

Article

# Novel Beverages of Yerba-Mate and Soy: Bioactive Compounds and Functional Properties

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**Abstract:** In this paper, two high-nutrition commodities that are produced in great amounts in Brazil were joined in a single functional product. Yerba mate (*Ilex paraguariensis*) is rich in bioactive compounds, while soybean is a high-quality protein source. The objective of this paper was to assess the psychochemical characteristics of two yerba-mate progenies (planted–PL and native–NT leaves) and then confirm whether the functional and nutritional properties of the main ingredients were conveyed to the beverage produced. The main raw material, yerba-mate leaves, and the drinks were assessed for bioactive compounds, antioxidant capacity, physicochemical properties, and nutritional value. Planted leaves showed higher concentration of 5-CQA, caffeic acid and rutin than the native plant, whereas caffeine and theobromine were detected in larger amounts in native leaves. The nutritional profile of the drinks was compared to commercial beverages—either yerba-mate-based or soy-based. They indeed provide more protein, fiber, and fats than traditional yerba-mate beverages (*chimarrão*, *tererê*, and mate tea). Soy drinks currently marketed, for their turn, have similar caloric value and higher contents of lipid and protein as compared to our product, but are poor in fibers. NT drink (DPPH—IC<sub>50</sub> 92.83 and ABTS—8.18 μM Trolox/mL) had higher antioxidant activity than PL (IC<sub>50</sub> 147.06 and 5.63 μM Trolox/mL) due to the greater volume fraction of yerba-mate extract. NT beverage has more 5-CQA and caffeine in the same intake of *tererê* and traditional mate tea. This healthy beverage contributes to an increasing income to the food industry and yerba-mate producers, and environmental gains that are related to the exploration of natural resources.

**Keywords:** yerba-mate; beverage; chemical composition; phenolic compounds; antioxidant activity

## 1. Introduction

Yerba-mate has been consumed for centuries as the traditional beverages *chimarrão* (infusion of dried leaves in hot water), *tererê* (infusion of dried leaves in cold water), and mate tea (infusion of toasted leaves in hot water), especially in countries where it is cultivated, such as Brazil, Argentina, and Paraguay [1]. In recent years, yerba-mate is gaining rapid market insertion in regions, such as South America, the United States, Europe, and Japan for two main reasons: the processing of yerba-mate does not require chemical treatments and the product contains a wide variety of bioactive compounds. Methylxanthines, saponins, and phenolic compounds are derived from the secondary plant metabolism and have been associated with many properties that benefit human health [2,3]. Biological tests involving aqueous extracts of yerba-mate have contributed to consolidate this plant as an important

ally in preventing the development of some diseases. Several *in vitro* and *in vivo* studies [4–8] have shown that regular consumption of yerba-mate drinks produces antioxidant and hypoglycemic effects, and also protects cell membranes, lipoproteins, and the DNA, helping to prevent the onset of diseases, such as atherosclerosis and cancer. Furthermore, yerba-mate contains vitamins and minerals, thus presenting a rich nutritional profile.

The yerba-mate drink enriched with soy hydrosoluble extract proposed here is also a way to explore the nutritional and functional potential of soybeans, which are nowadays more common in Eastern diets. Soybean (*Glycine max* (L.) Merrill), an herbaceous plant belonging to the family *Leguminosae*, subfamily *Papilionoideae*, and tribe *Phaseoleae*, is the most important oilseed produced in Brazil [9]. Soy is rich in good quality protein, has polyunsaturated fatty acids and phytochemicals, such as isoflavones, saponins, and phytates, and is a source of minerals such as copper, iron, phosphorus, magnesium, manganese, and B vitamins [10]. Therefore, the soy composition allows for its use as raw material for a wide range of products, including those that replace cow milk for lactose intolerant people and vegans.

The aim of this study was to evaluate the psychochemical properties of yerba-mate leaves of two different progenies (native and planted) and then assess their potential for the development of a rich-nutrition antioxidant beverage made from yerba-mate and soybeans. The beverages were assessed for chemical compounds (identification and quantification) and antioxidant activity, and their nutritional profile was compared to that of other beverages currently marketed.

## 2. Materials and Methods

### 2.1. Raw Materials and Beverages

Two yerba-mate progenies from a breeding study—12-year-old planted trees and 80-year-old native trees—were collected at Embrapa Florestas in Colombo, Paraná, Brazil (25°19′16″ south latitude and 49°09′31″ west longitude).

In a previous study [11], six yerba-mate extracts (YME) of each progeny were prepared from dried leaves and enriched with soy water-soluble extract (SWE) under different volume fractions. Two beverages were chosen on the basis of sensory analysis between the 12 resulting formulations, according to detailed methodology published in Frizon et al. [11]. One set of acceptance and purchase intent tests was conducted every month during six months of shelf-life study with 70 different panelists, totaling 420 panelists. The two best formulations assessed in the present paper were named PL and NT and were prepared according to the following volume fractions of YME and SWE: 2:1 (*v/v*) for PL and 3:1 (*v/v*) for NT.

### 2.2. Chemical Composition

The yerba-mate samples and the drinks were analyzed for moisture, ashes, lipids, and protein, according to the methods of the Association of Official Analytical Chemists [12]. Only the beverages were assessed for dietary fiber. The total carbohydrates were calculated by difference (100% – % moisture – % ashes – % lipids – % proteins – % fibers). The total soluble solids (TSS) were determined using a benchtop refractometer (RL3—Polskie Zaklady Optyczne S.A., Warsaw, Poland) with scale from 0 to 90 °Brix. The pH was measured by directly reading with a digital potentiometer (Hanna Instruments, São Paulo, Brazil). The energy value (kcal) was estimated using the conversion values of 4 kcal/g for proteins and total carbohydrates, and 9 kcal/g for lipids, according to Watt and Merrill [13].

#### 2.2.1. Phenolic Compounds

The extraction and determination of phenolic compounds from either yerba-mate leaves or beverages were performed, according to Dutra et al. [14]. Firstly, 100 mL of water: ethanol 1:1 (*v/v*) solution was added to 2 g of yerba-mate leaves and the mixture was left to rest for 12 h at room

temperature. Then, three 30 min-extractions were carried out with 25 mL of 50% hydro-ethanol solution under reflux at 85 °C. The extracts obtained in each of the three extractions were collected in a 250 mL-single-neck round-bottom flask and its volume was completed with the solvent. After filtration, the phenolic acids 5-caffeoylquinic acid (5-CQA) and caffeic acid and the flavonol rutin were determined by HPLC in an equipment (Agilent 1200 series, Waldbronn, Germany), controlled by the EZ Chrom Elite Software (edition 3.3.1.902) with automatic injection system and diode array detector. The column used was a Zorbax Eclipse XDB-C18 (4.6 × 150 mm, 5 µm). The mobile phase consisted of solvent A—water/formic acid (99.55:0.45 *v/v*)—and solvent B—methanol/formic acid (99.55:0.45 *v/v*). The gradient program started with 80% A: 20% B for 5 min rising to 58% A: 42% B in 7 min, returning to 80% A: 20% B in 16 min up to 20 min of chromatographic run. The temperature during analysis was 21 °C and the injection volume was 1 mL min<sup>-1</sup>. The detection was monitored at 325 nm for caffeic acid and 5-CQA and at 370 nm for rutin.

### 2.2.2. Methylxanthines

The methylxanthines caffeine, theophylline, and theobromine were extracted from 2 g of yerba-mate with sulfuric acid in a water bath, followed by neutralization with sodium hydroxide 40%, according to the methodology described by Dutra et al. [14]. After filtration, an aliquot of 5 µL of each sample was injected in the chromatograph and analyzed following the same method described in Section 2.2.1. Water/methanol (80:20 *v/v*) solution at an isocratic flow rate of 1 mL min<sup>-1</sup> was used as mobile phase. The detection was set at 272 nm for caffeine, theobromine, and theophylline.

## 2.3. Antioxidant Activity

### 2.3.1. ABTS<sup>•+</sup> Radical Method

The methodology described by Rufino et al. [15] was used for the determination of antioxidant activity by the ABTS<sup>•+</sup> radical method. Initially, the radical ABTS<sup>•+</sup> was formed from the reaction of 7 mM ABTS with 2.45 mM potassium persulfate, which were kept at room temperature in the dark for 16 h. Elapsed this time, 1 mL of this solution was diluted in sufficient ethanol to obtain an absorbance of 0.70 ± 0.05. To perform the analyses, 30 µL of the diluted sample were added to 3.0 mL of the ABTS<sup>•+</sup> radical and the absorbance was determined in a UV-1600 spectrophotometer at 734 nm after six minutes of reaction. As a standard solution, the synthetic antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used. All of the readings were performed in triplicate and the results were expressed as µM Trolox of antioxidant activity per g of sample.

### 2.3.2. DPPH<sup>•</sup> Radical Method

The DPPH<sup>•</sup> scavenging assay was determined, as published by Rufino et al. [15]. This radical leads to a decrease in absorbance when fixing a H<sup>•</sup> ion that was removed from the antioxidant. For analyzing the samples, an aliquot of 0.1 mL was added to 3.9 mL of the DPPH<sup>•</sup> radical (0.06 mM). Readings were taken with an UV-1600 spectrophotometer at 515 nm after 30 min of reaction. All of the determinations were performed in triplicate and included a control sample. The decline in absorbance of the samples was correlated with the control, allowing for setting the percentage of discoloration of the radical DPPH<sup>•</sup>. This made possible to calculate, after reaching the reaction equilibrium, the amount of antioxidant necessary to reduce 50% of the DPPH<sup>•</sup> radical. The antioxidant activity was expressed in µM Trolox/g yerba-mate and µM Trolox/mL beverage for the ABTS method, and in g/g DPPH for yerba-mate and mL/g DPPH for beverages for the DPPH method.

## 2.4. Physical Characterization

### Color

Color determinations were carried out using the colorimeter HunterLab Digital PlusMiniScan XE (Hunter Associates Laboratory Inc., Reston, VA, USA) with D65 illuminant and 10° angle of vision. The mean values for L\* (lightness), a\* (redness to greenness), b\* (yellowness to blueness), chroma [(a\*<sup>2</sup> + b\*<sup>2</sup>)<sup>1/2</sup>], and hue angle [ $\tan^{-1}(b^*/a^*)$ ] were determined according to the CIE Laboratory system [16].

## 2.5. Statistical Analysis

All of the experimental determinations were carried out in triplicate. The statistical analysis was performed with the software Statistica for Windows, version 7.0 (StatSoft, São Paulo, Brazil), using analysis of variance (ANOVA) and Tukey test with a 95% confidence level ( $p < 0.05$ ).

## 3. Results and Discussion

### 3.1. Chemical Composition and Physico-Chemical Characteristics of Yerba-Mate (Native and Planted) and Beverages

The chemical composition and the physicochemical characteristics of yerba-mate (planted and native) used as raw material in the manufacturing of beverages PL and NT are shown in Table 1. The yerba-mate leaves did not differ statistically regarding ashes, proteins, and moisture content; however, the native progeny showed higher fat content than the planted one. The percentages of protein, fats, moisture, and ashes for both progenies were higher than the results found by Berté et al. [17] for dried yerba-mate (7.97, 4.25, 3.60 and 5.14 g/100 g, respectively). However, the content of protein reported by Esmelindo et al. [18] (14.49 g/100 g) was higher than the results that are presented here, while the fat content was lower (6.76 g/100 g). In fact, studies show that yerba-mate may vary in chemical composition as a result of climatic conditions, type and conditions of soil, plant variety, seasonality, age of leaves, harvest time [19], industrial processing [20], and extraction methods [21]. Furthermore, the papers currently available in the literature on yerba-mate regard the secondary compounds rather than the chemical composition of the leaves [22,23].

**Table 1.** Chemical composition and physicochemical parameters of yerba-mate leaves and beverages.

Parameter	Yerba-Mate Leaves		Beverage	
	PL	NT	PL	NT
Proteins (g.100 g <sup>-1</sup> )	9.52 ± 0.42 <sup>a</sup>	10.06 ± 0.12 <sup>a</sup>	0.33 ± 0.31 <sup>c</sup>	0.52 ± 0.39 <sup>b</sup>
Fats (g.100 g <sup>-1</sup> )	6.30 ± 0.06 <sup>b</sup>	8.13 ± 0.10 <sup>a</sup>	0.95 ± 0.19 <sup>d</sup>	1.33 ± 0.03 <sup>c</sup>
Moisture (g.100 g <sup>-1</sup> )	5.77 ± 0.07 <sup>c</sup>	5.70 ± 0.09 <sup>c</sup>	90.60 ± 0.21 <sup>a</sup>	88.19 ± 0.10 <sup>b</sup>
Ashes (g.100 g <sup>-1</sup> )	7.04 ± 0.31 <sup>a</sup>	7.01 ± 0.07 <sup>a</sup>	0.21 ± 0.01 <sup>b</sup>	0.22 ± 0.01 <sup>b</sup>
pH	-	-	4.36 ± 0.02 <sup>b</sup>	4.46 ± 0.01 <sup>a</sup>
Edible fibers (g.100 g <sup>-1</sup> )	-	-	0.41 ± 0.03 <sup>b</sup>	0.54 ± 0.04 <sup>a</sup>
Total soluble solids (°Brix)	-	-	8.11 ± 0.02 <sup>b</sup>	10.16 ± 0.10 <sup>a</sup>
Carbohydrates (g.100 mL)	-	-	7.50 ± 0.08 <sup>b</sup>	9.20 ± 0.10 <sup>a</sup>
Energy (kcal/100 mL)	-	-	36.20 ± 0.21 <sup>b</sup>	48.42 ± 0.23 <sup>a</sup>
Luminosity (L*)	36.95 ± 0.59 <sup>d</sup>	42.14 ± 0.72 <sup>c</sup>	60.19 ± 0.22 <sup>a</sup>	57.09 ± 1.33 <sup>b</sup>
a*	-7.78 ± 0.02 <sup>d</sup>	-6.32 ± 0.38 <sup>c</sup>	2.21 ± 0.50 <sup>b</sup>	8.01 ± 0.58 <sup>a</sup>
b*	20.64 ± 0.47 <sup>a</sup>	21.87 ± 0.96 <sup>a</sup>	16.33 ± 0.97 <sup>b</sup>	19.88 ± 0.73 <sup>a</sup>
Chroma (C*)	22.06 ± 0.43 <sup>a</sup>	22.77 ± 0.91 <sup>a</sup>	16.48 ± 0.97 <sup>b</sup>	21.43 ± 0.90 <sup>a</sup>
Hue angle (h)	110.66 ± 0.48 <sup>a</sup>	106.13 ± 1.21 <sup>b</sup>	82.29 ± 1.66 <sup>c</sup>	68.07 ± 0.71 <sup>d</sup>

Mean values in the same line followed by the same superscript letter (a-d) do not differ statistically according to the Tukey test ( $p < 0.05$ ). PL = planted leaves and NT = native leaves. Color coordinates: a\* = redness and b\* = yellowness. Green tones and red tones are denoted by -a and +a, respectively. Blue and yellow tones are represented by -b and +b, respectively.

Each drink showed different chemical composition and physicochemical parameters. While PL drink had a slightly higher moisture ( $90.60 \pm 0.21\%$  vs.  $88.19 \pm 12.10\%$ ), the NT formulation had higher levels of lipids, carbohydrates, and fiber, as well as higher pH, soluble solids concentration, and energy value. The protein and ash contents, however, are statistically the same for both beverages. These results are attributed to the difference in composition between the yerba-mate progenies and the different volume fractions of YME and SWE in the products. When considering that there are not similar drinks in the market (made of yerba-mate and soy), our beverages were compared to commercial soybean-based beverages (Purity, Ades, Mais Vita and Mupy) available in supermarkets of Curitiba (Southern Brazil). Said commercial beverages had higher contents of protein (2.50–2.60 g/100 g) and fats (1.35–1.50 g/100 g) and lower values of dietary fiber (zero) and carbohydrates (3.70–5.50 g/100 g) than the drinks evaluated in this work, whose main base is the yerba-mate extract.

The total soluble solids (TSS) of our beverages ranged from  $8.11 \pm 0.02$  °Brix (PL drink) to  $10.16 \pm 0.10$  °Brix (NT drink) (Table 1), corresponding to pH values of, respectively,  $4.36 \pm 0.02$  and  $4.46 \pm 0.01$ . These TSS are lower than the usual values found for soft drinks (11 °Brix), juices (12 °Brix), soy-based beverages (15 to 20 °Brix and pH 3.64 to 3.9, Potter et al. [24]), and yerba-mate drinks (12 °Brix and pH 3.4, Mello et al. [25]). The drinks studied in this paper exhibited lower TSS for a higher pH, which contributed to a softer and less acid flavor. In fact, Contreras [26] concluded that yerba-mate-based drinks with lower TSS (around 9 °Brix) had greater sensory acceptance.

Table 1 also shows the results for color ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ , and  $h$ ). The native leaves are considerably brighter than the planted ones. The beverages were brighter than the raw material, indicating an influence of the formulation and manufacturing process on color. Visually, the beverages have a whitish tone (Figure 1), similar to vegetable milks, such as almond or rice. There was a statistical significant difference between the beverages regarding  $L^*$ , however coordinate  $a^*$  (from green – to red +) was negative for both of them, indicating a tendency to green. The beverages also slightly tended to red, and showed different values from each other for the  $a^*$  coordinate ( $p < 0.05$ ). Coordinate  $b^*$  (from blue – to yellow +) showed a tendency to a yellowish tone for both yerba-mate progenies and beverages. There was no statistical difference ( $p < 0.05$ ) of chroma  $C^*$  between yerba-mate leaves and beverage NT.  $C^*$  indicates the deviation from the grey point in the  $L^*$  scale. Beverage PL showed a lower  $C^*$  value than NT and the leaves. Regarding the hue angle, the yerba-mate leaves tended to green ( $h$  near to  $180^\circ$ ), while beverages tended to red (NT) and yellow (PL), with hue angles between  $0$  and  $90^\circ$ .



Figure 1. Aspect of the beverage of yerba-mate with soy developed in this work.

### 3.2. Polyphenol Content and Antioxidant Activity

The concentration of phenolic compounds in the leaves and beverages and their ability to eliminate free radicals, as analyzed by ABTS and DPPH methods, are presented in Table 2.

**Table 2.** Phenolic compounds and antioxidant activity of yerba-mate leaves and beverages.

Parameter	Yerba-Mate Leaves (mg/g)		Beverage (mg/mL)	
	PL	NT	PL	NT
5-CQA	19.323 ± 0.243 <sup>a</sup>	17.939 ± 0.481 <sup>b</sup>	0.185 ± 0.002 <sup>c</sup>	0.251 ± 0.008 <sup>c</sup>
Caffeic acid	0.137 ± 0.006 <sup>a</sup>	nd	0.009 ± 0.000 <sup>b</sup>	0.005 ± 0.000 <sup>b</sup>
Rutin	8.615 ± 0.196 <sup>a</sup>	6.081 ± 0.141 <sup>b</sup>	0.052 ± 0.001 <sup>c</sup>	0.051 ± 0.001 <sup>c</sup>
ABTS *	424.66 ± 0.03 <sup>b</sup>	485.47 ± 0.03 <sup>a</sup>	5.63 ± 0.02 <sup>d</sup>	8.18 ± 0.02 <sup>c</sup>
DPPH (IC <sub>50</sub> )	39.72 ± 0.01 <sup>a</sup>	38.64 ± 0.01 <sup>b</sup>	147.06 ± 0.02 <sup>c</sup>	92.83 ± 0.01 <sup>d</sup>

\* ABTS is expressed in  $\mu\text{M}$  Trolox/g yerba-mate and  $\mu\text{M}$  Trolox/mL beverage. IC<sub>50</sub> is expressed in g/g DPPH for yerba-mate and in mL/g DPPH for beverages. nd = Not detected. PL = planted leaves and NT = native leaves. Mean values in the same line followed by the same superscript letter (<sup>a-d</sup>) do not differ statistically according to the Tukey test ( $p < 0.05$ ).

The results confirm that the planted yerba-mate showed higher concentration of 5-CQA (19.323 ± 0.243 mg/g), caffeic acid (0.137 ± 0.006 mg/g), and rutin (8.615 ± 0.196 mg/g) than the native plant (17.939 ± 0.481 mg/g, non-detectable amount, and 6.081 ± 0.141 mg/g). According to Dartora et al. [27], yerba-mate directly exposed to sunlight has higher levels of phenolic compounds as compared with the shaded plant. Blum-Silva et al. [28] reported that the concentration of phenolic compounds is higher in younger leaves. However, Strassmann et al. [29] found greater concentration of total phenolic compounds in aqueous extracts of cultivated yerba-mate leaves, either young or adult (149.68 and 135.40  $\mu\text{g/mL}$ , respectively) in comparison to native leaves (64.08  $\mu\text{g/mL}$  and 59.30  $\mu\text{g/mL}$ , respectively). This may explain the results obtained in the present work: the planted yerba-mate corresponds to young leaves predominantly located in open places, while native plants correspond to old leaves located in forests and shaded areas. In fact, some studies report the positive correlation between intensity of sun radiation and production of flavonoids [30,31].

Regarding the beverages, higher concentration of 5-CQA was found in the NT formulation (Table 2), which has a greater ratio of yerba-mate extract/soy extract. However, such difference was not statistically significant (0.251 ± 0.002 mg/L for NT versus 0.185 ± 0.008 mg/L for PL). The results for 5-CQA in 100 mL of beverage (18–25 mg) are in the same range reported by other authors: 6–32 mg/100 mL of mate tea [32,33], 30 mg in 100 mL of coffee [34], and 6–36 mg/100 mL of *chimarrão* [35]. Our results were however lower than for *tererê*, 26–37 mg/100 mL [33]. Even considering that our manufacturing process includes extraction, mixture of ingredients, and pasteurization, the 5-CQA found for beverage NT is higher than for the same portion of green tea, a product widely known as a rich source of bioactives: 25 mg/100 mL versus 16 mg/100 mL, value reported by Nevena et al. [36].

The rutin content of beverages PL and NT (0.052 ± 0.001 mg/mL and 0.051 ± 0.001 mg/mL, respectively) was similar to the values reported by Mezdari et al. [37] for commercial frozen pulp acerola (0.058 mg/100 mL), crushed fruit (0.047 mg/100 mL), and squeezed fruit (0.059 mg/100 mL). These results confirm that the beverage NT presented functional components, such as 5-CQA and rutin, in equivalent or higher concentrations respective to the beverages currently marketed.

The caffeic acid content found for PL ( $\approx 0.9$  mg/mL) and NT ( $\approx 0.5$  mg/mL) beverages is similar or higher than the amounts determined in *chimarrão* (0.57 ± 0.20 mg/mL) by Bastos et al. [35].

The antioxidant activity was measured by the methods DPPH and ABTS (Table 2), showing that a minimum amount of either planted or native yerba-mate is able to reduce the free radical DPPH by 50%. The larger the capacity of elimination of free radicals, the lower the IC<sub>50</sub> value given by the DPPH method. The ABTS assay was used to determine the antioxidant activity of the compounds whether lipophilic (carotenoids) or hydrophilic (ascorbic acid and phenolic acid). By determining the amount of

ABTS consumed due to reaction with the sample, expressed in Trolox equivalents (concentration units), it is possible to provide an estimate of the amount of radicals consumed by the antioxidant [38,39].

According to both methods, the NT drink (DPPH—IC<sub>50</sub> 92.83 ± 0.01 and ABTS 8.18 ± 0.02 μM Trolox/mL) has higher antioxidant activity than PL (DPPH—IC<sub>50</sub> 147.06 ± 0.02 and ABTS 5.63 ± 0.02 μM Trolox/mL) due to its greater volume fraction of yerba-mate extract. Using the DPPH method, Bixby et al. [40] found a direct positive correlation between phenolic compounds and antioxidant activity, with the greatest activity being obtained for the yerba-mate aqueous extracts compared with green tea and red wine. Anesini et al. [41] demonstrated that chlorogenic acid, caffeic acid and the flavonoid rutin present in the yerba-mate aqueous extracts positively contribute to the antioxidant activity. Several studies have attributed further protective effects to these compounds, such as hepatoprotective, analgesic, anti-inflammatory, and preventive of hyperglycemia and LDL oxidation [42,43].

### 3.3. Methylxanthines

The results for methylxanthines are shown in Table 3. Theophylline was not detected in any samples, whereas caffeine and theobromine were detected in larger amounts in the native yerba-mate (5.048 ± 0.021 mg/g and 0.484 ± 0.036 mg/g, respectively). Planted leaves had considerable lower contents of caffeine (1.642 ± 0.183 mg/g) and theobromine (0.043 ± 0.008 mg/g) than native yerba-mate. All of the values determined are in the range reported in the literature, even when different procedures of sample preparation are regarded: 0.20–21.46 mg/g for caffeine and 0.05–5.10 mg/g for theobromine [21,44]. Theobromine affects the smooth muscles and is used either in the prevention and symptomatic relief of bronchial asthma or in the treatment of bronchospasm, also having an effect on the increase of diuresis [45]. Caffeine, in turn, is responsible for bitterness and the psychoactive effect.

**Table 3.** Methylxanthines for yerba-mate and beverages.

Parameter	Yerba-Mate Leaves (mg/g)		Beverage (mg/mL)	
	PL	NT	PL	NT
Theobromine	0.043 ± 0.008 <sup>b</sup>	0.484 ± 0.036 <sup>a</sup>	<QL	<QL
Theophylline	nd	nd	nd	nd
Caffeine	1.642 ± 0.183 <sup>b</sup>	5.048 ± 0.221 <sup>a</sup>	0.049 ± 0.002 <sup>c</sup>	0.183 ± 0.003 <sup>c</sup>

Mean values in the same row followed by the same letter do not differ statistically according to the Tukey test ( $p < 0.05$ ). nd = Not detected. <QL = Quantification limit: caffeic acid (0.80 μg/mL), theobromine (0.69 μg/mL). PL = planted leaves and NT = native leaves.

Beverage NT showed considerably higher content of caffeine than PL (0.183 ± 0.003 mg/mL versus 0.049 ± 0.002 mg/mL). The caffeine content of our beverages (4.9–18.3 mg/100 mL) was higher than found by Meinhart et al. [46] in *chimarrão* (1.8–16.6 mg/100 mL) and in the same range determined in *chimarrão* by Bastos et al. [31] (3.1–29 mg/100 mL) and in *tererê* by Meinhart et al. [46] (2.9–35.8 mg/100 mL). The caffeine content of NT formulation in specific (0.18 mg/mL) was greater compared to *tererê* and traditional mate tea (0.17 and 0.07 mg/mL) studied by Bastos et al. [33], but lower than reported by the same authors in *chimarrão* (0.27 mg/mL).

To sum up, the novel beverages that are evaluated in the presented study are an important source not only of bioactives from yerba-mate, but also of fibers and high-quality protein ascribed to soybeans. In addition, it features a mild flavor, differently from the yerba-mate traditional beverages, which present a characteristic bitter taste that is not appealing to most consumers. Furthermore, the high amounts of bioactives that are found in *chimarrão* are somewhat counterbalanced by the incidence of oral and or pharyngeal cancers associated with the high ingestion temperatures, e.g., 65 °C, for long periods [46,47].

It is worth mentioning that the beverages studied here received purchase intent scores corresponding to “would probably buy” and acceptance scores varying from 6.1 to 7.0 in a 9 point-scale,

as published in Frizon et al. [11]. Although these scores are not optimum, these beverages are rich in antioxidant compounds and protein. They are also physically, chemically, and microbiologically stable during storage as revealed in a previous study by the same authors [11]. This highly nutritious drink is an excellent market opportunity for yerba mate and soybean producers, and contributes to the exploitation of valuable natural resources from developing countries. Also, the diversification may attract new consumers, especially those that are concerned with sustainable food production.

#### 4. Conclusions

The development of new products with nutraceutical properties is one of today's world trends that are related to food production and consumption. Furthermore, exploring new attributes from existing raw materials is of great relevance, given the key impact on economy, society, and environment. The product evaluated in this paper contributes to an increasing income to the industry, technological development, and environmental gains as far as the use of natural resources is concerned.

The yerba-mate with soy beverages have high antioxidant capacity: ABTS and DPPH results are respectively 5.63  $\mu\text{M}$  Trolox/g and 147.06 mL/g DPPH for the PL progeny and 8.18  $\mu\text{M}$  Trolox/g and 92.86 mL/g DPPH for NT. Differences between both formulations are ascribed to the composition of phenolic compounds and methylxanthines of the yerba-mate leaves, which is a consequence of growing conditions, plant species, and sunlight incidence. The phenolic compounds and methylxanthines more abundant in the drinks developed are 5-CQA (0.25 and 0.19 mg/mL for NT and PL, respectively), rutin (0.05 g/mL for both progenies) and caffeine (0.18 and 0.105 mg/mL for NT and PL, respectively). Such compounds provide the beverages with stimulant and antioxidant properties. Furthermore, yerba-mate contains vitamins and minerals, presenting a broad nutritional profile for human health, while soy is rich in isoflavones and good quality protein.

Each 100 mL of the formulation NT offers about 48 kcal, 0.5 g dietary fiber, 0.5 g protein, 1.3 g fat, 25 mg 5-CQA, 18 mg caffeine, 5 mg rutin, and 0.5 mg caffeic acid. Soy beverages (without yerba-mate) currently marketed were used as a reference for comparison purposes: they have similar caloric value (39–45 kcal/100 mL), higher lipid content (1.4–1.5 g/100 mL) and protein content (2.5–2.6 g/100 mL) as compared to the product developed in this work. However, conventional soy drinks do not have fibers in its composition as do the products that are presented in this paper. Furthermore, the content of bioactive compounds of our product is similar or higher to either green tea or yerba-mate traditional beverages, such as *chimarrão*, *tererê*, and mate tea.

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