars.

The protein content ranged from 5.68 to 7.06 g·100 g⁻¹ at time 0 and significantly decreased with increasing concentration of marolo pulp flour until formulation F3, which is justified by the higher content of proteins in oatmeal (13.29 g·100 g⁻¹) according to Fujita and Figueroa [30], relative to marolo pulp flour (2.92 g·100 g⁻¹) [3]. During storage of 6 months, the protein content was significantly reduced in all formulations studied, which can be demonstrated, possibly by the occurrence of the Maillard reaction during storage. The Maillard reaction may occur during thermal processing and/or long-term storage of foods containing protein and reducing sugar [31]. Compared to other studies, the protein content of the food bar was similar to that found by da Silva et al. [3], for cereal bars with marolo flour, ranging from 6.12 to 7.25 g·100 g⁻¹ and Munhoz et al. [29], for cereal bars with bocaiuva, ranging from 7.69 to 8.33 g·100 g⁻¹.

The different formulations and the 6-month storage do not significantly interfere with the dietary fiber content of food bars. The dietary fiber showed an average of 4.1 g·100 g⁻¹ for insoluble dietary fiber, 3 g·100 g⁻¹ for soluble dietary fiber,

and $7.1 \text{ g} \cdot 100 \text{ g}^{-1}$ for total dietary, corresponding to 18.68% of the recommended daily intake of total dietary fiber for men and 28.4% for women, both aged 19–50 years [32]. According to Brazilian legislation [33], for a product to be considered “rich” in fiber, it is necessary that its formulation contain 6 g of fiber per 100 g for solids. Therefore, the food bars evaluated in this study can be considered rich in fiber. There are several physiological benefits related to the consumption of dietary fiber, which among others are the reduction of the risk of irritable bowel syndrome, diverticulitis, colon cancer, the satiety effect (serving as treatment to help control obesity), control of sugar and cholesterol levels in the blood, increased stool bulk, improved intestinal transit, and normalization of intestinal microflora [34].

The total carbohydrates content averaged $76.88 \text{ g} \cdot 100 \text{ g}^{-1}$. This parameter was not significantly different in different formulations and during storage of 6 months. Total carbohydrates content was the nutrient with highest concentration due to the high percentage of cereals and glucose syrup used in the formulations of the food bars. da Silva et al. [3] found similar values ranging from 79.7 to $80.8 \text{ g} \cdot 100 \text{ g}^{-1}$ for total carbohydrate content in cereal bars with marolo flour.

The energy value averaged $396.4 \text{ kcal} \cdot 100 \text{ g}^{-1}$ and was not significantly influenced by different formulations and during storage of 6 months. Lower values were found by Dutcosky et al. [27] which reported values between 291.24 and $364.36 \text{ kcal} \cdot 100 \text{ g}^{-1}$ for energetic value of food bars with added prebiotics and Guimarães and Silva [28] reported energy values from 349.61 to $358.77 \text{ kcal} \cdot 100 \text{ g}^{-1}$ for food bars with dried murici. This difference possibly occurs due to differences in the composition and quantity of ingredients used in the formulation of each food bar, especially in quantity and diversification of cereals and the amount of glucose syrup.

Figure 1 shows the results of the analyses for water activity, soluble solids, titratable acidity, pH, vitamin C, and carotenoid of the food bars during storage of 6 months.

The soluble solids content, titratable acidity, pH, vitamin C, and carotenoid were influenced significantly ($p < 0.05$), by the interaction between the formulation factors (F1, F2, F3, F4, and F5) and storage time. Water activity showed a significant difference only in isolated factors, formulation, and time (Figure 1).

The results for the water activity are below 0.65 and indicate food safety. For any type of bacteria, the minimum Aw value required for growth is 0.75, whereas osmophile yeast (which resist mediums with high sugar concentration) and xerophile fungi (which survive in mediums with little water) are able to grow on Aw from 0.61 to 0.65, respectively, using glucose and sugars as substrate [35]. Thus, it is possible to ensure microbiological safety of food bars during storage, under appropriate conditions of temperature and packaging.

For the soluble solids content it was observed that the addition of marolo pulp flour, in increasing quantities, increased soluble solids content of food bars. The control formulation (F1) and the formulation with addition of 20% marolo pulp flour (F2) had higher storage stability compared to formulations containing higher concentrations of marolo

pulp flour. Paiva et al. [36] analyzed cereal bars with residues of rice, soy, pineapple, and pequi nuts reported soluble solids similar to this study, with levels between 55 and 65%.

The titratable acidity of the samples increased significantly and proportionally to the concentration of marolo pulp flour in food bars. The pH showed the opposite behavior of acidity; that is, it was reduced with increasing concentration of marolo pulp flour in food bars. The increase in acidity with a consequent decrease in pH occurred, probably, due to the increase in organic acids such as malic acid, the main organic acid present in the marolo pulp flour [37].

For vitamin C content and total carotenoids, a significant increase was observed according to increase in the concentration of marolo pulp flour. The largest value found for vitamin C was for the F5 formulation with 50% marolo pulp flour, with $106.29 \text{ mg} \cdot 100 \text{ g}^{-1}$. According to values established by FAO [38] for the recommended daily intake for adults (45 mg/d) it was found that a portion of 25 g (commercial size) of F5 formulation has 59.2% of RDI. The highest value for total carotenoids was also for the F5 formulation with $0.29 \text{ mg} \cdot 100 \text{ g}^{-1}$.

During the 6-month storage all samples had significant degradation in vitamin C and carotenoid content. The degradation of ascorbic acid and carotenoid can be attributed mainly to aerobic oxidation reaction at the beginning of storage, and anaerobic oxidation during storage as the package used is impermeable to oxygen. According to Nagy [39], after the free oxygen in the packaging is consumed, anaerobic reactions predominate and, among them, degradation, but at a lower speed than under aerobic conditions.

Table 3 shows the results of the mineral composition analysis of food bars.

The calcium content ranged from 860.1 to $5128.1 \text{ mg} \cdot \text{kg}^{-1}$ and magnesium ranged from 689 to $1679.4 \text{ mg} \cdot \text{kg}^{-1}$. The phosphorus, copper, manganese, and zinc were significantly reduced with the increase in concentration of flour marolo pulp in food bars.

According to Brazilian legislation [33] “source food in vitamins and minerals” is one with at least 15% of the reference Recommended Daily Intake (RDI) per serving of solid food, and “food rich in vitamins and minerals” is one that contains at least 30% of the reference RDI per serving of solid food.

According to values established by the IOM [32] and FAO [38] for the recommended daily intake for adults, it was found that a portion of 25 g (commercial size) of formulation F2 has 21 and 161% of RDI of manganese and copper, respectively. For the formulation F3 values are 19 and 277% of RDI of manganese and copper, respectively. 25 g of F4 formulation showed 16 and 224% of the RDI of manganese and copper, respectively, and formulation F5 showed 16 and 202% of RDI of magnesium and copper. Therefore, the F2, F3, and F4 formulations are sources of manganese and rich in copper, and the F5 formulation is a source of magnesium and also rich in copper.

Magnesium is the fourth most abundant mineral in the body and is essential for maintenance of good health [40]. It helps to maintain normal muscle function and heart

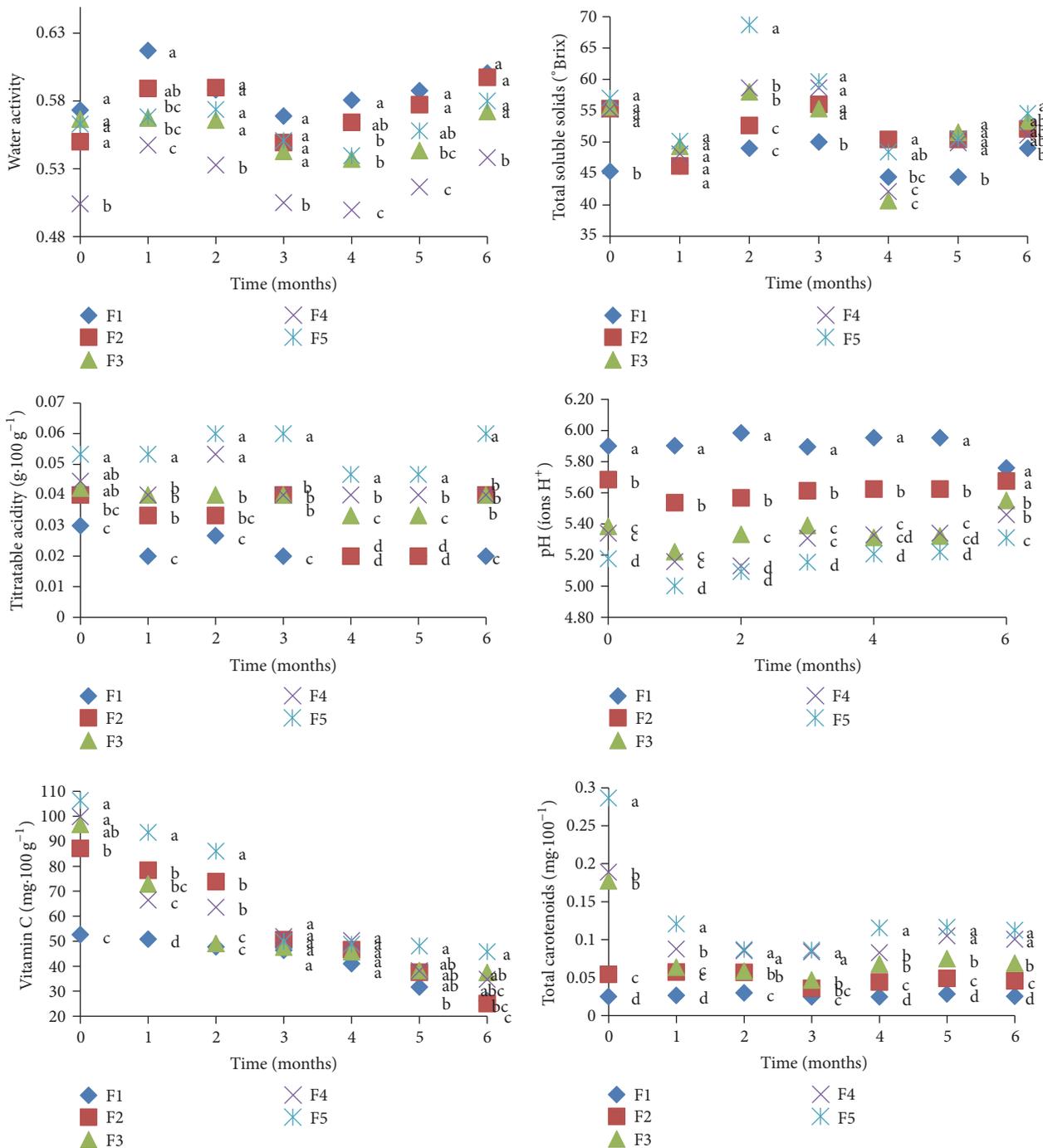


FIGURE 1: Mean values of determination of water activity, total soluble solids content, titratable acidity, pH, vitamin C, and total carotenoid of food bars with different marolo flour concentrations, stored for 6 months. Means followed by the same letter in each timing represent statistical similarities, between the concentrations, at 5% probability by Tukey test. F1: Control Food Bar. F2, F3, F4, and F5: food bars prepared with, respectively, 20, 30, 40, and 50% of marolo flour in substitution of oatmeal.

rate, contributes to maintaining a healthy immune system, maintains bones strong, and helps to regulate the levels of blood sugar. There is a growing interest in magnesium role in the prevention of disorders such as hypertension, cardiovascular disease, and diabetes [41].

In Figures 2, 3, and 4 are shown the results of antioxidant activity of food bar with marolo pulp flour, during the 6-month storage, by ABTS, DPPH, and FRAP, respectively.

The antioxidant activity determined by ABTS and FRAP methods in ethanol and aqueous extracts was influenced

TABLE 3: Mineral composition of food bars with marolo pulp flour.

Minerals (mg·Kg ⁻¹)	Formulation				
	F1	F2	F3	F4	F5
Calcium	1121.1 ± 0.45 ^c	925.1 ± 0.47 ^d	860.1 ± 0.78 ^e	1228.6 ± 0.15 ^b	5128.1 ± 0.31 ^a
Magnesium	767.1 ± 0.15 ^b	746 ± 0.45 ^c	711.8 ± 0.50 ^d	689.2 ± 0.44 ^e	1679.4 ± 0.26 ^a
Phosphor	4478.6 ± 0.57 ^a	3181.1 ± 0.31 ^b	2755.2 ± 0.46 ^d	2783.8 ± 0.46 ^c	2033.2 ± 0.30 ^e
Copper	140.7 ± 0.40 ^a	58.2 ± 0.31 ^e	99.9 ± 0.25 ^b	80.9 ± 0.15 ^c	72.9 ± 0.40 ^d
Iron	38.9 ± 0.30 ^b	26.3 ± 0.26 ^c	24.9 ± 0.31 ^d	22.8 ± 0.35 ^e	40.8 ± 0.50 ^a
Manganese	25.1 ± 0.25 ^a	19.4 ± 0.20 ^b	17.5 ± 0.40 ^c	15.6 ± 0.47 ^d	12 ± 0.59 ^e
Zinc	7.1 ± 0.35 ^a	6.4 ± 0.45 ^c	6.6 ± 0.40 ^b	5.8 ± 0.21 ^e	6.3 ± 0.15 ^d

Mean values ± standard deviation of three repetitions. Means followed by the same letter on the line do not differ statistically by Tukey test at 5% probability; F1: Control Food Bar; F2, F3, F4, and F5: food bars prepared with, respectively, 20, 30, 40, and 50% of marolo flour in substitution of oatmeal.

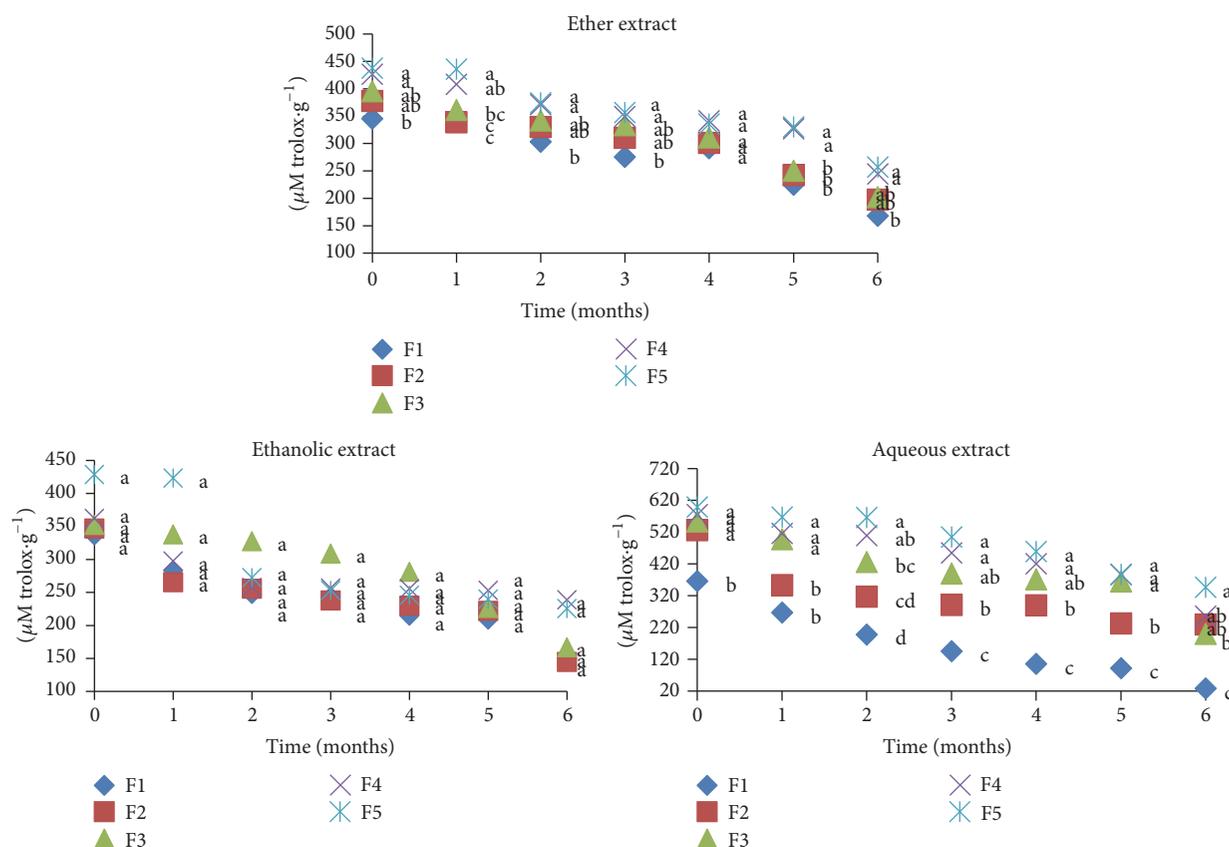


FIGURE 2: Mean values of determination of antioxidant activity by ABTS methods ($\mu\text{M trolox}\cdot\text{g}^{-1}$), present in food bars with different marolo flour concentrations, stored for 6 months. Means followed by the same letter in each timing represent statistical similarities between the concentrations, at 5% probability by Tukey test. F1: Control Food Bar. F2, F3, F4, and F5: food bars prepared with, respectively, 20, 30, 40, and 50% of marolo flour in substitution of oatmeal.

significantly ($p < 0.05$) by the interaction of the formulation factors (F1, F2, F3, F4, and F5) and storage time. In the ether extract, only the single factor formulation showed no significant difference ($p < 0.05$) (Figures 2 and 3). For the DPPH method, the ether extract showed a significant difference ($p < 0.05$) only for isolated time factor; for the ethanol extract, the isolated factors formulation and time showed significant differences ($p < 0.05$) and for the aqueous extract there was no statistically significant difference (Figure 4).

The ABTS method (Figure 2) measures the antioxidant activity of both lipophilic and hydrophilic compounds. The aqueous extracts showed higher antioxidant activity at time 0 than the other extracts.

The FRAP method (Figure 3) measures antioxidants in water-soluble and ethanolic aqueous solutions. The aqueous extracts showed higher antioxidant activity at time 0 than the other extracts.

The DPPH method (Figure 4) has advantages when the analysed antioxidants are more soluble in organic solvents.

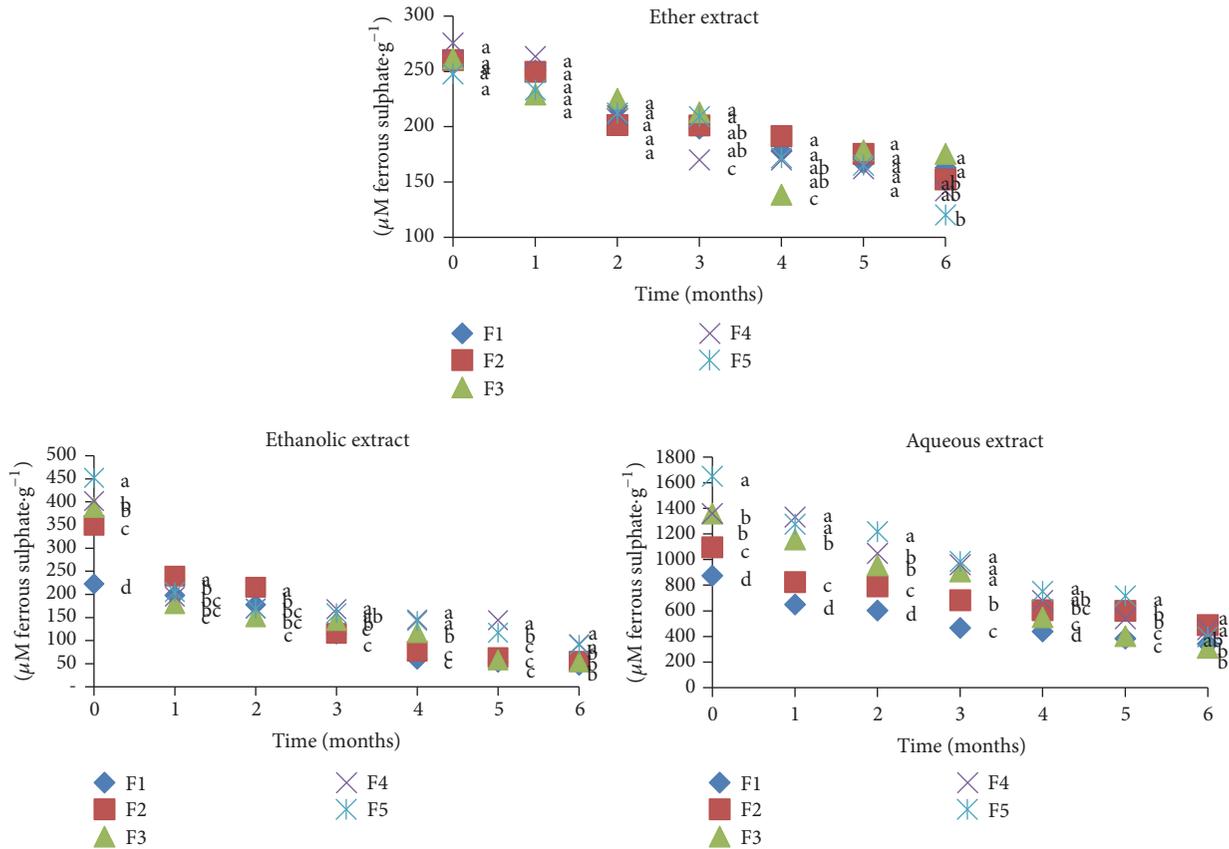


FIGURE 3: Mean values of determination of antioxidant activity by FRAP methods ($\mu\text{M ferrous sulphate}\cdot\text{g}^{-1}$), present in food bars with different marolo flour concentrations, stored for 6 months. Means followed by the same letter in each timing represent statistical similarities between the concentrations, at 5% probability by Tukey test. F1: Control Food Bar. F2, F3, F4, and F5: food bars prepared with, respectively, 20, 30, 40, and 50% of marolo flour in substitution of oatmeal.

The ethanolic extracts showed higher antioxidant activity at time 0 than the other extracts.

For the three methods and the three extracts evaluated, there is increased antioxidant activity with increasing concentration of marolo pulp flour. The formulation with 50% marolo pulp flour showed the highest antioxidant activity of the three methods.

The various solvents certify the maximum solubility of antioxidants in the sample. The use of three solvents of different polarities, ethyl ether, ethanol, and distilled water, enable solubilizing compounds which are more polar (aqueous extract), of intermediate polarity (ethanol extract), and nonpolar (ether extract), according to Borguini and Ferraz da Silva Torres [42]. Based on this statement, there is a predominance of polar antioxidants in food bars with marolo pulp flour, which showed higher results of antioxidant activity in the aqueous extracts, for two of the methods tested, ABTS and FRAP. Damiani et al. [37] studied the antioxidant activity of marolo fruits and also found higher antioxidant potential for the aqueous extract.

The antioxidant activity decreased with storage time for the three methods and for all the studied extracts. Typical compounds having antioxidant activity include the class of

phenols, phenolic acids and their derivatives, flavonoids, tocopherol, phospholipid, phytic acid, ascorbic acid, pigments, and sterols [43]. The reduced antioxidant activity in storage is explained by degradation, especially of compounds such as ascorbic acid and pigments such as the carotenoids present in the marolo pulp flour.

Figure 5 shows the results of the texture parameters for food bars stored for 6 months.

The texture, shear, cohesiveness, and chewiness parameters were influenced significantly ($p < 0.05$) by the interaction between formulation factors (F1, F2, F3, F4, and F5) and storage time. The elasticity parameter showed a significant difference only for the single factor time (Figure 5).

According to the forces applied to break the bars, the formulation with the addition of 50% marolo pulp flour demonstrated to be softer, averaging 51,4 N. The addition of marolo pulp flour increased the hardness of the food bar up to F4 formulation, and this can be explained by the smaller grain size of the marolo pulp flour relative to the oatmeal, which makes the food bars become more compact and increase the shear strength and hardness, but this fact does not apply to F5 formulation where marolo pulp flour proportions and flaked oatmeal were equal, which can be justified by the

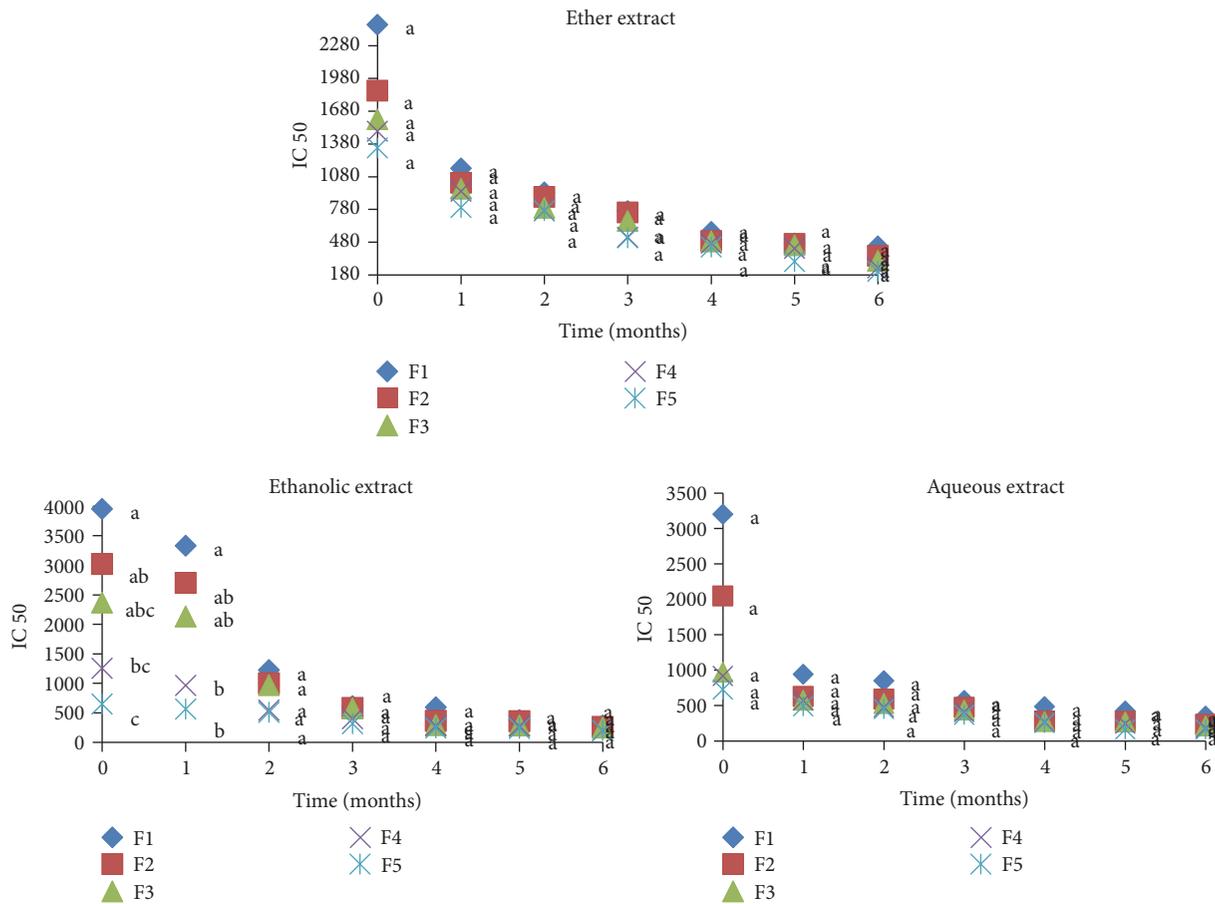


FIGURE 4: Mean values of determination of antioxidant activity by DPPH methods (IC 50), present in food bars with different marolo flour concentrations and stored for 6 months. Means followed by the same letter in each timing represent statistical similarities between the concentrations, at 5% probability by Tukey test. F1: Control Food Bar. F2, F3, F4, and F5: food bars prepared with, respectively, 20, 30, 40, and 50% of marolo flour in substitution of oatmeal.

higher soluble solids found in this formulation (48.44 to 68.66%), which makes the food bars become more softer and reduces the shear strength and hardness. da Silva et al. [44] found values ranging from 15.86 N to 17.87 N for shearing of food bars with marolo flour and jervá, respectively. High values for food bar hardness measurement are not always associated with low acceptance of the product. Generally, products formulated with high fiber content result in denser and harder products, which do not imply that they will have lower acceptance [45].

Elasticity is the rate at which a deformed material is returned to its initial condition after the deforming force is removed. It is the measure that the food reaches between the end of the first compression cycle and the second compression cycle [46]. The elasticity of the food bars showed no significant difference between the formulations in any of the studied times and averaged 0.45 for formulation F1, 0.41 for formulation F2, 0.44 for formulation F3, 0.46 for formulation F4, and 0.39 for formulation F5.

Cohesiveness is the measurement of the extension in which a material can be deformed before breaking and is

represented in the graph as the positive force area ratio of the second compression cycle by the first compression cycle [46]. Formulations with addition of 20% (F2) and 50% (F5) of marolo pulp flour had lower results for cohesiveness, averaging 0.10.

The chewiness is the energy required to disintegrate food to a state ready for swallowing: a product of cohesion, hardness, and elasticity [46]. The formulation with 50% marolo pulp flour showed lower results, with an average of 709.11, and, therefore, it requires less force to be chewed to the point of being swallowed up.

In instrumental texture analysis, the heterogeneity of the samples is a critical factor in the accuracy of results. The differences found in texture parameters between formulations during storage are due mainly to heterogeneity in the type of product considered, since food bars vary greatly in their combination of ingredients, size, and texture.

The results of the microbiological analysis of the food bars were presented in accordance with standards established by the Technical Regulation of Resolution RDC No. 12 [47], demonstrating the quality of the ingredients and the hygienic

TABLE 4: Sensory parameters evaluated by consumers from Goiania/GO/Brazil in food bars made with flour marolo pulp.

Sensory parameters	Formulation				
	F1	F2	F3	F4	F5
Appearance	7.01 ^d	7.36 ^c	7.63 ^c	8.06 ^b	8.45 ^a
Color	6.98 ^c	7.58 ^b	7.79 ^b	7.88 ^b	8.23 ^a
Aroma	6.98 ^d	7.23 ^c	7.57 ^b	7.73 ^b	8.01 ^a
Flavor	7.53 ^b	7.58 ^b	7.8 ^b	7.63 ^b	8.31 ^a
Texture	7.62 ^b	7.53 ^{bc}	7.37 ^{bc}	7.22 ^c	8.02 ^a

Means followed by the same letter in line do not differ statistically by Tukey test at 5% probability; F1: Control Food Bar; F2, F3, F4, and F5: Food bars prepared with, respectively, 20, 30, 40, and 50% of marolo flour in substitution of oatmeal.

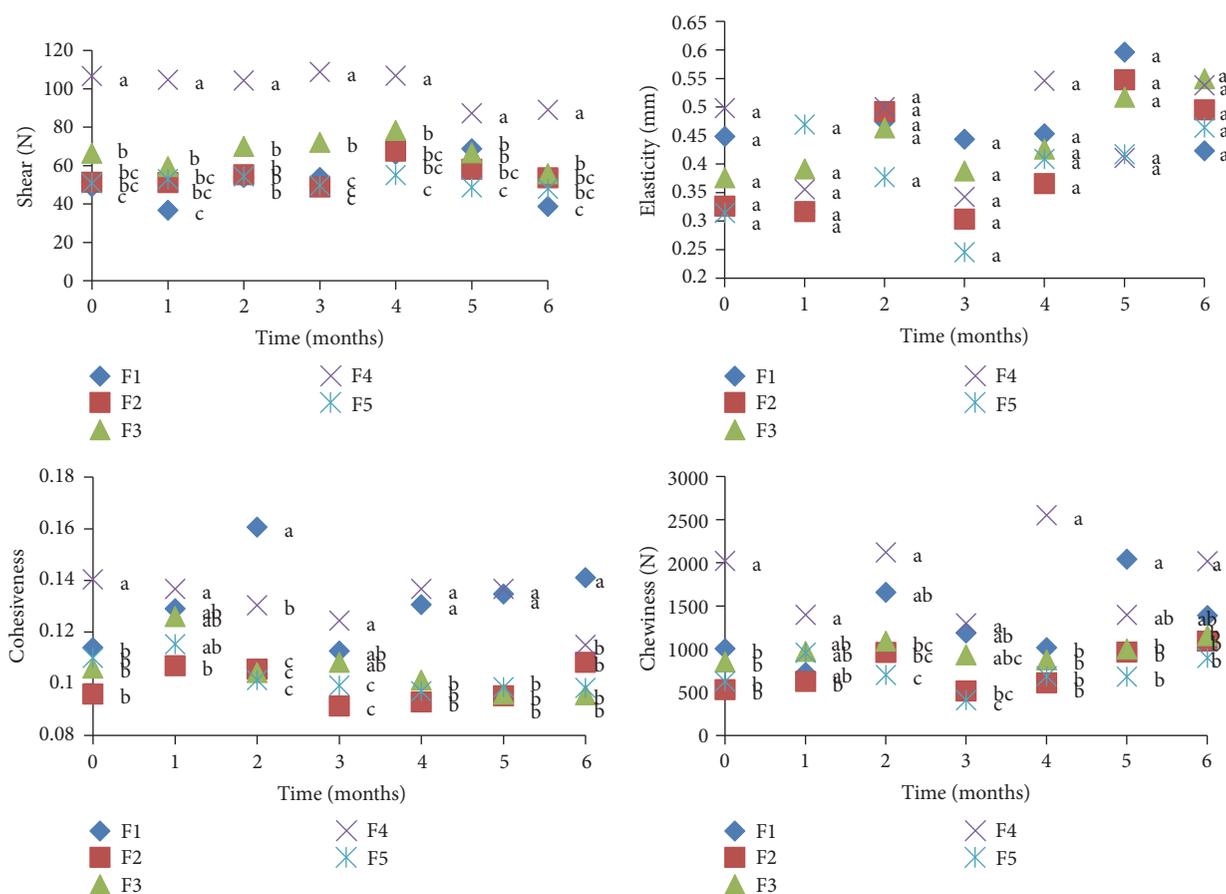


FIGURE 5: Mean values of determination of parameters of texture of food bars with different concentrations of marolo pulp flour stored for 6 months. Means followed by the same letter in each time represent statistical similarities between the concentrations, at 5% probability by Tukey test. F1: Control Food Bar. F2, F3, F4, and F5: food bars prepared with, respectively, 20, 30, 40, and 50% of marolo flour in substitution of oatmeal.

and sanitary control in the preparation of the food bars, allowing the food bars to be offered safely to the sensory analysis tasters.

Table 4 presents the marks awarded by the evaluators to the food bars formulations regarding sensorial preference. Sensory analysis was performed with 120 tasters, of which 45% were male and 55% female. Regarding age, 25% of the panelists were under 25 years of age, 38% were between 20 and 35 years of age, 28% were between 35 and 45 years of age, and 8% were over 45 years of age. As for consumption, 26%

of the panelists said they consumed food bars once a month, 25% consumed twice a month, 21% consumed once a week, and 6% consumed twice a week. As for the attributes evaluated, all formulations with added marolo pulp flour obtained an average higher than 7, indicating “like moderately” and being considered acceptable for consumption [48].

The food bars of formulation F5 with the addition of 50% marolo pulp flour presented higher averages for all attributes and was the favorite by the evaluators, presenting hedonic values between 8 and 9, indicating “like” and “like extremely,”

respectively. However, other formulations with added marolo pulp flour (F2, F3, and F4) showed average grades higher than 7, indicating “like moderately.”

Correlating the sensory analysis with the analysis of the nutritional composition, formulation F5 also showed higher levels of dietary fiber, vitamin C, carotenoids, minerals like calcium and magnesium, and higher antioxidant activity.

The panelists gave the best grade for texture to formulation F5, which was shown by instrumental determination to be among the more soft formulations.

Gomes et al. [49] when studying the sensory acceptance of food bars based on passion fruit rind, obtained grades similar to these from this study, ranged from 6.88 to 7.77 for flavor, from 7.28 to 7.57 for color, from 7.13 to 7.43 for texture, and from 7.10 to 7.53 for aroma.

4. Conclusion

The food bars developed with increasing proportions of marolo pulp flour were safe and acceptable during the study period. Demonstrated nutritional and commercial potential and can be considered as product rich in dietary fiber, having considerable amounts of vitamin C, total carotenoids, and antioxidant activity, besides showing to be rich in minerals such as calcium and magnesium.

Among the developed formulations, formulation F5, with addition of 50% marolo pulp flour, showed the best results for the physical, chemical, and nutritional analyzes as well as the best consumer acceptance.

Conflicts of Interest

The authors have declared that no conflicts of interest exist.

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