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Storage study of cereal bars formulated with banana peel flour

Bioactive compounds and texture properties

Storage study
of cereal bars

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Abstract

Purpose – This paper aims to examine the bioactive compounds and texture properties of cereal bars formulated with banana peel flour during storage.

Design/methodology/approach – Seven cereal bars were produced and stored during 11 months, under vacuum and protected from the light. The total phenolic compounds, the activity antioxidant by ABTS [2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] method, the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method expressed as EC50 (concentration required to reduce the original amount of free radicals by 50 per cent) and texture properties were evaluated over the storage period.

Findings – In general, total phenolic compounds decreased during storage (from 4.19 to 1.11 mg GAE. g-1f.w.). Although the total antioxidant activity (ABTS method) increased during the fifth month, it reduced during storage (from 3.41 to 0.30 $\mu\text{mol TE.g-1f.w.}$); and the EC50 was not modified in many formulations, though it decreased in other formulations during storage period (from 3913 to 19221 g fruit.g-1 DPPH). The force of rupture began to increase in the fourth month (reaching 62.4 N), and hardness began to increase in the ninth month (reaching 444 N). The formulation and time factors influenced the total phenolic compounds, total antioxidant activity (ABTS method), force of rupture and hardness, while EC50 was only influenced by the formulation (p -value = 0.001). A principal component analysis showed that time had little effect on the most important characteristics considered in description of the cereal bars.

Originality/value – Cereal bars can be consumed up to the third month of storage, considering the texture of the products. Moreover, the presence of bioactive compounds in cereal bars depends on the addition of banana peel flour, which it contributes to the insertion of total phenolic compounds and total antioxidant activity in cereal bars, aggregating functional properties in these products.

Keywords DPPH, Texture profile analysis, ABTS, Force of rupture, Total phenolics compounds

Paper type Research paper

1. Introduction

Because of the growing consumer demand for healthy, natural and convenient foods, attempts are being made to improve snack foods nutritional values by modifying their nutritive composition (Bhaskaran and Hardley, 2002; Gray *et al.*, 2003). Cereal bars are a popular and convenient food and, therefore, would be an ideal food format to deliver fruit-



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derived phenolic antioxidants (Sun-Waterhouse *et al.*, 2010; Damasceno *et al.*, 2016). Phenolics compounds, e.g. flavonoids and phenolics acids, belong to an important class of secondary metabolites and own recognized antioxidant activity. Antioxidant compounds neutralise free radicals and inhibit or interrupt the chain of propagation of oxidative reactions, converting free radicals into less harmful molecules and repairing the oxidative damage in human cells (Borges *et al.*, 2014). This class of compounds is also relevant to insertion in the elaboration of cereal bars.

The banana is one of the most extensively consumed fruits in the world and represents approximately 40 per cent of the world fruit trade. World banana production was 102 Mt in 2012, and Brazil was the sixth most important banana-producing country with a total production of 7 Mt (FAO, 2016). The banana peel represents 30-40 per cent of the total weight of the fruit, it is rich in minerals (Emaga *et al.*, 2007) and its quantity of total dietary fiber reaches 43.2 to 49.7 per cent (Mohapatra *et al.*, 2010). González-Montelongo *et al.* (2010) reported that banana peels contain large amounts of dopamine and l-dopamine, catecholamines that exhibit antioxidant activity, and depending on the variety, ripeness, and on the type of phenolic compound extraction used, the banana peel has from 3.1 to 380 mg GAE.g⁻¹ of total phenolic compounds (González-Montelongo *et al.*, 2010; Rebello *et al.*, 2014). In addition, banana peel contains low but permissible concentrations of toxic elements and may be recommended as dietary supplement, especially considering that banana peel contains lower quantities of toxic elements than other by-products, as pineapple peel (Kuppusamy *et al.*, 2017).

The application of banana peel flour in cereal bars was performed using a simplex centroid design for mixtures of banana peel flour, rice flakes and oat flour, and the products evaluated regarding sensory acceptability and profile (Carvalho and Conti-Silva, 2018). The cereal bars with 21 and 27.72 per cent of banana peel flour had good overall acceptability and aroma acceptability, respectively, even with different sensory profiles, indicating a relevant use to this by-product, which may favor the development of new products.

Many processed foods are multicomponent heterogeneous systems that are far from thermodynamic equilibrium (Mezzenga, 2007), and there is often a considerable time between its manufacture and consumption, during which a product is transported and stored. During this storage time, multiple chemical, physical and biological reactions occur serially and simultaneously, and some of these reactions can cause negative effects on the nutritional quality of foods (Loveday *et al.*, 2009). Likewise, the texture of cereal bars is affected along the shelf life (Imtiaz *et al.*, 2012), which can cause losses for manufacturers of cereal bars.

The banana is industrially processed when the peels are yellow with brown spots for producing a variety of products, as banana-raisin, chips, flour, jam, as well brandy and liqueur (Sebrae, 2008). In this way, the generation of banana peel from industries becomes a problem. Although studies about demand of banana peel were not found in the literature, its use to obtain products for human feed is an alternative to reduce the environment impact and aggregating nutritive value to the products, as well as be feasible at the sensory point-of-view, as seen in Carvalho and Conti-Silva (2018). However, time can affect physical and chemical characteristics of foods, and therefore, we aimed to study the storage of cereal bars formulated with banana peel flour regarding to bioactive compounds and texture properties.

2. Materials and methods

2.1 Materials

Samples of the Cavendish variety of banana (*Musa acuminata* L., *cv cavendshii*) provided directly by the producer of São José do Rio Preto, SP, Brazil, were matured in an ethylene

chamber while still at the production site. The bananas were stored in a cool, dry place until they reached the advanced stage of ripeness (when the peels had yellow and brown spots), it is at this stage of maturity that most bananas are industrially processed. The fruits were washed in running water, and then the peel was manually separated from the pulp.

Approximately 2.2 kg of banana peels were arranged in trays provided with small perforations to facilitate the passage of hot air. Then the trays were placed in a Pasiani oven with air circulation (Classic Model Turbo 240, Brazil). The oven was preheated (20 min) at 60°C, and the trays were then left inside overnight. The dried peels were crushed in a food process or until the banana peel flour was obtained, and the flour was then stored in polypropylene plastic bags at room temperature and in the dark.

The banana pulp was used to obtain dried banana, which was used then in the formulations of the cereal bars. Approximately 1.8 kg of banana pulp were cut longitudinally using stainless steel knives, and the pieces were placed on perforated trays and dried in the same oven (Pasiani). After the oven was preheated for 20 min at 60°C, and the trays were left inside for 24 h.

2.2 Cereal bar formulations

Seven cereal bar formulations were investigated, resulting of the mixture modeling methodology used in a previous study by our research group (Table I) (Carvalho and Conti-Silva, 2018). In this study, the simplex-centroid design for ternary mixtures was used to evaluate the effects of the interaction between the banana peel flour, rice flakes and oat flour on the sensory acceptability of the cereal bars, and the production of the cereal bars was performed as the same manner to the previous study.

2.3 Experimental design of effects of cereal bars storage

The processed cereal bars were stored for 11 months and were evaluated monthly to examine their bioactive compounds and texture properties. At zero time, three of the thirty-six cereal bars of each formulation that had been produced were evaluated. Then, three cereal bars of each formulation were then vacuum-packed together into low-density polyethylene bags, that were 195 mm (width) × 350 mm (length) × 10 μm (thick). A total of 11 bags of each formulation were packaged (one bag per month). The bags were kept in the dark at room temperature for 11 months; each month, one bag was randomly collected for analysis. At zero time and after each bag was opened, the rupture force of the three cereal bars was evaluated; next, ten pieces of the cereal bars were cut for the texture profile

Formulation	Proportion of each component in the cereal bar (% or g/100 g of cereal bar)		
	Banana peel flour	Rice flakes	Oat flour
1	42	0	0
2	21	21	0
3	21	0	21
4	14	14	14
5	27.72	7.14	7.14
6	7.14	27.72	7.14
7	7.14	7.14	27.72

Note: ¹banana peel flour + rice flakes + oat flour = 42 g

Table I.
Proportions¹ of
banana peel flour,
rice flakes and oat
flour in the cereal bar
formulations

analysis; finally, the remaining pieces were used in the bioactive compound analysis. Lights were maintained turned off during the bioactive compound analysis.

2.4 Analysis of bioactive compounds of the cereal bars

2.4.1 Antioxidant extraction. The procedure used was as follows: the extract of three fresh samples of each formulation from the pre-testing stage was obtained with 40 mL of methanol/water (50:50, v/v) in a magnetic stirrer at room temperature for 1 h. The samples were centrifuged at 25,400g (BR4i multifunction, Chanteau-Gontier, France) for 15 min, and the supernatant was recovered. Next, 40 mL of acetone/water (70:30, v/v) were added to the residue at room temperature, extracted for 1 h in a magnetic stirrer and centrifuged. The methanol and acetone extracts were combined, made to reach 100 mL using distilled water, and were then used to determine the quantity of total phenolic compounds and the antioxidant activity (Rufino *et al.*, 2010).

2.4.2 Estimation of total phenolic compounds. The quantity of total phenolic compounds of each extract was determined using the colorimetric method with the Folin-Ciocalteu reagent (Waterhouse, 2002). Results were expressed as milligrams of gallic acid equivalents per grams of fresh weight (mg GAE. g⁻¹f.w.).

2.4.3 Total antioxidant activity measured using trolox equivalent antioxidant capacity. The ABTS radical was generated by the reaction of 5 mL of aqueous ABTS 7 mM with 0.88 μ L of potassium persulphate (140 mM). The mixture was kept in the dark for 16 h and then diluted with ethanol until a solution with absorbance of 0.7 ± 0.05 at 734 nm was obtained (Rufino *et al.*, 2010). The ethanolic solutions of known trolox concentrations were used for calibration, and the results were expressed as μ mol of trolox equivalent per g of fresh weight (μ mol TE.g⁻¹f.w.).

2.4.4 Total antioxidant capacity measured using free radical scavenging via DPPH. Free radical scavenging activity was measured in triplicate using the method described by Rufino *et al.* (2010) with modifications. A 0.06-mM solution of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) in methanol was prepared, and an aliquot of 100 μ L of the antioxidant or the cereal bar extract solution was added to 3.9 mL of the DPPH solution. The antioxidant capacity was expressed as EC₅₀, and the values were expressed as g of fruit per g of DPPH (g fruit.g⁻¹ DPPH).

2.5 Analysis of texture properties of the cereal bars

Two analyses were used to measure cereal bar texture, using the TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, England):

- (1) Force of rupture: a three-point bending probe was used with a distance of 3.8 cm between the axles and a test speed of 1.0 mm.s⁻¹. For this test, three cereal bars were cut completely, and the maximum force (in newtons) was considered as the force of rupture (Kim *et al.*, 2009); and
- (2) Texture Profile Analysis (TPA): ten cereal bar samples, cut into of 20- × 20-mm pieces with the aid of a stainless steel knife, were used in the test. A cylindrical aluminum probe that was 25 mm in diameter and which operated at a speed of 1.0 mm.s⁻¹ was used; a time of 5 s was established between the two compressions; and the sample was compressed to 50 per cent of its original height. The hardness, cohesiveness, springiness, adhesiveness and chewiness parameters were obtained.

2.6 Statistical analysis

Principal component analysis (PCA) was applied to identify correlations between the bioactive compounds and the physical properties of the cereal bars at both the beginning and the ending of the storage study. Average quantities of bioactive compounds and of physical properties were placed in columns (variables), the different formulations of the cereal bars were placed in rows (cases) and the data were standardized before analysis. The PCA was performed based on a correlation matrix and without factor rotation. For both PCA (beginning and ending of the storage study), six of nine eigenvalues explained 100 per cent of data variation. However, two factors were extracted, as they explained more than 70 per cent of data variation, which means that PCA is an appropriate multivariate analysis to be applied to the data (Mardia *et al.*, 1979). Therefore, respectively to beginning and ending of the storage study, the first principal component explained 46.6 and 49.9 per cent of the data variation, and the second principal component explained 39.5 and 26.9 per cent, totaling 86.1 and 76.8 per cent. Moreover, variables (bioactive compounds and texture properties) were considered to correlate with principal component 1 or 2 when factorial charge was ≥ 0.70 or ≤ -0.70 , which indicates strong correlation with the principal component.

Analysis of variance at a significance level of 0.05 was applied with the factors “formulation” and “time” to investigate the influences of these factors on bioactive compounds and texture properties during the storage study of the cereal bars. The average of bioactive compounds and texture properties at the beginning and ending of storage study were compared using Student’s *t* test for paired samples (differences were considered significant when $p \leq 0.05$). Pearson’s correlation was also applied to discuss some results (significance level of 0.05). All statistical analyses were performed using Statistica 10.0 software (StatSoft Inc., Oklahoma, USA).

3. Results and discussion

PCA shows that time had little effect on the principal characteristics of the cereal bars: the formulations were described by almost the same variables at the beginning and at the ending of the storage period. At the beginning of the storage period, formulation 1 was described by a high quantity of total phenolic compounds and antioxidant activity (ABTS method), as well as by low values for EC₅₀ and hardness. The description of formulation 7, however, was the opposite of formulation 1: it was described by a low quantity of total phenolic compounds and by antioxidant activity measured using the ABTS method, as well as by high values of EC₅₀ and hardness. Formulation 4 was described by high chewiness and a low force of rupture, and formulations 2 and 6 were described as the opposite: by a high force of rupture and low chewiness, springiness and cohesiveness.

At the end of the storage period, formulation 1 was still described by a high quantity of total phenolic compounds and the antioxidant activity (ABTS method), as well as by low EC₅₀ values. Low hardness, however, did not describe formulation 1 by the ending of the storage period. Formulation 7 was also described in a slightly different manner, with high hardness and chewiness values and a low force of rupture. Formulation 2 also differed, with a high force of rupture and low adhesiveness, cohesiveness and springiness.

3.1 Bioactive compounds of the cereal bars

The quantity of total phenolic compounds ranged from 4.19 to 0.87 mg GAE.g⁻¹f.w. in formulations 1 and 7, respectively, and at time zero (Figure 1). Sun-Waterhouse *et al.* (2010) found values ranging from 2.87 to 0.60 mg CtE.g⁻¹ in cereal bars with inulin, phenolic extracts, and dietary fiber from apples, and similar values were also found in some raw fruits (Ignat *et al.*, 2011; Rufino *et al.*, 2010; Souza *et al.*, 2012). There was variation in the

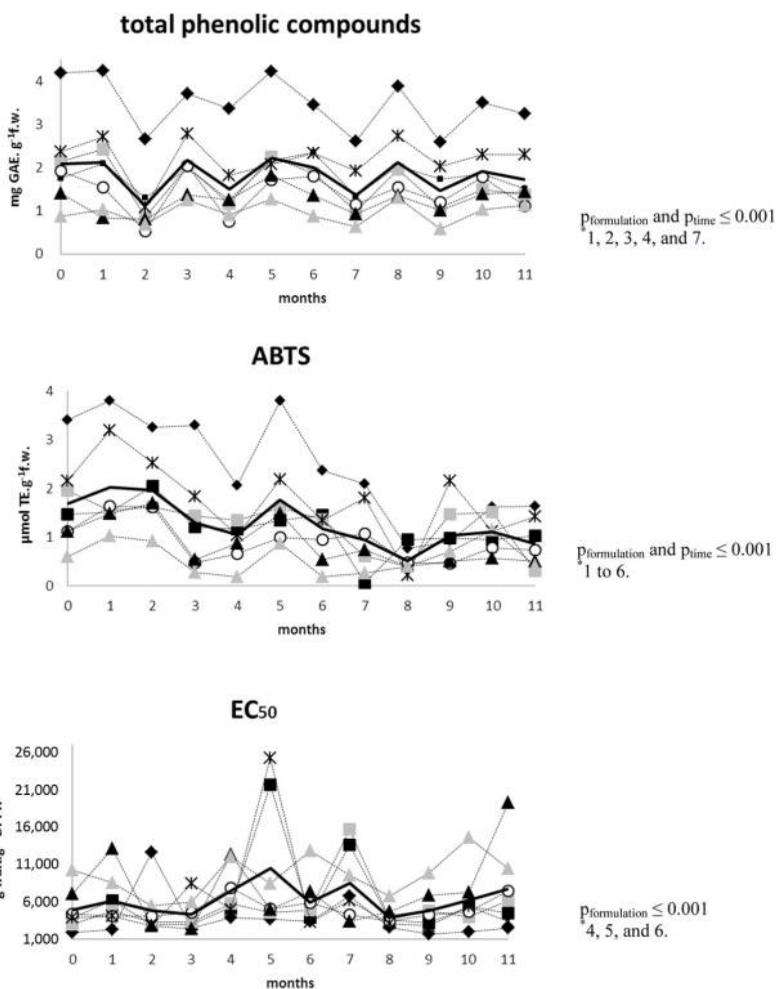


Figure 1. Total phenolic compounds, antioxidant activity by ABTS method and antioxidant activity by DPPH method expressed in EC₅₀ (concentration required to reduce the original amount of free radicals by 50%) of the cereal bars during storage study (11 months)

Notes: Quantities of banana peel flour in each cereal bar: ♦1 = 42 g; ■2 = 2 g; ■3 = 21 g; °4 = 14 g; *5 = 27.72 g; ▲6 = 7.14 g; ▲7 = 7.14 g. Lines with markers indicate each cereal bar along the storage study and the bold line represents the average of all of the formulations. *p*-values of formulation and time are resultant from two-way ANOVA. Asterisk (*) indicates samples that are different statistically ($p \leq 0.05$) between zero time and at eleven months, by paired Student *t* test

quantity of total phenolic compounds in all formulations during storage (Figure 1), and in general, the variation profile of all of the formulations over the 11-month period was similar to the average profile of the formulations as a group (bold line in Figure 1). Variation was influenced by the “formulation” and “time” factors (both *p*-values were ≤ 0.001). Total phenolic compounds decreased in the cereal bars 1, 2, 3 and 4 from zero time to 11 months,

while they increased in the formulation 7 ($p \leq 0.05$). Meethal *et al.* (2017) reported a decrease in the total phenolic compounds in cereal bars elaborated with jackfruit flour and stored in different packages throughout the storage period of the 28 days.

The total antioxidant activity was measured using the ABTS method. It ranged from 3.41 to 0.60 $\mu\text{mol TE}\cdot\text{g}^{-1}\cdot\text{f.w.}$ in formulations 1 and 7, respectively, at time zero (Figure 1). Yu *et al.* (2002) also found similar values, 2.32 to 3.22 $\mu\text{mol TE}\cdot\text{g}^{-1}\cdot\text{f.w.}$ in four samples of commercial cereal bars. During storage, a slight decrease in total antioxidant activity was observed until the fourth month; however, an increase in total antioxidant activity was observed in all of the formulations during the fifth month, with another subsequent reduction (Figure 1). This tendency was observed in almost all of the formulations, and also in the average profile of the formulations as a group (bold line in Figure 1). Variation was influenced by the “formulation” and “time” factors (both p -values were ≤ 0.001). Antioxidant activity decreased in the cereal bars 1 to 6 from zero time to eleven months ($p \leq 0.05$). An increase in total antioxidant activity is usually considered to be a product of the Maillard reaction (Klimczak *et al.*, 2007) which may have occurred when the cereal bars were heated. However, Meethal *et al.* (2017) reported a decrease in the antioxidant activity of cereal bars elaborated with jackfruit flour and stored in different packages throughout the storage period of the 28 days.

EC_{50} ranged from 1,858.68 to 10,244.05 $\text{g fruit}\cdot\text{g}^{-1}$ DPPH at time zero in formulations 1 and 7, respectively (Figure 1), and EC_{50} varied in all formulations during the storage period; however, this variation was influenced only by the “formulation” factor (p -value ≤ 0.001). The variation profile of the formulations over the 11-month period differed from the average profile of the formulations as a group (bold line in Figure 1). EC_{50} value is defined as the concentration of antioxidant that causes a 50 per cent decrease in DPPH absorbance (Chen *et al.*, 2013); thus, the higher the value, the lower the antioxidant activity. Therefore, the antioxidant activity increased in the cereal bar 5 from zero time to 11 months (decrease in the EC_{50}), while it decreased in the formulations 4 and 6 (increase in the EC_{50} ; $p \leq 0.05$).

Although formulations 2 and 3 contained the same quantity of banana peel flour (21 g/100 g), as did formulations 6 and 7 (7.14 g/100 g) (Table I), the quantity of total phenolic compounds and total antioxidant activity differed between these samples in almost every month of storage (Figure 1). This difference indicates that the other ingredients (rice flakes and oat flour) may have some effect on bioactive compounds, as they also exhibit antioxidant activity (Kilci and Gocmen, 2014; Wanyo *et al.*, 2014).

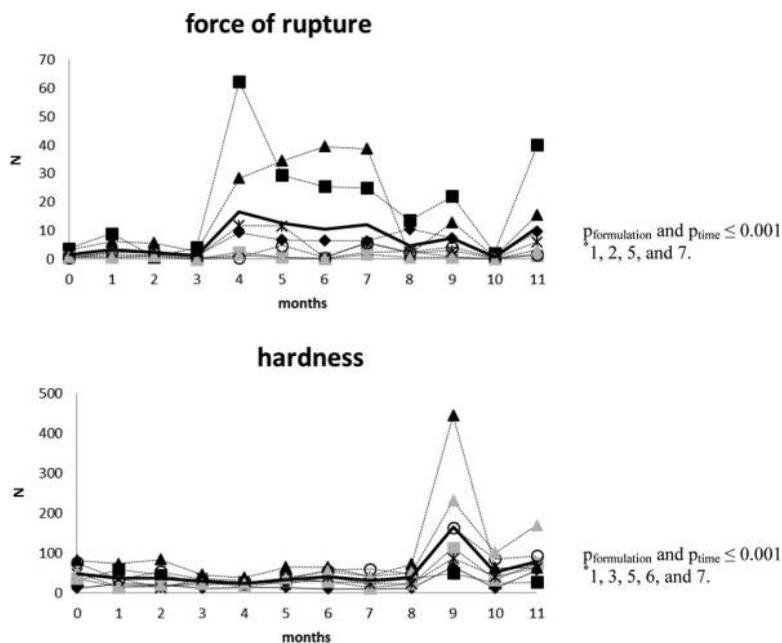
When measured using ABTS, formulation 1 (which included a higher quantity of banana peel flour) was found to have a higher quantity of total phenolic compounds, and formulations 6 and 7 (which included lower quantities of banana flour) had lower total antioxidant activity. Additionally, formulation 1 had lower EC_{50} values, while formulations 6 and 7 had higher EC_{50} values (Figure 1). Over the entire course of the storage period, the correlation coefficients between the quantities of banana peel flour and total phenolic compounds in the cereal bars ranged from 0.88 to 0.99 ($p \leq 0.05$). They ranged from 0.84 to 0.98 ($p \leq 0.05$) between the quantity of banana peel flour and the total antioxidant activity measured using the ABTS method (except for months 7 to 10, in which correlations were weak), and they ranged from -0.78 to -0.88 ($p \leq 0.05$) between the quantity of banana peel flour and EC_{50} values in some months (with weak correlations in other months). This finding indicates that the presence of bioactive compounds in cereal bars depended on the addition of banana peel flour, which contributes to total phenolic compounds and total antioxidant activity in cereal bars and which enhances the functional properties of the products. Yadav and Bathnagar (2016) studied the effect of storage of cereal bars produced

with defatted soy flour, and their results showed stability in moisture, free fatty acid and colour values attributes of the samples in different packages for a period of three months.

3.2 Texture properties of the cereal bars

At beginning of the storage period, the average texture properties of the cereal bars ranged from 0.45 to 3.70 N for force of rupture, from 12.2 to 81.7 N for hardness, from 0.10 to 0.24 for cohesiveness, from 0.31 to 0.75 for springiness, from 1.85 to 6.50 N.s for adhesiveness and from 1.01 to 10.16 N for chewiness. The “formulation” and “time” factors influenced only hardness, springiness and the force of rupture (p -values ≤ 0.001).

The force of rupture began to increase in all of the samples in the fourth month (Figure 2), particularly formulations 2 and 6, which had higher proportions of rice flakes in the composition (Table I). A higher force of rupture of cereal bars with rice flakes was not expected because the rice flakes are voluminous, which makes compacting the cereal bars more difficult and which therefore is likely to reduce the force of rupture. Despite the increase in force of rupture values starting in the fourth month, the values in the tenth month were similar to those at zero time. Over the course of the 11-month period, the variation profile of almost all of the formulations was similar to the average profile of the



Notes: Quantities of banana peel flour in each cereal bar: ♦1 = 42 g; ■2 = 21 g; ■3 = 21 g; ○4 = 14 g; *5 = 27.72 g; ▲6 = 7.14 g; ▲7 = 7.14 g. Lines with markers indicate each cereal bar along the storage study and the bold line represents the average of all of the formulations. p -values of formulation and time are resultant from two-way ANOVA. Asterisk (*) indicates samples that are different statistically ($p \leq 0.05$) between zero time and at 11 months, by paired Student t test

Figure 2.

Texture properties of the cereal bars during storage study (11 months)

formulations as a group (bold line in [Figure 2](#)), although an opposite behavior occurred in formulations 2 and 6 from the fourth month to the seventh month. Furthermore, the force of rupture increased in the cereal bars 1, 2, 5, and 7 from zero time to eleven months ($p \leq 0.05$).

The hardness of the cereal bars changed less over time than the force of rupture values, though its increase was more apparent in the ninth month, especially in formulations 3, 4, 6 and 7 ([Figure 2](#)). These formulations had higher amounts of oat flour ([Table I](#)), and the high capacity of oat to absorb water ([Sharma et al., 2014](#)) may have led to the increased hardness of the cereal bars. The hardness of all formulations changed similarly over the 11-month period, as did the average profile of all formulations. Moreover, the hardness increased in the cereal bars 1, 3, 5 and 7 from zero time to 11 months, while decreased in the formulation 6 ($p \leq 0.05$).

Chemical and physical interactions among the ingredients in cereal bars can occur over time and begin to affect the taste and texture of the product. The shelf life of a cereal bar is substantially determined by its texture. Moreover, the time from manufacturing to consumption of the snack bars may be influenced by the lack of a thermodynamic equilibrium, which is common in heterogeneous multicomponent systems in processed foods ([Loveday et al., 2009](#); [Mezzenga, 2007](#); [Sun-Waterhouse et al., 2010](#)). [Figure 2](#) helps to establish a cut-off point at the eighth month in terms of the texture properties, as, until this point, the hardness of the cereal bars is still similar to the beginning of the storage period, and the force the rupture is not significantly different from the initial values of some formulations.

4. Conclusion

Effects of time on phenolics compounds, antioxidant activity and texture properties were not the same for all formulations of cereal bars formulated with banana peel flour. The quantity of total phenolic compounds in some cereal bars decreased over the period of storage, though it increased in another formulation. Total antioxidant activity, which was measured using the ABTS method, increased in the fifth month, but it then decreased until the last month. In addition, total antioxidant activity decreased in almost all of the formulations over the course of the storage period. The antioxidant activity measured using the DPPH method was not modified in many formulations, though it decreased in some formulations. The texture properties of the cereal bars changed during storage, with an increased force of rupture starting in the fourth month and with increased hardness starting in the ninth month. Although time did affect total phenolic compounds, antioxidant activity and texture properties of cereal bars during storage, the PCA showed that time had little effect on the most important characteristics used to describe cereal bars: the formulations were described by almost the same characteristics at the beginning and at the end of the storage period. Considering the texture, we can observe that the cereal bar with the addition of banana peel flour can be consumed up to the third month of storage, due to the high increasing on the force of rupture at the fourth month, although studies about sensory acceptability should be performed to evaluate this increment. However, considering the bioactive compounds, consumption of the cereal bars can be done up to the fifth month, based on results for total phenolic compounds and total antioxidant activity measured through ABTS method. Finally, the presence of bioactive compounds in cereal bars depends on the addition of banana peel flour, which contributes to the insertion of total phenolic compounds and the antioxidant activity in cereal bars and therefore aggregates functional properties in the products.

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