LWT - Food Science and Technology 215 (2025) 117245

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Stability study of bioactive compounds from yerba mate extract encapsulated by ionic gelation and application of microparticles in fruit and cereal bars

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ARTICLE INFO

Keywords:
Yerba mate microparticle
Bioactive compound's stability
Food application
Fruit bars

ABSTRACT

Changes in consumer eating habits and consumption trends have shown a preference for foods combining convenience and healthiness. The objective of this work was to study the stability of yerba mate dehydrated microparticles and use them in fruit and cereal bars, aiming to increase the bioactive compounds as well as maintain their acceptance. Microparticles were produced by ionic gelation and dried in fluidized bed. Stability was analyzed according to colour changes and quantification of bioactive compounds for six weeks of storage at the temperatures of 25 °C and 35 °C. Fruit and cereal bar formulations added with yerba mate microparticles were developed, aiming to add bioactive compounds to such product. Stability was studied for 151 days using total phenolic compounds' content and colour changes as parameters. Microencapsulation has shown to be appropriate to preserve the colour and compounds of interest from yerba mate extract, providing longer protection from time and temperature effects. Bars were submitted to acceptance test which, with respect to general evaluation, showed that samples added with dried microparticles had over 80% acceptance. The increase of bioactive compounds associated with product acceptance suggests the use of yerba mate, whether free or encapsulated, in fruit and cereal bars is feasible.

1. Introduction

New trends together with changes in consumer lifestyle and eating habits have been indicating the preference for easy-to-prepare healthy meals (Sharma, Sachdev, & Kaur, 2014). Nowadays, snacks market aims to use plant-based ingredients, preferably with functional properties. This change is associated with conscious consumption by part of population who is increasingly concerned about health as well as animal well-being (Mordor Intelligence, 2023).

The search for a healthier lifestyle is connected to food choices and motivations, such as family, genetic, and cognitive factors, as well as cultural and social influences (European Commission, 2018). Trends of consuming industrial foods have shown the preference for higher nutritional value products due to worry about diet and including of whole grains, fibers, and fruits, aiming to increase immunity and cardiovascular and digestive health (Rego, Vialta, & Madi, 2020).

Most cereal bar consumers are women. The income level is not relevant for consumption, and cereal bars are frequently seen as a snack to alleviate hunger. Among consumer age groups, elderly associate this product to something healthy, while young people and adults to convenience. The decision to purchase cereal bars is usually encouraged by their flavor and nutritional value (Degáspari, Mottin, & Blinder, 2009). A limiting factor still observed in some categories is price. Clean label ingredients can cause the product to be not much accessible in low-income countries; however, South America is the fastest growing market. North America is the largest market of this sector (Mordor Intelligence, 2023). Another difficulty is to find the perfect balance in the product, comprising all the requirements considered as important, such

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https://doi.org/10.1016/j.lwt.2024.117245
Received 15 May 2024; Received in revised form 12 December 2024; Accepted 17 December 2024
Available online 18 December 2024

as flavor, appearance, and nutritional quality (Favaro et al., 2020).

By being a popular and practical product, cereal bars would be the type of product appropriate to provide phenolic compounds and fibers from fruits and cereals. Expanding their consumption beyond breakfast could promote a greater increase of functional ingredients in diet. Current cereal bar market includes from antioxidant and protein-rich options to the use of so-called superfruits, which distinguish these bars from others, making them more attractive to consumers (Sharma et al., 2014). Cereal bars present attractive characteristics to the consumer for their nutritional composition associated with healthy products and for the convenience of consumption (Cruz et al., 2024). However, the biggest challenge for obtaining a cereal bar with good acceptability is the combination of different ingredients with specific functionalities, such as vitamins, minerals, proteins, grains, fibers, thickening agents, sweeteners, and flavorings, while trying to achieve specific nutritional goals (Cruz et al., 2024). According to Kosicka-Gębska, and Sajda-kowskaJeżewska-ZychowiczGębskiGutkowska (2024) consumers are interested as a snack in the form of fruit and cereal bars with health-promoting properties. However, it is important for manufacturers to introduce an addition to their composition or reduce the content of certain unfavorable ingredients for health reasons, for example, sugar.

The yerba mate (*Ilex paraguariensis*) has been explored for having several health benefits, such as the prevention of cardiovascular diseases, the treatment of hepatitis B, in addition to presenting antioxidant and antimicrobial properties (Costa, E, Racanicci, & Santana, 2017; Pinto et al., 2021). It is also possible to highlight the reduction in the risk of degenerative diseases caused by oxidative stress (Baeza et al., 2016). The benefits offered by the yerba mate in general are due to its composition rich in polyphenols, such as phenolic acids, alkaloids and flavonoids, highlighting chlorogenic acids, caffeine, and rutin (Budin et al., 2023; Pilatti-Riccio et al., 2019) and also as methylxanthines and saponins (Alasmari, 2020). Health-promoting properties of yerba mate extract including nervous system stimulant effect, anti-obesity, antimutagenic, hypocholesterolemia and hepatoprotective properties, and antioxidative effect (inhibition of lipid peroxidation in plasma or LDL oxidation) have been also reported (Akbarmehr et al., 2023). These reports imply on positive nutritional proprieties of yerba mate and suggests its important antioxidant effects in human health (Akbarmehr et al., 2023).

One of the methods to add these components to food would be by microencapsulation, which could even be used to mask possible flavors and odors. The purpose is to maintain compound stability, aiming the best delivery and promoting their controlled release (Choudhury, Meghwal, & Das, 2021; Vasisht, 2014; Sobel, Versic, & Gaonkar, 2014). The use of such technique in food products may result in higher quality foods (Silva et al., 2014).

Encapsulation by ionic gelation produces hydrogel spheres, which is advantageous in terms of biocompatibility and due to its adjustable properties to control asset release profile (Skicki, 2021; Silveira et al., 2023). On the other hand, the hydrophilic property impairs the encapsulation of compounds that tend be lost to an aqueous medium. As the high moisture content of particles obtained by ionic gelation may cause limited stability and lead to incompatibility with the food matrix, the strategy of subsequent drying of these particles can be a viable alternative for greater stability and better commercialization possibilities of these particles (Budin et al., 2023). Since 2021, approximately 11,700 research and review articles related to this method have been published according to the statistics of Google Scholar. Interesgly, the number of articles has increased year by year, reaching a peak in 2021 with 2090 publications. This shows the researchers' interest in the ionic gelation method (Hoang et al., 2022).

This work had the purpose of studying the stability of yerba mate dehydrated microparticles and their subsequent use in fruit and cereal bars, aiming to increase the bioactive compound content as well as maintain their acceptance and stability of added phenolic compounds.

2. Material and methods

Hydroalcoholic extract of yerba mate (*Ilex paraguariensis*) 64°GL was used, supplied by the company Heide Extratos Vegetais, located in the city of Pinhais/PR - Brazil. The extract was produced with the leaves of *Ilex paraguariensis* in an alcoholic vehicle (ethyl alcohol).

Microparticles were produced by ionic gelation from double emulsion (W/O/W), according to adapted methodology (Aizpurua-Olaizola et al., 2016; Belscak-Cvitanovic et al., 2016; Isusi, Karbstein e Van der Schaaf, 2019; Moura et al., 2018). In this process, a simple W/O, emulsion (water in oil) in a ratio of 35:65 was produced, where the yerba mate extract was added by dripping into a mixture of Liza® canola oil – 60g, (Cargill Agrícola S.A., Mairinque/Brazil) and PGPR – 5g (polyglycerol polyricinoleate) surfactant (Concepta Ingredients, São Paulo/-Brazil). From this emulsion, a W/O/W double emulsion (water in oil in water) was prepared, with the addition of 2% pectin solution, in a ratio of 20:80 (20g simple emulsion to 80g pectin solution). With the aid of Encapsulator equipment model B-390 (Büchi, Flawill, Switzerland), microparticles were produced by dripping the double emulsion into a 3% calcium chloride solution. Subsequently, particles were dried by fluidized bed (company LabMaq – Riberão Preto/SP), which used Fluid Bed Dryer FBD 1.0 equipment. Details about this study can be found in Budin et al. (2023). In order to conduct the process, particles were firstly sieved and tamized (mesh of 1.4 mm and 710 μm). Drying conditions were: sample amount 500g, inlet temperature of 50 °C, outlet temperature of 30 °C, time of 40 min, air flow rate 1.2–2.0 m^3/min and compressed air flow rate 25–40L/min.

2.1. Dried particles stability

To study stability, the particles were divided into ten portions of 4 g and placed in polyethylene bags. Five portions were destined for storage at 25 °C/55 %RH, and the other five portions at 35 °C/55 %RH, both protected from light. During 5 weeks, a sample of each condition was removed from storage and carried out the following analyses:

2.1.1. Colour analysis

A portable colourimeter CR-400 (Konica Minolta Sensing Inc., Osaka, Japan) was used for evaluation by CIE Lab system, L*, a*, and b* parameters (Konica-Minolta Sensing Inc., Osaka, Japan). Where L* represents luminosity, which varies from 0 (black) to 100 (white), a* varying between + 60 (red) and −60 (green) and b*, from +60 (yellow) to −60 (green), all these values are represented in the colour space chromaticity diagram. Chroma (C*) were calculated according to Equation (1).

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (1)$$

Stability was measured by total colour difference calculated according to Equation (2).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

where: ΔE*: total colour difference; ΔL*: difference between luminosity; Δa*: difference between the chromatic coordinate a*; Δb*: difference between the chromatic coordinate b*.

2.1.2. Total phenolic compounds

Phenolic compounds of particles were determined by the FolinCiocalteau spectrophotometric method according to Erkan-Koç et al. (2015). The methodology required the construction of a 5-point standard curve of gallic acid (40, 80, 120, 160, and 200 μg/mL). A blank was also prepared, which had all used reagents except for the sample, which was replaced by distilled water. Samples and standard curve readings were made in a UV/Visible spectrophotometer (Agilent Technologies, Cary 60 MY13110012, United States of America) at a wavelength of 750

nm. The results were shown in mgGAE/100 g.

2.1.3. Antioxidant activity by DPPH method

Performed according to method described by Jiménez-Zamora et al. (2016). This method is based on the capture of the DPPH radical (2, 2-diphenyl-1-picrylhydrazyl) by the antioxidants present in the sample. The radical has an intense violet colour, which undergoes reduction and discolouration depending on the concentration of antioxidant compounds to which it is subjected. In order to conduct this assay, yerba mate extract diluted in alcohol 70% was used. A 6-point Trolox standard curve (50, 100, 200, 400, 600, and 800 μM) was made. Both samples and standard curve were read at a wavelength of 515 nm in a UV/Visible spectrophotometer. The results were shown in μmol TE/g (Trolox Equivalent).

2.1.4. Antioxidant activity by ABTS method

Performed according to method described by Jiménez-Zamora et al. (2016) In this method, an ABTS radical solution (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and 2.6 mM potassium persulfate are used. When it comes into contact with the sample, capture occurs. of the radical, due to the antioxidant compounds present there. During the reduction reaction, the solution loses its colour, which from dark green becomes lighter depending on the concentration of antioxidants. A 5-point Trolox standard curve (100, 500, 1000, 1500, and 2000 μM) was made. The samples of yerba mate extract diluted in alcohol 70% and standard curve were read after 6 min of incubation in the absence of light at a wavelength of 734 nm. For absorbance reading, an UV/Visible spectrophotometer was used. The results were shown in μmol TE/g (Trolox Equivalent).

2.1.5. Microparticle degradation kinetics

The degradation of quality standards studied was calculated based on kinetic models described by Moura et al. (2018). Adjustments in zero- and first-order reaction models were analyzed. The coefficient of determination of regression (r^2) was used as criterion for choosing the best adjustment of model to experimental data, from which reaction constant (k), activation energy (E), Q_{10} and half-life time ($t_{1/2}$) were obtained by using equations (3) and (6), respectively:

$$ln\left(\frac{C}{C_0}\right) = -kt \tag{3}$$

$$Q_{10} = \frac{k_T}{k_{T-10}} \tag{4}$$

$$t_{1/2} = \ln 2 / k \tag{5}$$

$$E = 0,46 \times T^2 \times \log Q_{10} \tag{6}$$

where: T (Kelvin), Q_{10} (ΔT°C), E (cal. g.mol^{-1}), k (day^{-1}).

2.2. Production of fruit and cereal bars added with yerba mate microparticles

The microparticles were incorporated to a standard dehydrated fruit (red berries - Blackberry (*Rubus* subg. *Rubus*) and raspberry *(Rubus idaeus* L.), cherry (*Prunus avium*), apple (*Malus* domestica *Borkh)* and strawberry (*Fragaria* × *ananassa*), by Liotécnica Tecnologia de Alimentos, Embu das Artes, Brazil) and cereal bar formulation (Table 1). They were prepared at *Cereal Chocotec*, ITAL.

Four formulations were evaluated, which corresponded to four product variations while maintaining phenolic compound ratio:

- Fruit and cereal bar (standard): SB;
- Fruit and cereal bar added with yerba mate extract: EB;
- Fruit and cereal bar added with wet yerba mate particles: WPB
- Fruit and cereal bar added with dry yerba mate particles: DPB

Table 1
Fruit and cereal bar formulation.

Ingredients	SB		EB		WPB		DPB	
	g	%	g	%	g	%	g	%
Rice flakes (ball type)	429	9	429	8	429	8	429	8
Oat flakes	334	7	334	6	334	6	334	6
Corn flakes	286	6	286	6	286	5	286	6
Dehydrated strawberry	334	7	334	6	334	6	334	6
Dehydrated red berries	382	8	382	7	382	7	382	7
Dehydrated cherry	515	10	515	10	515	9	515	10
Dehydrated apple	134	3	134	3	134	2	134	3
Cashew nut	286	6	286	6	286	5	286	6
Fibersol®	768	15	768	15	768	14	768	15
Gum Arabic	85	2	85	2	85	2	85	2
Fructose	148	3	148	3	148	3	148	3
Maltodextrin	371	7	371	7	371	7	371	7
Soy lecithin	21	0	21	0	21	0	21	0
Palm fat	148	3	148	3	148	3	148	3
Glycerin	106	2	106	2	106	2	106	2
Salt	16	0	16	0	16	0	16	0
Water	637	13	637	12	637	12	637	12
Yerba mate extract	–	–	200	4	–	–	–	–
Wet yerba mate particles	–	–	–	–	500	9	–	–
Dry yerba mate particles	–	–	–	–	–	–	200	4
Total	5000	100	5200	100	5500	100	5200	100

SB: fruit and cereal bar (standard); EB: fruit and cereal bar added with yerba mate extract; WPB: fruit and cereal bar added with wet yerba mate particles; DPB: fruit and cereal bar added with dry yerba mate particles.

Fruit and cereal bar production was composed of the following steps: homogenization of ingredients, laminating, and cut, respectively conducted in a mixer, laminator, and cut equipment of Braslaer brand (Fig. 1). Then, the bars were stored in metal packaging. The storage was made at room temperature in a clean, dry, well-ventilated place protected from light.

2.3. Centesimal composition of fruit and cereal bars

2.3.1. Moisture and volatile compounds

It was measured according to AOAC – Association of Official Analytical Chemists (2012) regulation.

2.3.2. Ash content

It was measured according to IAL methodology (2005) by the method of waste incineration.

It was measured by the prior acid hydrolysis determined as per IAL – Adolfo Lutz Institute (2005).

2.3.3. Protein

It was measured according to AOAC – Association of Official Analytical Chemists (2012) regulation, by Kjeldahl method.

The analysis of the protein content of fruit bar samples (item 2.3) was calculated by multiplying the total nitrogen by a specific factor for converting nitrogen into protein. This analysis does not differentiate between nitrogen of protein and non-protein origin (nucleic acids, free amino acids, amino sugars, amines, amides, free BCAA, caffeine, monosodium glutamate, ammonium ions, porphyrins, taurine, urea, vitamins, among others), in addition of not allowing the origin of the protein used to be identified. For correct interpretation of the protein result, all sources of nitrogen in the product must be considered.

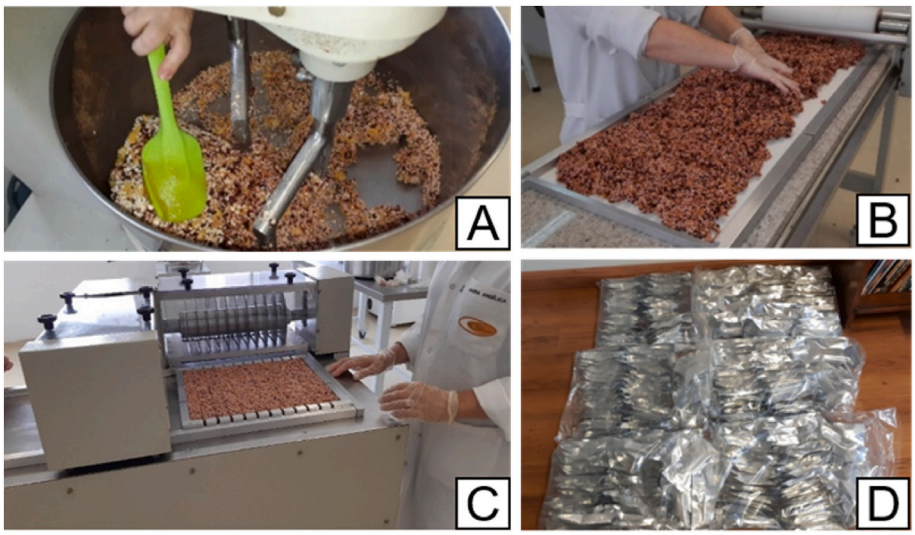

Fig. 1. Steps of fruit and cereal bar preparation. A) Homogenization, B) Laminating, C) Cut, and D) Storage. Source: Authors.

2.3.4. Carbohydrates

Carbohydrates present in samples were calculated by difference:

$$Carbohydrates = 100 - (g.100\ g^{-1} moisture + g.100\ g^{-1} ashes + g.100\ g^{-1} total\ fat + g.100\ g^{-1} proteins) \quad (7)$$

2.3.5. Energy value

It was calculated according to Kalil (1975), Passmore, Nicol, Rao, Beaton, and Demaeyer (1975), and USDA – United States Department of Agriculture (1963), in which protein and carbohydrate percentages were multiplied by factor 4 (kcal/g) added to total fat content multiplied by factor 9 (kcal/g).

2.4. Microbiological analyses of fruit and cereal bars

2.4.1. Analysis of the presence of Salmonella sp.

Methodology based on AOAC – Association of Official Analytical Chemists (2012), which used Bax System for the detection of *Salmonella*.

2.4.2. Count of Escherichia coli

The count of *E. coli* was performed as per ABNT (2022) NBR ISO 16649:2001, by using TBX method.

2.4.3. Count of mold and yeast

The count of mold and yeast was conducted according to Salfinger and Tortorello (2015) using Compendium APHA.

2.5. Characterization of fruit and cereal bars

2.5.1. Water activity of fruit and cereal bars

Representative samples of the bars were initially crushed and packaged in white plastic capsules. It was measured by using a Decagon hygrometer (USA) and methodology adapted from Downes and Ito (2001) and Troller and Scott (1992). This technique is based on measuring the "dew point", which will indicate the amount of free water in the sample.

2.5.2. Colour analysis of fruit and cereal bars

Colour analysis was measured as described earlier in section 2.1.

2.5.3. Fruit and cereal bars texture

Hardness was measured in a SMS brand texture analyzer TA-XT Plus (Stable Micro System, Godalming, United Kingdom) operating with *software Exponent Lite* version 5.1.1.1.0, probe HDP/KS5 (Kramer Shear Cell), at the following conditions: compressive strength measurement, pre-test speed: 1.0 mm/s; test speed: 2.0 mm/s; post-test speed: 10.0 mm/s; distance: 40.0 mm.

2.5.4. Active ingredients extraction from fruit and cereal bars

In order to determine total phenolic compounds and antioxidant activity, extract obtained from bars was used. An amount of 7 g from the sample and 7 g of Celite was weighted, then they were mixed and 30 mL of ethyl alcohol 70% were added. The mixture remained in ultrasonic bath for 4 min and was homogenized in disperser (Turratec - Tecnal model TE102, Piracicaba, Brazil) at 14.000 rpm for other 4 min. Vacuum filtration in Kitassato equipment attached to a Buchner funnel was performed, and filtering was collected in a 100 mL volumetric flask. Residue in the filter paper was recovered and a new extraction was made, exactly as the first one. The process was repeated once more, resulting in three extractions. The volumetric flask with the extract was completed with ethyl alcohol 70%. The following analyzes were carried out on this extract:

2.5.5. Total phenolic compounds

It was analyzed according 2.1 section.

2.5.6. Antioxidant activity by DPPH method

It was analyzed according 2.1 section.

2.5.7. Antioxidant activity by ABTS method

It was analyzed according 2.1 section.

2.6. Sensory acceptance

The sensory project was evaluated by the research ethic committee of Hospital Municipal Dr. Mário Gatti – HMDMG, Campinas, Brazil and was approved under CAAE Number: 38438520.8.0000.5453.

Samples were sent to obtain mean acceptance results in general and particularly about appearance, texture, flavor, Sweetening, and aftertaste, as well as preference.

Sensory acceptance was determined by means of a test with consumers, according to ABNT (2016) NBR ISO 11136; Meilgaard et al. (2007), and a rank test for preference based on ABNT (2015) NBR ISO 8587, as described below:

In order to evaluate the four samples of red berries bar with cereal (standard, added with extract, added with wet particles, and added with dry particles), 81 fruit bar consumers were recruited, without

restrictions regarding gender, age and frequency of consumption. At the beginning of the test, before evaluating the samples, consumers answered questions about fruit bar consumption habits and personal characteristics related to age and definition of social class according to the Brazilian Economic Classification Criteria 2021 (ABEP – ASSOCIAÇÃO BRASILEIRA DE EMPRESAS DE PESQUISA, 2021).

The samples were evaluated with respect to:

- General acceptance and particularly for appearance, texture, flavor, Sweetening, and aftertaste, by using the 9-point hedonic scale (9 = liked extremely, 5 = neither liked nor disliked and 1 = disliked extremely).
- Quantity of fruits, firmness, crispness, and intensity of red berry flavor by using optimal 5-point scale (5 = much more/firmer/crispier/more intense than I like it; 3 = the way I like it; 1 = much less/less firm/less crispy/less intense than I like it).

Consumers were instructed to order the four samples according to their preference, and to describe the reasons for ranking the most preferred sample in 1st place and the least preferred one in 4th place. The samples were identified by random 3-digit codes and presented in a sequential monadic test, as per a balanced complete block design. *Compusense Cloud* system was used for data collection and analysis.

Data were subjected to analysis of variance and Tukey's test for comparison of means. The statistic program XLSTAT 2021.5.1.1228 was used for the penalty analysis, which evaluated the effects of intensity with respect to: quantity of fruits, firmness, crispness, and red berry flavor intensity, whether suboptimal or above optimal, on general acceptance of the samples.

When evaluating the preference, results regarding the sum of ranking positions were treated based on Friedman's test and Fischer's test for comparison among the samples at a 5% error level.

2.7. Stability of fruit and cereal bars

For stability study, fruit and cereal bars were stored at 25 °C/55% moisture and 35 °C/55% moisture, protected from light. Analyses were made once a month for approximately 151 days, with phenolic compound and colour analysis conducted as shown below.

Degradation of studied quality standards was calculated based on the kinetic models obtained by Moura et al. (2018). Adjustments of zero- and first-order reaction models were evaluated. The coefficient of determination (r^2) of regression was used as criterion for choosing the best model adjustment to experimental data, of which the constant of reaction (k) and Q_{10} were obtained by using equations (3)–(5).

2.8. Statistical analysis

The authors were responsible for sampling and collecting the material, whose identification was provided to the analysis laboratories. The results apply exclusively to the sample(s) analyzed, and the use of ITAL's name is prohibited, under penalty of compensation, to qualify production over which it did not exercise control. All analyzes were carried out in laboratories approved under ISO 9001 and in some cases also in laboratories accredited under ISO 17025.

All the experiments were perfomed at least in triplicate and submitted to variance analysis for determining significant differences between the averages were determined using one-way (ANOVA) analysis and Tukey test comparisons ($p < 0.05$) performed with Statistica 7 software.

3. Results and discussion

3.1. Study of stability of dehydrated yerba mate microparticles

3.1.1. Colour analysis

As observed in Table 2, Luminosity (L*) of microparticles showed a significative decrease in both storage temperatures, 25 °C and 35 °C. Chromatic coordinate b* also showed a significative reduction in yellow over time.

Oliveira (2015) studied stability of sorghum grain for 180 days with storage at three temperatures (4°, 25°, and 40 °C). Over the course of days, L* increased in the three temperatures. At 25 °C, Luminosity was increased from 23.36 (day zero) to 30.09 (day 180). The b* coordinate also showed an increasing trend, ranging from 18.10 on the first day to 22.82 on the last day of the experiment. Such trend was contrary to the one observed in this study. Since they are different types of products, *in natura* grain and microparticle, the latter also underwent several steps, which may have contributed to different behaviors during storage.

On the other hand, Machado et al. (2019), when analyzing stability for 120 days of pasteurized and frozen *Physalis peruviana* L. pulp, have not observed any significant differences in Luminosity or chromatic coordinate b* during storage. In this case, maintenance of colour may be associated to sample freezing. For dried yerba mate microparticles, storage at a different condition may have accelerated the decrease in Luminosity and coordinate b*.

According to Fenoglio et al. (2021) when planning to add microparticles of yerba mate extract produced by spray and freeze dryer to a real food matrix, it would be interesting not to change your natural colour. Microparticles could change how consumers would perceive the product as "less natural" or "more processed'' if changes in colour are detected.

With respect to chromaticity (C*), firstly there was a significative reduction in microparticles saturation and, after some days, such trend remained constant.

Total colour difference (ΔE) showed an increase in both temperatures, with a greater slope during storage at 35 °C (Fig. 2). At this temperature, microparticles had their colour more affected through the days. The higher temperature increased the degradation and oxidation of the compounds present in the particles, mainly the yerba mate extract, which is naturally darker, and the vegetable oil, which was used to prepare the emulsion. At a higher temperature, for a certain period of time, more significant changes occurred in these particles. And these changes in the sample are progressive, accentuating the difference in relation to the previous period.

The ΔE value was adjusted for Arrhenius and the best-fitting model was the zero order one. Where $Q_{10} = 1.20$ and activation energy (E_a) = 3.23 kJ/mol, Q_{10} represents that, for each 10 °C increase, microparticles will show a 1.20-fold increase in their total colour difference. By studying the stability of free yerba mate extract, Budin et al. (2023) achieved $Q_{10} = 1.86$ and $E_a = 11.18$ kJ/mol. Thus, encapsulation has promoted the preservation of extract colour, as in its free state there were greater changes due to time and storage temperatures.

3.1.2. Total phenolic compounds

The time of storage of dried yerba mate microparticles resulted in a significative decrease in total phenolic compounds at both temperatures analyzed (Table 3).

These compounds, as they are naturally sensitive, are subject to degradation due to storage temperature. Studies show that the lower this temperature, the greater the preservation of phenolic compounds (Campos et al., 2009). Fenoglio et al. (2021) found that yerba mate extract in spray-dried powders showed the highest polyphenol content and adequate oxidative stability, also achieving the lowest moisture content compared to the freeze-dried samples.

In the case of microencapsulation of yerba mate extract, Akbarmehr et al. (2023) found that all spray-dried powders presented significantly ($p < 0.05$) less content of phenolic compounds and antioxidant capacity in relation to pure yerba mate. The phenolic compounds of the encapsulated samples ranged between 2.5 and 3.3 mg GAE/g powder and the authors concluded that the use of hydrolyzate as transporter played a significant role in maintaining the phenolic compounds and antioxidant activity of yerba mate extract during spray drying process. In the present

Table 2
Standards of colour of dried yerba mate microparticles during stability study with storage at two temperatures.

Storage temperature	Time (days)	Parameter			
		L*	b*	C*	ΔE
25°C	0	$39.19^{a} \pm 1.11$	$27.97^{a} \pm 0.70$	$28.28^{a} \pm 0.69$	–
	7	$34.05^{b} \pm 1.65$	$24.03^{b} \pm 1.24$	$25.02^{c} \pm 1.27$	$7.04^{d} \pm 1.62$
	14	$33.41^{b} \pm 2.10$	$23.98^{b} \pm 1.71$	$25.47^{c} \pm 1.72$	$8.28^{d} \pm 1.81$
	21	$32.63^{c} \pm 1.57$	$25.10^{b} \pm 1.61$	$26.97^{b} \pm 1.63$	$9.14^{c} \pm 1.32$
	28	$29.48^{c} \pm 1.26$	$20.04^{c} \pm 1.14$	$21.80^{e} \pm 1.22$	$13.29^{b} \pm 1.42$
	35	$26.44^{d} \pm 1.27$	$21.90^{c} \pm 1.52$	$24.28^{d} \pm 1.63$	$15.46^{a} \pm 1.21$
35°C	0	$39.19^{a} \pm 1.11$	$27.97^{a} \pm 0.70$	$28.28^{a} \pm 0.69$	–
	7	$29.40^{b} \pm 0.51$	$20.80^{c} \pm 0.45$	$22.57^{c} \pm 0.48$	$12.97^{d} \pm 0.50$
	14	$30.03^{b} \pm 0.81$	$21.81^{b} \pm 0.65$	$23.76^{b} \pm 0.70$	$12.22^{d} \pm 0.81$
	21	$29.19^{bc} \pm 1.25$	$21.74^{b} \pm 0.67$	$23.97^{b} \pm 0.72$	$13.18^{c} \pm 0.98$
	28	$28.23^{c} \pm 1.34$	$19.67^{d} \pm 1.22$	$21.76^{d} \pm 1.36$	$14.67^{b} \pm 1.35$
	35	$25.25^{d} \pm 2.00$	$20.08^{c} \pm 2.63$	$22.64^{c} \pm 2.93$	$17.20^{a} \pm 1.44$

Means followed by different letters are significantly different among each other at $p < 0.05$ by Tukey's Test.

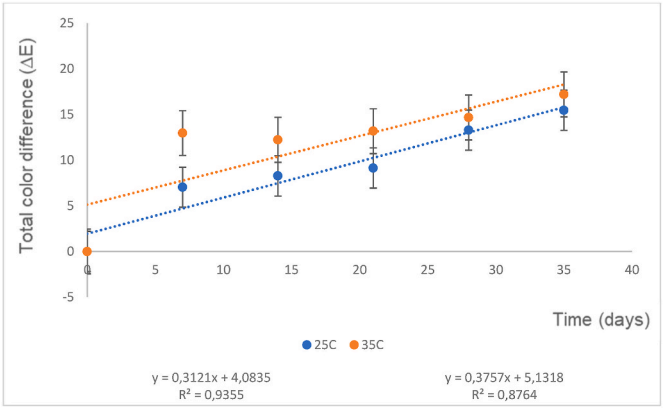

Fig. 2. Total colour difference (ΔE) of dried yerba mate microparticles during storage at two temperatures.

Table 3
Evaluation of Ln phenolic compounds present in dried yerba mate microparticles during storage at two temperatures.

Parameter	Time (days)	Storage temperature	
		Ln 25 °C	Ln 35 °C
Ln Phenolic compounds (mg GAE/100 g)	0	$6.15^{a} \pm 0.02$	$6.15^{a} \pm 0.02$
	7	$5.87^{b} \pm 0.01$	$5.69^{b} \pm 0.03$
	14	$5.77^{d} \pm 0.03$	$5.58^{d} \pm 0.03$
	21	$5.78^{d} \pm 0.03$	$5.65^{c} \pm 0.03$
	28	$5.81^{c} \pm 0.01$	$5.66^{c} \pm 0.00$
	35	$5.82^{c} \pm 0.01$	$5.72^{b} \pm 0.01$

Means followed by different letters are significantly different among each other at $p < 0.05$ by Tukey's Test.

study, the content of phenolic compounds in yerba mate microparticles obtained by ionic gelation followed by drying in a fluidized bed were slightly higher, ranging from 4.69 to 3.37 mg GAE/g when stored at 25 °C for 35 days.

When analyzing stability of phenolic compounds in microspheres with blackberry leaves (*Morus nigra* L.) extract stored at 4 °C and 25 °C for 81 days, Schafranski (2019) observed that in dehydrated microspheres there was no significant difference at a 5% level in total phenolic compound concentration up to 21st day of storage. Also, no difference was observed between 36th and 66th day. Dehydrated microspheres showed a concentration ranging from 74.3 mg GAE g^{-1} (day zero) to 62.6 mg GAE g^{-1} (81 days). Storage at the lowest temperature has shown to be more effective, with greater preservation of phenolic compounds, from 74.3 mg GAE g^{-1} (day zero) a 66.3 mg GAE g^{-1} (81 days). The same was concluded in this study: phenolic compounds present in particles stored at lower temperatures have lower degradation than those stored at higher temperatures.

Jacques et al. (2010) studied the phenolic compound stability in frozen blackberry (*Rubus fruticosus*) pulp. The pulps stored at −10 °C ranged from 1938.70 mg GAE 100 g^{-1} of fruit (day zero) to 1490.05 mg GAE 100 g^{-1} of fruit (6 months). The pulps showed a higher loss of phenolic compounds than samples kept at −80 °C, with 1938.70 mg GAE 100 g^{-1} of fruit on day zero and 1780.02 mg GAE 100 g^{-1} of fruit in 6 months. This result also supports that, the lower the storage temperature, the greater the phenolic compound preservation.

The results achieved in stability study at the two temperatures (25 °C and 35 °C) fitted better to a first order model, as shown in Fig. 3.

The 35 °C temperature had a grater slope. From these results, values of $Q_{10} = 1.16$ and $E_a = 2.63$ kJ/mol were found. Q_{10} shows the effect of temperature to preserve phenolic compounds, from which it can be concluded that each 10 °C increase causes 1.16-fold higher degradation. Budin et al. (2023) have found values of $Q_{10} = 1.27$ and $E_a = 14.81$ kJ/mol for hydroalcoholic extract of yerba mate. Therefore, encapsulation can be crucial to preserve phenolic compounds since free extract tends to have a faster degradation at higher temperatures.

3.1.3. Antioxidant activity of dry particles by DPPH and ABTS methods

Stability of dried yerba mate microparticles antioxidant activity was measured by two different methodologies using reagents. In both methodologies (ABTS and DPPH) used to quantify the antioxidant activity, the first-order model had a better fit.

As shown in Table 4, antioxidant activity of microparticles had a constant decrease during storage, and such behavior was evident in both

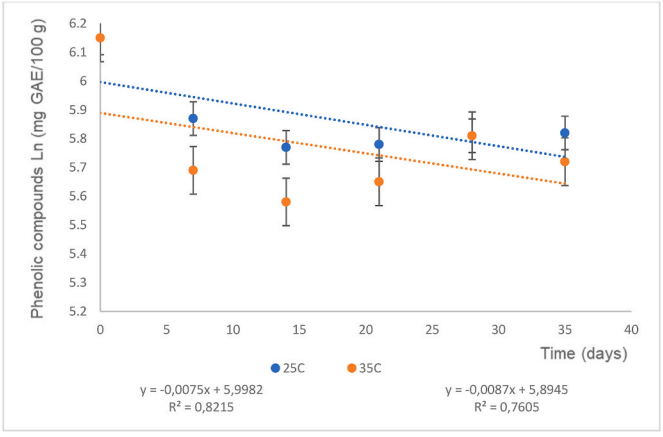

Fig. 3. Graph of Ln of phenolic compounds of dried yerba mate microparticles according to storage time.

methods used.

In the production of microparticles by spray dryer Akbarmehr et al. (2023), they obtained maintenance of antioxidant activity by DPPH was 78.1% 76.4 and by ABTS it was 88.0%. In the present study, 87% of the antioxidant activity was maintained by DPPH and 92% by ABTS, when stored for 35 days at 25 °C.

Nunes et al. (2015) studied stability for 21 days of antioxidant compounds from commercial fruit-based beverages, such as blueberry smoothie also containing apple, banana, grape, and raspberry. The experiment was conducted at the same temperature conditions of the point of sale, not being exactly specified. Determination was made by DPPH and ranged from 103 mg TE/100 mL on the day of product purchase to 20 mg TE/100 mL on 21st day of storage, which corresponds to the product expiration date. It was observed that the product had a substantial decrease in its antioxidant activity during storage.

Machado et al. (2019) analyzed the antioxidant activity by ABTS of pasteurized frozen *Physalis* pulp stored at −18 °C for 120 days. On Day zero, the sample had 2.44 μmol TE/g. Significant losses of antioxidant activity has occurred with time. After 120 days of storage, no antioxidant activity was detected in the pulps.

Antioxidant activity by ABTS (Fig. 4) resulted in $Q_{10} = 1.27$ and $E_a = 4.24$ kJ/mol. Therefore, for each 10 °C increase, the antioxidant activity of microparticles decreases 1.27 time. Considering the stability evaluated by DPPH analyses (Fig. 5), values of $Q_{10} = 1.12$ and $E_a = 2.01$ were found. The Q_{10} results achieved in both analyses were close, with results slightly over 1. By comparing the values observed by Budin et al. (2023) with respect to free yerba mate extract ($Q_{10} = 0.92$ and $E_a = 3.45$ kJ/mol), it can be observed the antioxidant activity by DPPH had a slight greater decrease due to higher temperatures.

3.2. Evaluation of fruit and cereal bars

Fruit and cereal bars, in its four variations, had similar values of proteins (Table 5). Dry particle bar had a greater amount of ashes, followed by samples with wet microparticles and with extract, which did not show statistic differences.

Dry particle bar was considered less moisten; the highest moisture content was found in the sample with wet microparticles. In order to the microparticle moisture would not interfere with the final product, an adjustment in the formulation and process would be required so the final moisture content in this sample would achieve a value similar to other formulations.

Fat content was significantly higher in samples added with dry microparticles, followed by wet microparticle bar. This result is justified by the use of ionic gelation technique, which is made from an emulsion in which most part is composed of vegetable oil. When these microparticles are dry, all the oil content is evidenced since this ingredient is present at a higher amount in simple emulsion. The fat content has also influenced the caloric value of the bars: the dry microparticle bar was considered as the most caloric one.

Table 4
Evaluation by Ln of ABTS and Ln of DPPH of antioxidant activity of dried yerba mate microparticles during storage at two temperatures.

Antioxidant temperature	Time (days)	Storage temperature	
		Ln ABTS (μmol TE/g)	Ln DPPH (μmol TE/g)
25 °C	0	$3.17^{a} \pm 0.05$	$2.97^{a} \pm 0.01$
	7	$2.98^{b} \pm 0.06$	$2.61^{c} \pm 0.01$
	14	$3.01^{b} \pm 0.02$	$2.63^{b} \pm 0.01$
	21	$2.90^{c} \pm 0.06$	$2.58^{c} \pm 0.03$
	28	$2.94^{c} \pm 0.11$	$2.63^{b} \pm 0.01$
	35	$2.93^{c} \pm 0.02$	$2.59^{c} \pm 0.01$
35 °C	0	$3.17^{a} \pm 0.05$	$2.97^{a} \pm 0.01$
	7	$2.71^{d} \pm 0.04$	$2.35^{d} \pm 0.01$
	14	$2.77^{c} \pm 0.02$	$2.48^{b} \pm 0.02$
	21	$2.82^{b} \pm 0.01$	$2.45^{c} \pm 0.01$
	28	$2.80^{b} \pm 0.04$	$2.44^{c} \pm 0.06$
	35	$2.74^{c} \pm 0.05$	$2.51^{b} \pm 0.02$

Means followed by different letters are significantly different among each other at $p < 0.05$ by Tukey's Test.

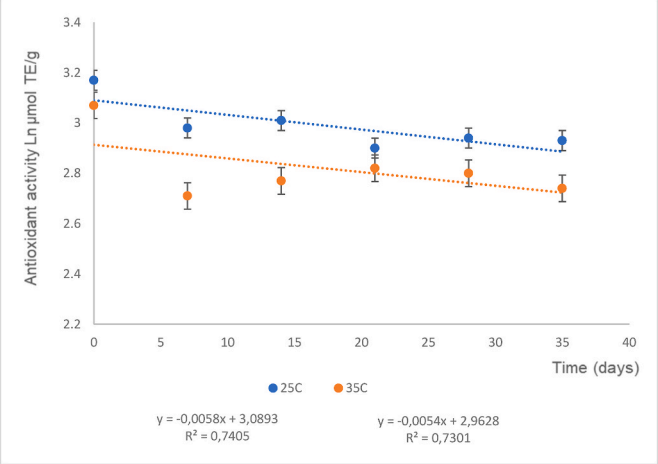

Fig. 4. Graph of Ln of antioxidant activity by ABTS method of dried yerba mate microparticles according to storage time.

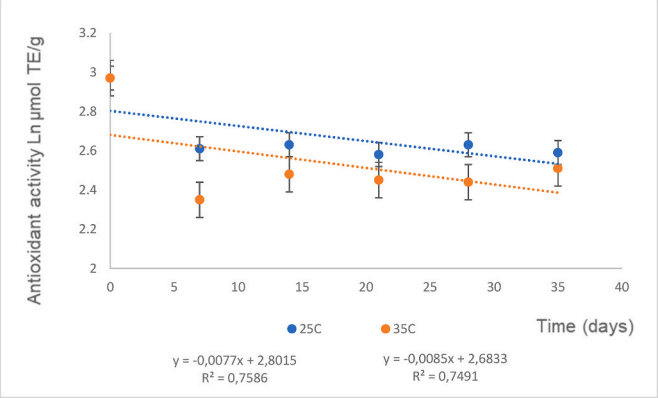

Fig. 5. Antioxidant activity by Ln of DPPH of dried yerba mate microparticles in the course of storage time.

When characterizing dehydrated fruit granola bars, Siqueira and Starling (2016) found a moisture content of 14.8 g/100 g, which is a value close to the one found in standard bars (13.59 g/100 g), bars with extract (13.52 g/100 g) and bars with dry microparticles (13.19 g/100 g).

Silva et al. (2009) produced cereal bars added with industrial waste of passion fruit. Bar containing 30% of waste in its formulation had 4.3 g/100 g of protein, value close to the one found in fruit and cereal bars in this work. With respect to fat amount, 7.8 g/100 g of lipid was found, which is close to samples not added with microparticles whether dry or wet, since these had higher values due to oil fraction in the emulsion.

By adding 10% of powdered yerba mate, *chimarrão* type, into cereal bars, Chiesa et al. (2012) produced products with caloric value of 415.09 kcal/100 g, 9.13% proteins and 8.77% lipids. The bars had caloric value and protein content above the values determined in the four types of samples analyzed in this study (Table 5). Even though these are products with similar propositions, there are differences in the nature of incorporated active ingredient and in bar formulation, factors which generate distinct values of centesimal composition.

When evaluating the proximate composition of cereal bars enriched with pineapple peel flour (Damasceno et al., 2016), the following levels were found: 8.2–9.4% moisture, 2.6–3.2% ash, 6.5–7.3% fat, 6.3–6.9%

Table 5
Centesimal composition of fruit and cereal bars and microbiological analyses.

	Determination	SB	EB	WPB	DPB
Centesimal composition	Moisture and volatile compounds (g/100g)	13.59 [b] ± 0.22	13.52 [b] ± 0.01	17.61 [a] ± 0.16	13.19 [c] ± 0.10
	Ashes (g/100g)	1.25[c] ± 0.05	1.31 [b] ± 0.13	1.30 [b] ± 0.01	1.39 [a] ± 0.01
	Total fat (g/100g)	7.56[c] ± 0.09	6.88 [d] ± 0.13	9.75 [b] ± 0.20	11.34 [a] ± 0.14
	Protein (Nx6,25) (g/100g)	4.38[a] ± 0.00	4.05 [a] ± 0.02	4.08 [a] ± 0.03	4.23 [a] ± 0.02
	Total carbohydrates (g/100g)	73,22 [a]± 0.67	74,24 [a] ± 0.67	67,26 [ab] ± 0.67	69,85 [a]± 0.67
	Energetic value (kcal/100g)	378 [b] ± 5.75	375 [b] ± 2.43	373 [b] ± 1.60	398 [a]± 2.22
Microbiological analyses	*Salmonella* (in 25g)	absent	absent	absent	absent
	Escherichia coli (CFU/g)	<10	<10	<10	<10
	Mold count (CFU/g)	10 (es)[1]	<10	<10	10(es)[a]
	Yeast count (CFU/g)	<10	<10	<10	1×10^2(es)[a]

Mean ± Standard deviation; SB: fruit and cereal bar (standard); EB: fruit and cereal bar added with yerba mate extract; WPB: fruit and cereal bar added with wet yerba mate particles; DPB: fruit and cereal bar added with dry yerba mate particles. CFU = colony-forming unit.

[a] Estimated count, below the limit of quantification of the method. Means followed by different letters are significantly different among each other at $p < 0.05$ by Tukey's Test.

of 15.0 proteins and 71.3–72.5% carbohydrates. In evaluating the centesimal composition of cereal bars enriched with ora-pro-nóbis flour (Cruz et al., 2024), the following levels were found: 8.9–12.9% moisture, 3.3–3.4 % ash, 3.9–15.9% fat, 13.5 to 15.0 protein and 56.8–66.3% carbohydrates. In the present study, fruit and cereal bars had higher moisture, carbohydrates, fat and lower ash and protein content (Table 5). The presence of fruits increased the moisture and juiciness of the fruit and cereal bar studied.

Microbiological evaluation of fruit and cereal bars was conducted based on Normative Instruction no. 60 (Brasil, 2019), which establishes the microbiological standards for foods including cereal bars added or not with other ingredients. The legislation anticipates the maximum limit of *E.coli* for this category of food is 10^2 CFU/g, and of mold and yeast is 10^4 CFU/g. With respect to *Salmonella*, the result must be "absence" for this type of microorganism (Brasil, 2019). Therefore, fruit and cereal bars fall within the microbiological standard required by the legislation and safe for consumption from a microbiological point of view.

Table 6 shows the characterization of standard fruit and cereal bar, as well as bars with extract, with wet particles and with dry particles.

3.2.1. Water activity in fruit and cereal bars

The sample with extract had lower activity of water, followed by standard and dry particle bars. Formulation with wet particles resulted in the highest index of activity of water.

The formulation containing yerba mate extract in its free form may have demonstrated this behavior due to the desiccant property that ethanol can exert. The extract used was composed of 64% ethyl alcohol, and when it came into contact with the other ingredients in the bar, it may have removed a portion of the free water present, reducing the water activity of this sample. And in the case of the bar added with wet particles, the water activity was higher due to the ionic gelation technique, in which the high presence of water is inherent due to the method itself, which consisted of a double microencapsulated W/O/W emulsion. This sample did not go through any other process, the particles were simply aggregated in this way along with the bar's ingredients. This did not happen with the sample with dry particles, which, before being added to the formulation, went through the drying process, reducing their humidity and water activity.

Gutkoski et al. (2007) elaborated cereal bars with different sugar and oat contents, affecting soluble solid and fiber contents. In bars with 75°Brix and 16% fiber, the activity of water was 0.661. In those with 70°Brix and 20%, activity of water was 0.686. The results achieved were close to bars with wet and dried yerba mate particles.

Vieira et al. (2019) developed cereal bars using flour from agro-industrial waste. Bars added with mixed flour from dry pineapple and cashew waste. Samples with 10% and 20% flour had activity of water of 0.61. In the standard sample with no added flour the value was 0.62, which is equal to the value found in this study for standard bar. Bars with particles also showed values around 0.6.

Damasceno et al. (2016) evaluated the aa of cereal bars added 0–9% pineapple peel flour and found values of 0.66–0.72. Similar values to those found in this study.

Cruz et al. (2024) evaluated the physical and chemical characteristics of cereal bars enriched with ora-pro-nóbis flour and obtained aw values of 0.40–0.54. These values are lower than those obtained in this study, however the aw values in this study are also below the range established for the growth of most microorganisms (0.90 and 0.99) and thus might have good microbiological stability, in addition to also influencing nutritional and sensory stability.

3.2.2. Colour analysis of fruit and cereal bars

Bars with dry particles were considered lighter than standard bar (with no particles or extract); however, L* value was not significantly different at 5% level for bars with wet particles and extract.

Standard sample was considered as darker than bar added with dry

Table 6
Characterization of fruit and cereal bars.

Analyses		Results			
		SB	EB	WPB	DPB
Activity of water		0.620 ± 0.01[b]	0.574 ± 0.01[c]	0.687 ± 0.02[a]	0.626 ± 0.00[b]
Colour	**L***	45.91 ± 5.35[b]	49.02 ± 3.88[ab]	47.09 ± 4.80[ab]	50.24 ± 3.76[a]
	a*	13.03 ± 1.39[ab]	11.26 ± 1.29[b]	13.76 ± 1.78[a]	12.14 ± 2.55[bc]
	b*	17.09 ± 3.40[b]	20.78 ± 4.96[a]	18.83 ± 3.93[ab]	20.39 ± 4.54[ab]
Texture (N)		445.0 ± 14.3[b]	600.3 ± 6.7[a]	208.7 ± 20.8[d]	288.6 ± 21.0[c]
TPC (mg GAE/100 g)		89.44 ± 1.68[d]	214.46 ± 2.62[a]	142.21 ± 0.67[c]	167.67 ± 5.39[b]
A.A. (μmol TE/g)	**ABTS**	1.61 ± 0.37[c]	7.51 ± 0.37[a]	3.27 ± 0.53[b]	6.57 ± 0.53[a]
	DPPH	2.54 ± 0.33[d]	9.39 ± 0.48[a]	6.34 ± 0.11[c]	7.56 ± 0.29[b]

Mean ± Standard Deviation; n = 4, where n = number of repetitions used for all analyses, except for "instrumental colour" (n = 20). SB: fruit and cereal bar (standard); EB: fruit and cereal bar added with yerba mate extract; WPB: fruit and cereal bar added with wet yerba mate particles; DPB: fruit and cereal bar added with dry yerba mate particles. Means followed by different letters in the same line are significantly different among each other at $p < 0.05$ by Tukey's Test. TPC = total phenolic compounds; GAE = gallic acid equivalent; A.A. = antioxidant activity; TE = Trolox equivalent.

particles, showing no significant differences from bars with extract and wet particles.

Considering chromatic coordinate a*, which comprises from red to green, bars with wet particles had a greater trend to red than bars with extract, but there was no difference from standard sample. Likewise, bar with extract was not different from the standard sample or bar added with dry particles.

In coordinate b*, which extends from yellow to blue, bar with extract was significantly different from standard, but not from samples with particles (WPB and DPB).

In general, all samples had colour tending to a mixture of yellow and red (Fig. 6). This occurs due to the presence of red berries homogenized in bars, as well as nuts and cereals having a yellowish colour.

In the evaluation of cereal bars containing ora-pro-nóbis flour and mango jelly, the colour values obtained by Cruz et al. (2024) were L* 30.9 to 28.2, a* 4.60 to 5.60 and b* 8.9 to 16.2, all values lower than those found in Table 6, showing that the fruit and cereal bars in this study have more colour and can have greater sensory appeal.

Bueno et al. (2020) submitted bars with grape and *jabuticaba* flour to cooling and baking. Bars with grape skin flour had luminosity of 47.66 (cooling) and 44.81 (baking), while bars with *jabuticaba* flour had L* value of 43.49 (cooling) and 40.28 (baking). Samples with grape flour were considered lighter than those added with *jabuticaba* flour. These values were close to the ones found in this work.

Segundo Fenoglio et al. (2021) colour is a relevant feature for consumer acceptability since it creates visual attractiveness. The effect of spray drying on the encapsulation of yerba mate extract reduced the greenness of the powders, which may be a desirable feature when planning add the active ingredient to a real food matrix without changing your natural colour.

3.2.3. Fruit and cereal texture

Table 6 shows the results for bar hardness. All the samples were substantially different among each other at $p < 0.05$ and MSD (minimal significant difference) was 30.4. Bar with liquid yerba mate extract had the highest level of hardness, followed by standard sample. Lower values of hardness were found in bars with microparticles; the bar added with wet particles had the lowest hardness level. Incorporating particles whether wet or dry has caused a reduction in hardness, while extract has significantly increased it.

Due to the composition of the extract used being 64% ethyl alcohol, when it comes into contact with the other ingredients in the bar, it may have removed part of the free water present, thus increasing the hardness of this sample. In the case of the bar added with wet particles, the presence of water is inherent to the microencapsulation method itself, which consisted of a double emulsion. Consequently, this sample had less texture. When the bar was added with dry particles, the amount of water added to the bar was smaller, leading to a greater texture than the bar with wet particles.

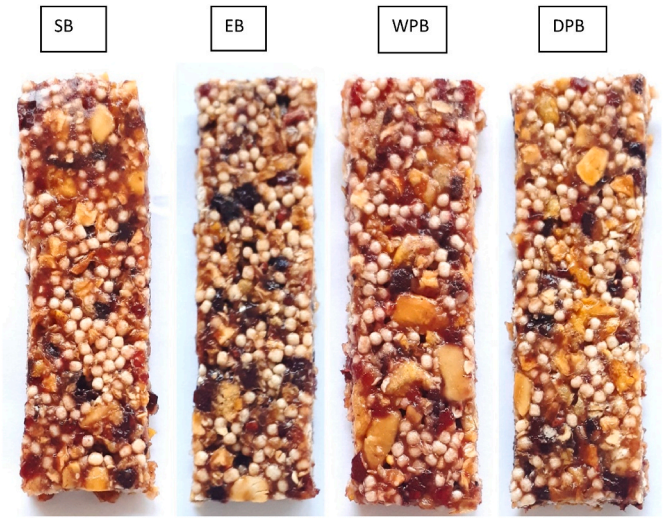

Fig. 6. Standard fruit and cereal bar (SB), bars with extract (EB), added with wet yerba mate particles (WPB), and added with dry yerba mate particles (DPB).

Damasceno et al. (2016) evaluated the texture of cereal bars added with 0–9% pineapple skin flour and found values of 99.6–157.9N. This can be explained by the ability of flour to absorb the sugar syrup, allowing the ingredients to agglomerate, and the cereal bar becoming more compressed.

Gomes (2017) produced fruit bars added with mango peel and seed flour of *Espada* and Tommy Atkins varieties. Bars were added with 50% of flour and hardness results achieved were 169.98 N, 134.12 N, 171.44 N, and 179.66 N for *Espada* mango peel bar, Tommy peel bar, *Espada* seed bar, and Tommy seed bar, respectively. Bars with seed flour had higher hardness values. However, it was observed that adding flour has caused to reduce the hardness, since standard bar had a value of 226.85 N.

Machado (2018) used grape skin flour to add fibers and antioxidants into cereal bars. She evaluated 6 formulations, of which 3 were added with 10, 15, and 20% of thick flour and 3 were added with 10, 15, and 20% of thin flour. Bar cut resistance ranged from 0.98 to 1,65 N. Sample with 20% thin flour had hardness of 1.42 N, and sample with 10% thick flour had a value of 1.65 N. These values were considered much below the ones found in this study.

Bueno et al. (2020) observed the following hardness results: 15.5 N and 18.60 N for grape flour bars submitted to cooling and baking, respectively; and 22.79 N and 18.98 N for *jabuticaba* skin flour bars submitted to cooling and baking, respectively. Bars made of *jabuticaba* flour had higher hardness values. Although these values were higher, the hardness in these bars were below the value observed in this study.

By comparing these values with the one found in bars of this study, we observe these are evidently lower, being closer to the results found in samples with particles. The difference between the formulations found in the literature and those presented in this work may have led to significant differences in texture values. These variations are normal in the market for this type of product as there are many combinations of ingredients available.

3.2.4. Total phenolic compounds

Bars had an increase in total phenolic compound content as yerba mate was added, whether as free extract or wet or dry microparticles elaborated from such extract (Table 6).

Standard sample with no addiction of yerba mate had the lowest amount of phenolic compounds. Among the samples having yerba mate in their composition, the bar with wet particles had the lowest level of added phenolic compounds of 142.21 mg/100 g. Sample with dry particles had a significant increase, with 167.67 mg/100 g of GAE.

Fruit and cereal bar added with free yerba mate extract had the highest total phenolic compound content. In the extract, quantification of active ingredients may have been higher since compounds are diluted in a free vehicle (hydroalcoholic vehicle). High temperatures were not used for bar production and packaging protected the bars from light. These factors may have been decisive for polyphenol preservation. For particle-containing samples, in addition to the process for their production which is composed of several steps, the active ingredients of interest also had to be extracted for analysis, which may not have been totally recovered by extractions. However, the purpose of using microparticles is protecting something which, whether free or in its natural state, can be easily degraded.

In bar formulations, proportional amounts were added which were theoretically corresponding to phenolic compound delivery caused by adding the extract, or wet or dry yerba mate particles (Table 7). For dry particles, addition was slightly higher but even so the quantification

remained below the expected. One reason could be related to potential losses during extraction, which consequently would cause a reduction in total phenolic compound concentration in respective sample.

Based on standard sample, the bar with extract had 139.78% increase of total phenolic compounds. The bar with dry particles also had a substantial increase of 87.50%.

Machado (2018) used flour made of grape peel, a waste from red wine production, to develop cereal bars enriched with fibers and antioxidants. Bars with 10% grape peel flour and coarse granulometry had 95.77 mg/100 g of total phenolic compounds, and formulation with 15% of the same flour had a value of 172.15 mg/100 g. This result was close to the one found in fruit and cereal bars added with dry particles.

Considering that the process adopted to obtain fruit and cereal bars in the present study was at temperatures below cooking, it is expected that no relevant losses have occurred of phenolic compounds in the final product and that this process has been essential for sensory improvement and health safety of new products developed, such as fruit and cereal bars.

3.2.5. *Antioxidant activity by ABTS and DPPH methods*

Among samples added with yerba mate, the bar with wet particles had the lowest antioxidant activity by both methods.

The results regarding DPPH analysis had different values at 5% significance level for all the samples.

According to ABTS analysis, bars added with yerba mate extract and dry particles were the ones resulting in higher antioxidant activity; values were not considered different at 5% significance level.

When studying cereal bars added with grape peel flour from red wine process, Machado (2018) also determined antioxidant activity by ABTS. Bars having 10% and 15% of grape peel thick flour showed antioxidant activity of 5.82 and 5.61 μmol Trolox/g, respectively. When these results were compared with antioxidant activity observed in bar added with yerba mate dry microparticles of 6.57 μmol/g, these values are similar, being slightly higher in the latter.

Fenoglio et al. (2021) highlighted that the antioxidant capacity of the yerba mate extract obtained by atomization was maintained, as was found in this study.

3.2.6. *Sensory analysis*

In general, standard fruit bar had a mean score corresponding to "I liked it" as per the scale used, being significantly more accepted at 5% level than fruit bar samples with extract and wet particles, which had a mean score between "I liked it a little" and "I liked it", and close to "I liked it", respectively (Table 8). The fruit bar sample with dry particles which also had a mean score between "I liked it a little" and "I liked it" was similar to standard fruit bar and bar with extract. The bar sample with wet particle was significantly less accepted compared to the other at 5% level.

With respect to the appearance, the standard sample and those with extract and dry particles had mean scores corresponding to "I liked it" in the scale used, not being significantly different among each other at 5% level. These were more accepted than the sample with wet particles, which had a mean score of "I liked it a little".

Regarding the texture, standard sample and the one with dry particles had a mean score of "I liked it", not being substantially different between each other, and were more likely to be accepted than other samples at 5% level. Texture of sample with yerba mate extract with a mean score close to "I liked it a little" was significantly more accepted than sample with wet particles, which had a mean score close to "I did not like or dislike it".

In terms of flavor, standard sample had a mean score corresponding to "I liked it" and was substantially more accepted at 5% level than samples of fruit bar added with extract and with wet particles, having a mean score close to "I liked a little" and not different from each other. Sample with dry particles had an intermediate mean and was similar to other samples.

Concerning the Sweetening, standard sample had higher acceptability with a mean score of "I liked it" and significantly different at 5% level from sample with extract, which had a mean score between "I liked a little" and "I liked it". Samples with wet and dry particles with intermediate mean scores were similar to each other, from standard sample or sample with extract.

For aftertaste, standard sample with mean score close to "I liked it" was significantly different at 5% level from sample with extract, which had a mean score of "I liked it". Samples with wet particles and dry particles and intermediate mean scores had no differences among each other, the standard sample or sample with extract.

When preference was considered, standard sample and sample with dry particles were the most preferred ones, not being substantially different among each other. The least preferred ones were samples with extract fruit bar and samples with wet particles, although the samples with extract and dry particles had no differences compared to each other.

The scale taken for acceptability percentages as acceptance (rates 9

Table 7
Influence of adding extract, wet and dry yerba mate particles to increase total phenolic compound content in fruit and cereal bars.

		EB	WPB	DPB
Corresponding amount (%) in formulation	Extract	4	–	–
	Wet particle	–	9	–
	Dry particle	–	–	4
Total phenolic compound content (mg GAE/100 g)[a]		125.02 ± 2.38	52.77 ± 0.58	78.23 ± 4.82
Phenolic compound increase (%)[b]		139.78	59.00	87.50

EB: fruit and cereal bar added with yerba mate extract; WPB: fruit and cereal bar added with wet yerba mate particles; DPB: fruit and cereal bar added with dry yerba mate particles. GAE = gallic acid equivalent.

[a] Total phenolic compound content found in bars, not considering standard bar value.

[b] Contribution made by adding the extract, wet and dry yerba mate particles in total phenolic compound content increase in fruit and cereal bars.

Table 8
Results achieved when evaluating the acceptability and preference of fruit and cereal bars.

Acceptability[a]	*Fruit and cereal bar*				
	SB	EB	WPB	DPB	MSD[c]
General mode	7.2 ± 1.3^{a}	6.4 ± 1.9^{b}	5.8 ± 1.9^{c}	6.6 ± 1.8^{ab}	0.65
Appearance	7.3 ± 1.3^{a}	7.1 ± 1.5^{a}	5.8 ± 1.8^{b}	7.1 ± 1.3^{a}	0.49
Texture	7.0 ± 1.5^{a}	6.2 ± 1.7^{b}	4.9 ± 2.1^{c}	6.9 ± 1.5^{a}	0.67
Flavor	7.1 ± 1.5^{a}	6.2 ± 2.0^{b}	6.2 ± 2.0^{b}	6.5 ± 1.8^{ab}	0.71
Sweetening	7.0 ± 1.3^{a}	6.4 ± 1.9^{b}	6.5 ± 1.5^{ab}	6.5 ± 1.6^{ab}	0.55
Aftertaste	6.9 ± 1.7^{a}	6.0 ± 2.0^{b}	6.2 ± 1.8^{ab}	6.3 ± 2.0^{ab}	0.68
Preference – sum of ranking positions [b]	164^{c}	213^{ab}	240^{a}	193^{bc}	32.21

SB: fruit and cereal bar (standard); EB: fruit and cereal bar added with yerba mate extract; WPB: fruit and cereal bar added with wet yerba mate particles; DPB: fruit and cereal bar added with dry yerba mate particles.

[a] Results expressed as mean ± standard deviation of 81 evaluations. For each attribute, means followed by equal letters were not significantly different at 5% error level by Tukey's test.

[b] The higher the sum of ranking positions, the lower the preference by this sample.

[c] MSD: Minimal significant difference at 5% error level by Tukey's test for acceptability and Fischer's test for preference.

to 6), indifference (rate 5), and rejection (rates 4 to 1) associated with fruit and cereal bar samples by hedonic scale was used to evaluate the samples in general and for appearance, texture, flavor sweetening, and aftertaste.

For general evaluation, samples of standard fruit bar, bar with extract, and bar with dry particles had acceptance rates above 80%, while the bar sample with wet particles achieved 69% acceptance and 25% rejection of consumers.

Appearance was the attribute to which the standard fruit bar, bar with extract, and bar with dry particles had the highest acceptance rates (minimum of 88.9%); the bar with wet particles had only 63% acceptance and 25% rejection.

Regarding texture evaluation, the standard sample, sample with extract, and sample with dry particles had acceptance rates above 74%, particularly the standard sample which achieved 90% acceptance. The texture of sample with wet particles was not much appreciated, being rejected by 46.9% of consumers.

With respect to flavor, the standard sample excelled the others, with acceptance rates of 90%. For Sweetening, all samples had acceptance rates of at least 74% of consumers.

In aftertaste evaluation, the sample with extract had the highest rejection rate (25%), followed by sample with dry particles, sample with wet particles, and standard sample, with rejection rates of 25.9%, 21.0%, and 8.6%, respectively.

The scale for rating percentages was above optimal (rates 5 and 4), optimal (rate 3), and suboptimal (rates 2 and 1) for amount of red berries (A), firmness (B), crispness (C), and intensity of red berry flavor (D). Penalty analysis of each sample was used to calculate the difference between the mean of the group considering each attribute with optimal intensity and the mean of the groups considering such attribute more or less intense than optimal.

In terms of intensity of red berry flavor, all the samples were considered as having an optimal quantity of red berries by at least 61% of consumers. However, 33% of consumers considered the sample with extract had less fruit than the optimal amount, resulting in a 1.5-point reduction in general acceptability.

As for firmness, 79% of consumers considered the sample with wet particles less firm than the optimal, resulting in a 1.6-point reduction in general acceptability. For 53,1% of consumers, the sample with extract was firmer than the optimal, however there was no significant impact in acceptance or reduction of at least 1 point of general acceptability.

For crispness, 84% of consumers considered the sample with wet particles less crispy than the optimal, which was the same opinion as 28.4% about the sample with extract. Both samples had a 1.7-point reduction in general acceptability. Although 40% and 24.7% of consumers had considered samples with dry particles and the standard, respectively, as less crispy than the optimal, there was no significant impact in acceptance or reduction of at least 1 point of general acceptability.

Red berry flavor in samples with extract, with wet particles and with dry particles was regarded as less intense than the optimal by 35.8%, 28.4%, and 23.5% of consumers, respectively, with a significant reduction of 1.9, 1.4, and 1.6 point in general acceptability, respectively.

Chiesa, Schlabitz e Souza (2012) elaborated cereal bars added with different contents of powder yerba mate. Bar containing 5% powder was considered with good acceptance by tasters. According to Index of Acceptability (IA) results, cereal bars with 5% and 10% of yerba mate achieved values close to 70%, being considered as accepted by tasters and thus with potential to be commercialized. When yerba mate content increased to 20%, a sharp drop in IA to 52.7% was observed, showing that higher contents of powder would cause increased rejection of the product. Among the attributes evaluated, flavor had a greater impact when yerba mate was incorporated into the product. Particularly about "flavor" with respect to the presence of yerba mate, fruit and cereal bars of this study were considered as accepted by the tasters. It should be emphasized that bar added with dry microparticles achieved 79% acceptance among the consumers.

Silva et al. (2009) produced cereal bars added with industrial waste of passion fruit: dry and grounded albedo and peel. When 30% of waste were incorporated into the bars, general impression and intention to purchase were not affected. When considering fruit and cereal bar added with dried yerba mate microparticles, high levels of appearance and general acceptance by the tasters of 90.1% and 80.3% were observed.

If global acceptability values are considered, the results presented in Table 8 (6.4 a 7.2) were similar to those of Cruz et al. (2024) for ora-pro-nóbis flour bar and mango jelly (6.9 a 7.5). Similar global acceptability values (6.5–7.5) were obtained by Damasceno et al. (2016) in the evaluation of a cereal bar with pineapple peel flour.

Kosicka-Gebska et al. (2022) showed in their work that fruit and cereal bars were significantly more likely to be consumed by consumers in the Health Oriented and Involved clusters, mainly due to the desire to eat nutritious foods product and take care of your health. Although consumers tend to accept more healthiest carriers in functional foods, it appeared that the presence of cereals and fruits in bars may be more meaningful to these people than the confectionery product itself.

3.3. Stability study of fruit and cereal bars

Table 9 shows results of kinetic parameters of colour analysis for the four fruit and cereal bars. The results were best fitted in zero-order model for colour analysis and first-order model for phenolic compounds. Degradation rates (k) of total colour change are extremely low specially for EB. Q_{10} and Ea values are similar, with results slightly higher for bars with wet and dry particles probably due to the presence of rapeseed oil used in microencapsulation. In addition, degradation rates (k) of phenolic compounds are higher for SB and EB bars, but Q_{10} and Ea values are lower. These results show that changing the temperature from 25 °C to 35 °C has caused a greater change in bars with wet and dry particles, once again due to the presence of oil in microparticles.

Stability of phenolic compounds and colour of pectin gum added with hibiscus extract microparticles was evaluated by Moura et al. (2019). These authors observed the pectin gum had a significant increase in total phenolic compound content during storage for 62 days. Total phenolic compound quantification was conducted by Folin-Ciocalteau method. According to Erkan-KoÇ et al. (2015), some components, such as citric acid and reducing sugars, have an interfering effect on this analysis. The results of this method can be reliable only after these interfering compounds are removed, and such removal can be performed by sample purification only. For the pectin gum, degradation compounds of sugar from hydrolysis reaction and Maillard reaction were maintained, which may have increased the total polyphenol content. Similar results were observed for fruit and cereal bars.

Carvalho and Conti-Silva (2018) evaluated the stability of cereal bars added with banana peel flour stored for 11 months, under vacuum and

Table 9
Results of kinetic parameters of instrumental colour (ΔE) and phenolic compounds of fruit and cereal bars.

ΔE	k25 (dia^{-1})	k35 (dia^{-1})	Q_{10}	Ea (KJ/mol)
SB	0.0244	0.0426	1.75	9.89
EB	0.0056	0.0116	2.07	12.92
WPB	0.0148	0.0362	2.45	15.87
DPB	0.0195	0.0489	2.51	16.31
Phenolic compounds	k25	k35	Q_{10}	Ea (KJ/mol)
SB	0.1625	0.2054	1.26	4.16
EB	0.2459	0.3496	1.42	6.24
WPB	0.0034	0.0543	15.97	49.16
DPB	0.0113	0.1564	13.84	46.62

SB: fruit and cereal bar (standard); EB: fruit and cereal bar added with yerba mate extract; WPB: fruit and cereal bar added with wet yerba mate particles; DPB: fruit and cereal bar added with dry yerba mate particles.

protected from light. According to the authors, total phenolic compounds decreased during storage (from 4.19 to 1.11 mg GAE/g). Although total antioxidant activity (ABTS method) increased during the fifth month, it reduced during storage (from 3.41 to 0.30 μmol TE/g). The results presented in this study differ from those of these authors, showing that the presence of fruit in the cereal bar may have compromised the readings of phenolic compounds due to the presence of reducing sugars that interfere with the analysis.

Fenoglio et al. (2021) demonstrated that the antioxidant capacity of yerba mate extract encapsulated by spray dryer was proven in a real food matrix, where a notable increase in oxidative stability was obtained by adding this active additive to mayonnaise. These results suggest that microencapsulated yerba mate extract can be used as a functional additive for new foods and beverages. In the present study, the antioxidant capacity of yerba mate extract applied to fruit bars was also confirmed, making it a promising ingredient for application in foods and beverages.

Mean colour variation (mean ΔE) of pectin gum with particles during 55 days of storage at 25 °C and in the absence of light ranged from 6.5 to 8.25 (Moura et al., 2019). For fruit and cereal bars, ΔE values found in this study were, on average, 6.5 in the 77 th day of storage and 9.6 in the 151 th day of storage.

Food colouring is directly related to the acceptability of food products (Kosicka-Gebska et al., 2022). However, the instrumental evaluation of the colour of composite products such as fruit and cereal bars may present high total colour change (ΔE) values, but they are often imperceptible to humans, especially if the product is consumed infrequently.

4. Conclusion

According to the research carried out in this study, microencapsulation of bioactive compounds by ionic gelation followed by drying in a fluidized bed can be considered a practical and economical solution to preserve functional food compounds.

Stability study of microparticles showed there was preservation of colour and total phenolic compounds. A substantial increase in phenolic compound content was observed in fruit and cereal bars added with extract (139.78%) and dry particles (87.50%).

Bars added with extract and dry particles had acceptance above 80%, while bar with wet particles was the least accepted one, with rejection of 25%. The provided increase of bioactive compounds along with product acceptance suggests the use of yerba mate extract, whether free or encapsulated, in fruit and cereal bars is feasible.

Fruit and cereal bar stability was considered as good with respect to the colour; however, for phenolic compounds the change in the storage temperature can have a greater impact on the bars added with microparticles.

CRediT authorship contribution statement

Ana Caroline Budin: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Cristiane Silvano Wensing:** Methodology, Formal analysis. **Carla Léa Vianna Cruz:** Methodology, Formal analysis. **Cristiane Rodrigues Gomes Ruffi:** Methodology, Formal analysis. **Aline Oliveira Garcia:** Software, Methodology, Formal analysis. **Sílvia Cristina Sobottka Rolim de Moura:** Writing – review & editing, Supervision, Software, Methodology, Investigation, Funding acquisition, Conceptualization.

Data availability statement

The data are available in a repository, but subject to prior request to the authors.

Funding

This research was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil (postgraduate scholarships) and State of Sao Paulo Research Foundation - FAPESP, Brazil (Grant 2019/19647-0).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Silvia Cristina Sobottka Rolim de Moura reports financial support was provided by State of Sao Paulo Research Foundation - FAPESP, Brazil. Silvia Cristina Sobottka Rolim de Moura reports a relationship with-Institute of Food Technology, Brazil that includes: non-financial support.

Acknowledgments

The authors would like to thank the company Heide Extratos Vegetais, Pinhais-PR for supplying yerba mate extract.

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