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Bread with Yerba Mate Aqueous Extract (*Ilex paraguariensis* A.St.-Hil.)

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ABSTRACT

Ilex paraguariensis A.St.-Hil., (yerba mate) has been gaining space in face of the larger demand for alternative raw materials in food production. The study aimed to develop a bread formulation with an yerba mate aqueous extract replacing water, for making it more nourishing and functional. From a basic bread formulation, excluding fungal inhibitors and malt extract, two new formulations were prepared containing yerba mate aqueous extract replacing water. One of the formulations received an extract prepared with 75 g of dehydrated, crushed leaves diluted in 500 mL of water. The other was obtained with 150 g diluted in the same amount of water. Both formulations were submitted to sensory evaluation based on taste, aroma, texture, color and global acceptance criteria. The results were submitted to an Analysis of Variance (ANOVA). Even without any significant differences between the two formulations, 86 and 80% of participants graded the bread with the more and less concentrated extract, between 7.0 and 9.0, respectively. The Acceptability Index was over 70%. Evaluation of purchase intention indicated a preference for the bread with the more concentrated extract (76%). These results show that yerba mate constitutes an alternative raw material in the composition of new food products.

Key words: Acceptability index, food products, new products, sensory evaluation

INTRODUCTION

A considerable portion of the population, worried about their health, has experienced behavioral changes, especially regarding the search for healthier foods (Azevedo *et al*., 2011). To cater to this new option, the search for alternative raw materials in the production of food has grown and among the options, are some economically important vegetable species (Ganem, 2011).

Ilex paraguariensis A.St.-Hil, native to Brazil, Argentina and Uruguay and popularly known as yerba mate, has become an alternative in the production of healthier foods, exceeding its use in the chimarrão, tea and tereré to which it was restricted for decades (Sindicato das Industrias do Mate no Estado do Rio Grande do Sul, 2013) (Mate Industries Union of Rio Grande do Sul). The plants from which the leaves are obtained are kept in their natural environment (natural handling) or cultivated in uniform plantations (cultivated handling).

Because of its antioxidant, stimulant and diuretic properties, as well as its action as a DNA protector in degenerative processes, such as, cardiovascular problems, many studies have emphasized its use in foods (Machado *et al*., 2007). Recently, its consumption has expanded and as a consequence, products containing yerba mate as a preservative and natural coloring, drugs, cosmetics, ice creams, cereal bars and more recently, the fiber and vitamin C beverage Coca Cola® (Pimentel *et al*., 2006; Culliney, 2013) released in Japan are standing out in the market.

Bread, one of the foods the Brazilian population most consumes, reaching a consumption of 33.5 $year^{-1}$ $person^{-1}$ (Associacao Brasileira da Industria de Panificacao e Confeitaria, 2010) (Brazilian Bakery and Confectionery Industry Association) is still not among the products containing yerba mate. This is a product that is part of daily consumption for Brazilian populations, helping the growth, development and maintenance of health and life (Servico Brasileiro de Apoio as Micro e Pequenas Empresas, 2013) (Brazilian Support Service to Micro and Small Companies). Today, with a larger variety of flavors, shapes and versions, industrialized breads are consolidated in the market while attracting different consumer profiles (Danelli *et al*., 2010).

Resolution #263, from September 22, 2005, defines bread as, the product obtained from wheat flour and/or other flours, with added liquid, that may contain other ingredients, as long as they do not mischaracterize the product. Thus, the study aims to define a bread formulation with *Ilex paraguariensis* (yerba mate) extract intending to make the bread more nourishing and/or functional and through, sensory analysis, to evaluate the acceptability of the final product.

MATERIALS AND METHODS

The study was conducted at Univates throughout 18 months, from January, 2013 to June, 2014.

Collection and preparation of the leaves: *I. paraguariensis* were collected from 10 individuals from the "cultivated" handling system in the property of Juliana Montagner (director of Ervateira Ximango), located at Ilópolis, Rio Grande do Sul (geographical coordinates 28°55'24"S and 52°07'47"W). The option for the extract obtained from plants in "cultivated" handling was influenced by the high protein value found, since the chemical analysis (unpublished data) indicated 20.9% of proteins, a much higher percentage than the one presented in the extract obtained from natural handling plant leaves (11.1%).

After collection, the leaves were transported in isothermal boxes to the Univates Chemical Analysis Laboratory to be sanitized in running water. After that, they were dried in an incubator with air circulation at 45±5°C for 12 h until they were completely dehydrated. After drying, the leaves were crushed and kept in an amber container until the aqueous extract was ready. The extraction of solubles was performed following a methodology adapted from Ribeiro *et al*. (2012). The 150 g of crushed yerba mate leaves were weighted in an electronic scale with a maximum capacity of 200 g. The leaves were arranged in one side on the bottom of a 1000 mL beaker. Afterwards, to compact the surface, 50 mL of cold water were poured over the crushed leaves and soon after, 750 mL of hot water were added (65±5°C), keeping them immersed for 20 min, after the extract was filtered. The entire procedure was repeated with half the amount of leaves with the intention of generating the reduced concentration extract.

Preparation of formulations: From the basic Sebess (2010) formulation, two bread formulations were developed (Table 1) with the extract obtained from different amounts of crushed yerba mate leaves diluted in the same amount of water. In formulation 1 (F1), the extract obtained from

Table 1: Ingredients used in the Basic Formulation (BF) of the bread and the developed formulations containing extract of yerba mate (*Ilex paraguariensis* A.St.-Hil.) leaves

Ingredients	BF1	F1	F2
Wheat flour (kg)	01	01	01
Water (L)	0.600	-	-
Yerba mate aqueous extract (L)	-	0.600	0.600
Sugar (kg)	0.100	0.080	0.080
Butter (kg)	0.100	0.050	0.050
Malt extract	0.100	-	-
Baker's yeast (kg)	0.030	0.030	0.030
Salt (kg)	0.020	0.020	0.020
Calcium propionate (kg)	0.010	-	-

FB[1]: Basic formulation of the bread (Sebess, 2010); F1: Formulation of the bread containing extract obtained from 150 g of leaves of yerba mate (*Ilex paraguariensis* A.St.-Hil.), F2: Formulation of the bread containing extract obtained from 75 g of leaves of yerba mate (*Ilex paraguariensis* A.St.-Hil.)

150 g of leaves was used and in formulation 2 (F2), the extract from 75 g of leaves replacing water. Aside from replacing the extract for water, the fungal inhibitor (calcium propionate) and the enhancer malt extract were withdrawn so as to not interfere with the perception of the characteristics of yerba mate.

For the preparation, all ingredients were weighted in a digital electronic scale with a maximum capacity of 5.0 kg. After weighting, wheat flour, salt, sugar, yeast and butter were placed in the dough mixer and the mixing process was started, with the slow addition of the yerba mate aqueous extract. After mixing for 5 min, in order to dissolve salt and sugar, the dough rested for another 5 min. Subsequently, the dough was mixed 2 min more. After homogenization, the dough was formed into a cylinder, going through the equipment for 20 times and then, it was positioned in the divisor to round 30 g cylinder-shaped bread rolls. The product was taken to the fermentation chamber at 27°C and 95% humidity until its initial size was doubled (40 min). Next, the bread was baked at 190ºC for 30 min, with steam injections in the first 10 sec. After complete cooling, the breads were packed in transparent plastic bags, with 10 units each.

Sensory analysis and of texture: The sensory analysis was performed at the Sensory Analysis Laboratory of the UNIVATES University Center, after approval of the project by the Research Ethics Committee of the UNIVATES University Center (opinion #21727313.9.0000.5310).

The samples were evaluated by 60 untrained tasters. They were positioned in individual booths with natural white lighting and received a glass of mineral water, a plate with one sample (30 g) of each formulation, coded with three random digits (924 for F1 and 571 for F2), a pen, the Free Informed Consent Term (Appendix 1), along with the Sensory Analysis Form (Appendix 2).

After tasting both formulations, each participant filled out the evaluation form regarding the sensory characteristics of the samples. The tasters evaluated the aroma, taste, color, texture and global acceptance of the product through a 9-point structured scale. They were also inquired about their intention to purchase the production, attributing amounts from 1-5 points (1-would certainly not buy, 2-would probably not buy, 3-might or might not buy, 4-would probably buy, 5-would certainly buy).

The breads were also subject to a test regarding the texture of the core, for this characteristic is very emphasized in quality and might be considered the most influential sensory characteristic in acceptance by consumers (Da Silva *et al*., 2009). The test was performed at FURG's (Rio Grande Federal University) Food Laboratory, using the texturometer equipment TA-XT2 (Stable Micro Systems, UK), which measures the necessary strength to shear bread slices versus time, according

to a the method (74-09.01). The texturometer was equipped with a 36 mm diameter cylindrical probe in the following work conditions: pre-test speed: 1.0 mm sec^{-1}, test speed: 1.7 mm sec^{-1}, post-test speed: 10.0 mm sec^{-1}, compression: 40% and thickness of slice: 25 mm.

Shelf life estimate: Both formulations of the bread were conditioned in common polyethylene plastic bags and kept in a BOD incubator at 20±1°C, accompanied by fungal growth, daily, for 20 days. The shelf life estimate helps demonstrate the period in days the breads keep their quality (Cardoso and Rubensan, 2011). Their expiration date was noted and the bread was discarded after the first sign of fungi in the samples. Shelf life was determined considering visual aspects of the product.

The evaluation results were submitted to an Analysis of Variance (ANOVA). Since the tasters were not trained, the F test was applied only for the samples.

RESULTS AND DISCUSSION

Sensory analysis and of texture: With very similar visual characteristics, tasters exhibited a higher preference for the F1 formulation (extract obtained from 150 g of yerba mate), since considering the evaluated attributes, it received a higher percentage of grades 7, 8 and 9 for most of the evaluated attributes (Fig. 1). However, there was not a significant difference between the percentages of each attribute for the F1 and F2 formulations ($F<F_{critical}$) (Table 2). The taste attribute of the F1 formulation was the evaluated variable presenting the greatest difference in relation to F2 (extract obtained from 75 g of yerba mate). While 56% of the tasters marked F1 with a grade 8, considering the taste criterion, only 34% attributed the same grade to F2 (Fig. 1a). For the aroma attribute, F1 also ranked higher, since grade 8 was the most voted one (Fig. 1b). For the color attribute (Fig. 1d), grades 7, 8 and 9 of F1 had a higher percentage of votes. As for the texture attribute (Fig. 1c), F2 received higher grades. Both the marks 7 and 8 obtained a higher percentage of choice compared to F1.

According to Silva *et al*. (1998), foods prepared based on ingredients that belong to the eating habits of a certain population are more accepted, since feeding behavior results from an environmental, psychological, socio-cultural and economic relationship. In this context, the F1 formulation, with a higher concentration of yerba mate extract, obtained higher marks in most of the evaluated attributes and was pointed as the favorite by the tasters. Only for the texture attribute the F1 did not obtain the highest percentage of marks however, the values for both formulations are very close.

The distribution histogram of the grades for the global impression attribute showed that most of the tasters assigned a grade 8 (liked very much) to both bread formulations (Fig. 2). No grades below 4 were assigned (slightly disliked), indicating good acceptance for both bread formulations by the tasters. Among the total number of tasters, 86% assigned grades between 7 and 9 to the bread with the more concentrated extract (F1) (moderately liked, liked very much, liked really very much, respectively). A similar result was verified by Azevedo *et al*. (2011) when sensory evaluating sliced bread with 5% of powdered whey, comparing it to a market brand. According to the obtained grades, the authors verified that both sliced bread samples were accepted, however, the grades

Table 2: Comparison between the ANOVA results between F and F critical for the attributes taste, aroma, color, texture and acceptance for bread containing aqueous *Ilex paraguariensis* A.St.-Hil. extract, formulated from 150 g (F1) and 75 g (F2) of crushed leaves

Attribute	Taste	Aroma	Color	Texture	Acceptance
F of the samples	1.6267	0.0087	1.7567	0.7582	1.8786
F critical			4.038392634		

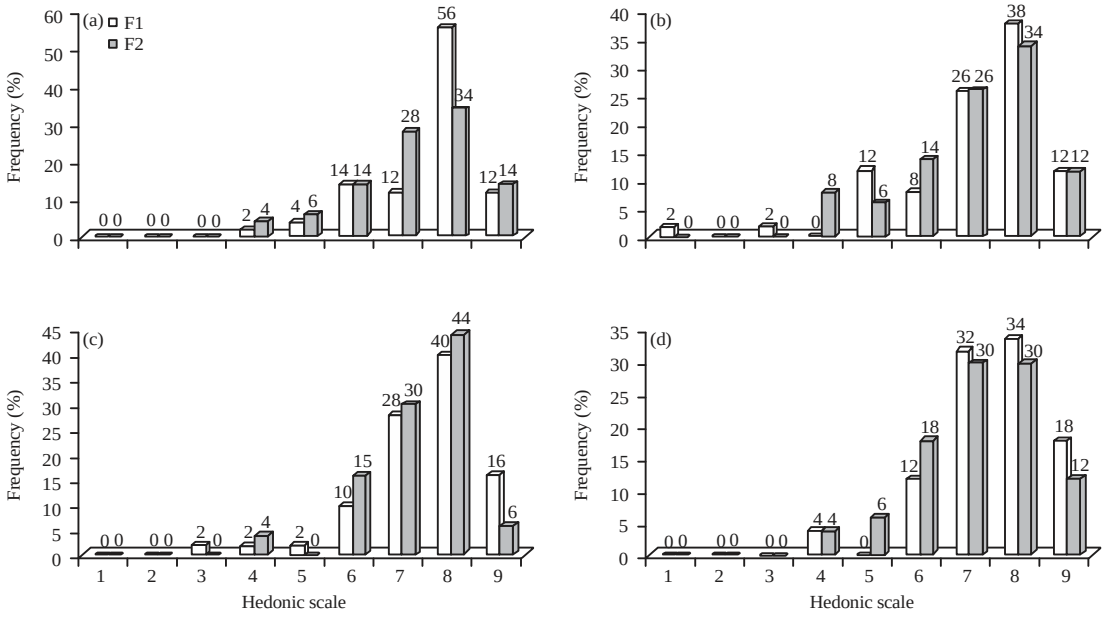

Fig. 1(a-d): Frequency distribution of grades assigned by the tasters for each one of the attributes of formulations F1 and F2 of bread with *Ilex paraguariensis* extract (a) Evaluation of the (a) Taste, (b) Aroma, (c) Texture and (D) Color attribute

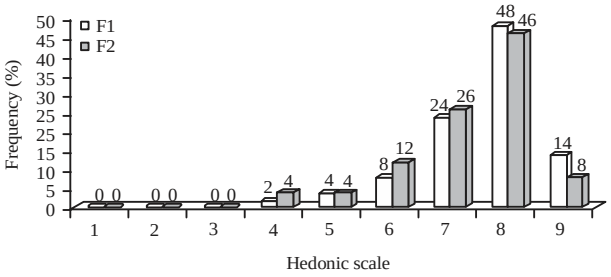

Fig. 2: Global acceptance frequency values for both bread formulations (F1, containing aqueous extract obtained from 150 g of crushed leaves and F2, containing extract obtained from 75 g of *Ilex paraguariensis* crushed leaves)

averages of the bread with 5% of powdered whey presented were above 7, showing a higher sensory acceptability than commercial sliced bread. The study showed the viability of using whey, which, as well as the yerba mate extract, can be easily incorporated to the formulation of foods, especially breads.

The same was found by Vieira *et al*. (2008) and Berte *et al*. (2011), when formulating, respectively, candies and jelly with yerba mate. The sensory tests indicated that yerba mate candies were acceptable and approved regarding purchase intention. Jelly, which contained, besides yerba mate, inulin, obtained a purchase preference higher than 70%.

Regarding the acceptability index of bread with yerba mate aqueous extract, the results showed values above 70% for both formulations in all the attributes (Fig. 3). According to Ambrosio *et al*. (2006), an approval above 70% indicates the product was well accepted. The F1 formulation, which

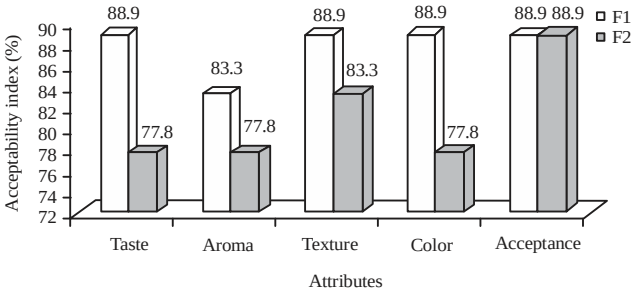

Fig. 3: Global acceptance index by attribute for both bread formulations containing aqueous extract obtained from 150 g (F1 Formulation) and 75 g (F2 Formulation) of *Ilex paraguariensis* crushed leaves

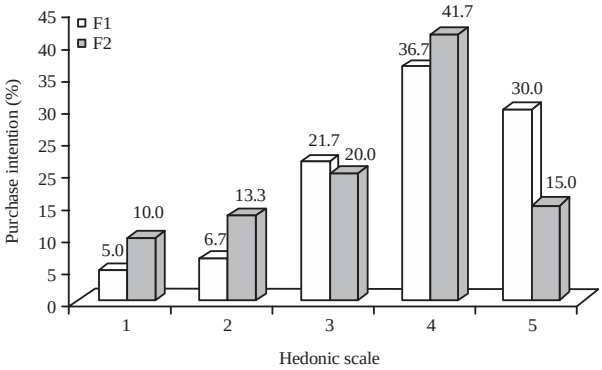

Fig. 4: Histogram with mean values obtained for purchase intention for both bread formulations containing aqueous extract obtained from 150 g (F1 Formulation) and 75 g (F2 Formulation) of *Ilex paraguariensis* crushed leaves (1 = would certainly not buy, 2 = would probably not buy, 3 = might buy or might not buy, 4 = would probably buy, 5 = would certainly buy)

corresponds to the bread with a higher concentration of aqueous yerba mate extract, stood out even more in the preference of the tasters, since the approval percentages were higher than 80% in all the attributes, different from what was found in other researches where yerba mate (*Ilex paraguariensis*) was applied: development of cereal bar (Chiesa *et al*., 2012), carbonated drinks (Mello *et al*., 2009) and jelly with added fiber (Berte *et al*., 2011). In the studies cited, the acceptability of the product was inversely proportional to the amount of the extract. As for the present study, the increase in the amount of yerba mate and consequently, the amount of fibers did not reduce the acceptability of the F1 bread because the addition of fiber in bread results in a non-favorable appearance of the product: dark coloring, unpleasant texture and reduced volume (Nunes, 2008).

For purchase intention, F1 received 67% and F2 received 57% of the 4 and 5 responses that correspond to Would probably buy and Would certainly would buy, indicating a positive attitude of the tasters regarding the possibility of purchasing the bread in both formulations. However, also for purchase intention, F1 obtained higher values than F2 (Fig. 4).

To Mello *et al*. (2009), the characteristic bitter taste of yerba mate is one of the biggest difficulties for using the plant in food product formulations. This was found in the Chiesa *et al*. (2012) study, for which cereal bars containing powdered yerba mate were developed. Through a

Table 3: Count, sum, mean and variance of results of the texture analysis for the F1 and F2 formulations of the bread

Summary	Count	Sum	Mean	Variance
F1	3	7.291636541	2.430545514	0.071043143
F2	3	7.688119401	2.562706467	0.10273142

F1: Formulation of the bread containing extract obtained from 150 g of leaves of yerba mate (*Ilex paraguariensis* A.St.-Hil.), F2: Formulation of the bread containing extract obtained from 75 g of leaves of yerba mate (*Ilex paraguariensis* A.St.-Hil.)

purchase intention survey about the cereal bar, the authors verified a reduction in preference for purchasing the cereal bar when the amount of the plant in the formulation increased.

Firmness analysis showed similar values between the formulations (Table 3), with the mean value for F1 (2.43 N) lower than the F2 mean (2.56 N). Similar values for texture analysis were found by Nabeshima *et al*. (2005) when compared the technological and sensory characteristics of sliced breads fortified with three different iron sources (reduced iron, iron pyrophosphate and micro encapsulated ferrous sulfate monohydrate) in the concentration of 4.2 mg of iron/100 g of flour. The authors observed that the breads containing iron pyrophosphate were the softer ones (312.77 g f or 3.07 N), while the highest values were obtained with the breads that contained reduced iron.

Adding other ingredients to bread, such as flax seeds, emulsifiers and salt tend to provoke an increase in bread firmness (Altamirano-Fortoul and Rosell, 2011). Borges *et al*. (2011) added 10% of flax seed flour to the formulation of French rolls and verified an increase in firmness from 1.40-7.32 N. These results were opposite to the findings in this study, since the bread with the higher amount of yerba mate extract presented lower firmness. However, the result of the instrumental firmness analysis of both formulations was different from the sensory analysis. For the tasters, the best results were perceived in the F2 formulation but the instrumental analysis showed a softer texture for the F1 formulation. According to Carr *et al*. (2006) and Oliveira *et al*. (2007), firmness values between 0.8 and 8.5 N evidence good sensory acceptance.

Shelf life estimate: In life cycle analysis, according to the changes in sensory attributes performed with both yerba mate bread formulations, we observed that sensory formulations started in the fourth day of storage for the F2 formulation, including the formation of mold. Thus, we established the expiration date for F2 as 3 days. However, for the F1 formulation, with a higher concentration of the extract, mold appeared only on the 12th day. The life cycle found for F1 was much higher than the reported in other studies (EMATER, 2000; Carvalho *et al*., 2011; Esteller *et al*., 2004). According to EMATER (2000), the expiration of whole wheat homemade breads vary from three to seven days if packed at room temperature. Carvalho *et al*. (2011) observed that with cowpea whole breads at room temperature, the sensory changes started in the 4th storage day, including the appearance of mold, corresponding to an expiration of three days. Esteller *et al*. (2004) replaced sucrose in bread formulation for other four types of sugar (liquid and crystal fructose, anhydrous dextrose, honey and inverted sugar). The type of sugar for the formulation nine to ten days, the same period when compared to traditional bread with sucrose.

The longest life cycle estimate for the yerba mate bread is strongly associated with the chemical properties of yerba mate, due to its high polyphenol content. According to Colpo (2012), polyphenolic compounds in plants like yerba mate are involved in a great variety of functions, including antimicrobial and antifungal actions, also acting as a natural preservative (Berte *et al*., 2011).

CONCLUSIONS

The bread containing a more concentrated aqueous yerba mate extract (150 g of leaves) was accepted by more than 80% in all the studied attributes. Since, there was not a significant

difference between the two formulations in regards to the evaluated parameters and acceptance by consumers, we may conclude that the amounts evaluated can be used in bread production with no loss to the characteristics of the final product. Shelf life was higher for the formulation with the addition of extract obtained from a higher amount of yerba mate (150 g), amounting to 12 days. For its antifungal properties, yerba mate in the higher concentration acted as a natural preservative for the breads.

We suggest more researches regarding the properties of bread with an aqueous yerba mate extract during its consumption by people. The applicability of the plan is viable for foods and values the species, because it exhibits healthy compounds, as well as adding the properties of the plant to the most popular food in Brazilian tables.

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