

Article

Innovative Green Tea Mate: Physicochemical Profile and Sensory Aspects

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Abstract: *Yerba mate* (*Ilex paraguariensis*) is a plant originating from South America. From this plant, mate a drink is produced. Brazil is the first mate producer in the world. The diffusion of mate is mainly limited to the native regions of South America, as it is consumed with *cuia* and *bombilla*. Trying to expand mate culture and export it towards Europe makes it necessary to offer this drink in a new guise that sets it apart from traditional preparation patterns. In this instance, the introduction of green tea mate may represent a solution. This innovative product comes from *I. paraguariensis* sprouts, which are further processed and consumed as green tea from *Camellia sinensis*. The present study aimed to investigate the physico-chemical and nutraceutical characteristics and consumer perceptions towards the visual aspect of the innovative green tea mate (L1, L2, and L3) compared to commercial traditional Brazilian mate (CI and CB) and roasted mate (L). Moreover, a preliminary sensory evaluation with untrained panellists was carried out. Significant results were detected, highlighting the higher content of bioactive compounds in the innovative product. Total Phenolic Content (TPC) almost doubled in green tea mate (L1 117.14 mg GAE/g d.p., L2 128.10 mg GAE/g d.p., L3 126.21 mg GAE/g d.p.) compared to the other samples (CI 71.91 mg GAE/g d.p., CB 54.23 mg GAE/g d.p., L 34.16 mg GAE/g d.p.) ($p < 0.05$). Considering caffeine, batches L1 and L3 had a content of 3.68 mg/g d.p. and 3.58 mg/g d.p., respectively, significantly higher when compared to the amount retrieved in the CB sample, 1.57 mg/g d.p. ($p < 0.05$). The consumer survey demonstrated the interest of consumers towards the new product, as it was perceived similar to more commonly consumed teas, while from a sensory point of view, a vegetal and hay flavour were more defining for the product.

Keywords: *Ilex paraguariensis*; mate; colour; chlorophyll; alkaloids; HPLC quantification; bioactive compounds; consumer survey; sensory analysis



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1. Introduction

Ilex paraguariensis A. St. Hil., known as “mate” (or *yerba mate* in Spanish and *erva-mate* in Portuguese), is a plant of the *Aquifoliaceae* family, native to South America. A drink that is very popular in some South American countries (e.g., Paraguay, Uruguay, Brazil, Argentina, and Chile) derives from the mate leaves [1].

Brazil is the main *yerba mate* producer, with 880 thousand metric tonnes produced in 2019, followed by Argentina (837 thousand metric tonnes produced) and Paraguay (171 thousand metric tonnes produced). The Brazilian states with the highest production of *yerba mate* are Santa Catarina, Rio Grande do Sul, and Paraná [2]. In 2018, Paraná concentrated 87% of all the *yerba mate* production in the country. 393 thousand metric tonnes of the total production (about 345.09 thousand) are exported, mainly from the Central-South region [2].

The *yerba mate* cultivation sector has a strong social impact, contributing to the political emancipation of the state of Paraná. The revenues from the economic activity contributed to the construction of schools and the creation of the university in 1912 [3].

The origin of this traditional drink dates back to the Guaraní people and is obtained from the whole plant (both leaves and stems), harvested manually or mechanically. The processing phase follows a specific and well-established procedure, involving a stage in direct contact with fire to inactivate enzymes, a drying step, and a grinding phase [4].

The traditional infusion (*mate* in Spanish or *chimarrão* in Portuguese) is prepared in a hollowed gourd, filled up with dried product, and then poured into hot water (70–80 °C). The infusion can also be prepared with cold water, and it is known as “*tereré*” [5,6]. Another alternative diffused in Paraguay, Brazil, and Argentina is “*mate cocido*” (“*chá mate*” in Portuguese), consumed as a classic tea.

Outside South America, it is commonly consumed in Lebanon, Syria, and other parts of the Middle East, mainly in relation to immigration and emigration dynamics [7].

Even today, mate consumption is limited to the regions where it is produced, primarily due to its traditional consumption, which is related to the customs, culture, and identity of a few regions of South America and involves the use of traditional tools.

The plant is well-known for its antioxidant, antimicrobial, digestive, nervous system stimulating, and diuretic properties [8,9]. Some studies also cite possible anti-inflammatory effects, vasodilating, anti-obesity, anti-diabetic, and anti-cancer properties [8,10–12]. These health-promoting characteristics may be drivers to expand the consumption of *yerba mate* and, consequently, its market [13].

Dried *yerba mate* leaves contain around 200 compounds, including minerals, xanthines, flavonoids, anthocyanidins and procyanidins, phenolic acids, rutins and saponins, and high levels of antioxidants [14].

All the cited properties make this species potentially useful as a raw material for food preservatives, food supplements, colourants, hygiene products, and cosmetic products [15].

Literature reports that the phenolic composition of *yerba mate* is comprehensive of dimeric caffeic acid (1.73 mg/g d.m., 1.68 mg/g d.m., 1.65 mg/g d.m. corresponding to different commercial brands), caffeoylquinic acids (43.38 mg/g d.m., 44.70 mg/g d.m., 47.57 mg/g d.m.), feruloylquinic acids (0.15 mg/g d.m., 0.48 mg/g d.m., 0.53 mg/g d.m.), di- and tricaffeoylquinic acids (30.65 mg/g d.m., 33.82 mg/g d.m., 30.92 mg/g d.m.) and flavonoids (4.73 mg/g d.m., 4.85 mg/g d.m., 5.01 mg/g d.m.) [16–18].

In addition, mate contains significant amounts of caffeine [14,19]. The study by Bastos et al. [20] determined the caffeine and 5-caffeoylquinic acid (5-cqa) content in *chimarrão*, *tereré*, and tea mate. The high levels of alkaloids in *I. paraguariensis* leaves justify their use in energy drinks or as an alternative to coffee, which is more widely known and consumed in Europe and the USA [21]. The amount of compounds depends on many conditions, such as cultivars and growing soil [22].

In the context of product innovation towards market expansion, the development of a new food product based on *I. paraguariensis*, green mate tea, is interesting. This novelty product would be sold in teabags or loose, entering the market as a substitute for classic *Camellia sinensis* tea, overcoming the barriers, outside the traditional context, associated with the conventional consumption of *yerba mate*. As green tea, only the sprouts of the *I. paraguariensis* plant are used to develop the green tea mate product. Indeed, plant shoots are recognised as richer in bioactive compounds than mature leaves [23]. The high antioxidant capacity of sprouts can be exploited effectively in various pharmaceutical and nutritional fields. For example, it has been reported the possible use as a food supplement in antioxidant capsules [24].

Resuming, traditional mate drink consumption is strictly connected to the use of specific instruments (*cuia*, the cup, made out of a dried gourd or wood, and *bombilla*, the straw used to drink, which has a filtering bottom ending, avoiding the passage of mate leaves), rituals, and customs, usually related to social and convivial moments. Outside of mate-native countries, people may not be comfortable with the typical preparation; thus,

green tea mate, made from *I. paraguariensis* sprouts and consumed as classic tea, could certainly be more practical. This work aims to characterise the innovative green tea mate product compared to the traditional Brazilian one and a commercial roasted mate sample. To understand the product potential differences and similarities with other infuse and tea products, colour analysis was carried out on both the dried sample and the infusion. Also, the chlorophyll content was spectrophotometrically measured to complete the physico-chemical characterisation of the products. In addition to this, a consumer survey was built to understand the visual perception of the product from both mate consumers' and non-consumers' points of view during purchase and consumption. Finally, a preliminary sensory evaluation was carried out to complete the description and produced a summarised vocabulary for the new *yerba mate* tea, being conscious of the limitations deriving from the non-standardised method but aiming to introduce the innovative green tea mate to consumers.

2. Materials and Methods

2.1. Sample Preparation

The study compared six samples of *I. paraguariensis*: two commercial mate samples, one from Italy (Barão, Barão de Cotegipe, Rio Grande do Sul, Brazil; CI) bought in a shop selling imported products and one from Brazil (Ximango, Ilópolis, Rio Grande do Sul, Brazil) (CB), a commercial roasted mate (Leão alimentos e bebidas, Fazenda Rio Grande, Paraná, Brazil) from Brazil (L), and three batches of innovative green tea mate (L1, L2, and L3), obtained from the plant shoots and directly collected in Brazil from the producer company (Mon Jullì, Ilópolis, Rio Grande do Sul, Brazil). L1, L2, and L3 were processed in the same way but collected at different times. CB and CI were processed according to the traditional Brazilian technique (Figure 1a) [25] and intended for traditional consumption using *cuia* and *bombilla*. For the drink preparation, the *cuia* should be filled with the dry product up to three quarters, then covered, turned upside down, and shaken to create a layer on top of the *cuia*. Using a spoon, a hole is created, and hot water is poured inside. At the end, the mate is drunk with the *bombilla*. The production process for roasted mate followed the same preparation as for traditional mate, but a further high temperature treatment is present (roasting) (Figure 1b) [26]. Finally, the innovative mate was managed as for green tea (Figure 1c) [27]. The preparation of the last two infusions follows the typical tea drink preparation: a few grammes (3–5 g) of dried product are added with a cup of water; after 3–5 min, the drink is ready. It is important to say that these are guidelines that are commonly described on the packaging at purchase points; however, the concentration and the infusion time may change according to personal taste. All the packed samples were stored at $-20\text{ }^{\circ}\text{C}$ to avoid possible modifications. The sampled mate was previously grounded to obtain a powder. The powder was collected in 50 mL tubes (VWR International, Milan, Italy), accurately closed with Parafilm[®], and stored at $-20\text{ }^{\circ}\text{C}$ until use for the analyses. Exceptionally, the colour analysis was performed on the not-shredded product. All the analyses were performed in triplicate.

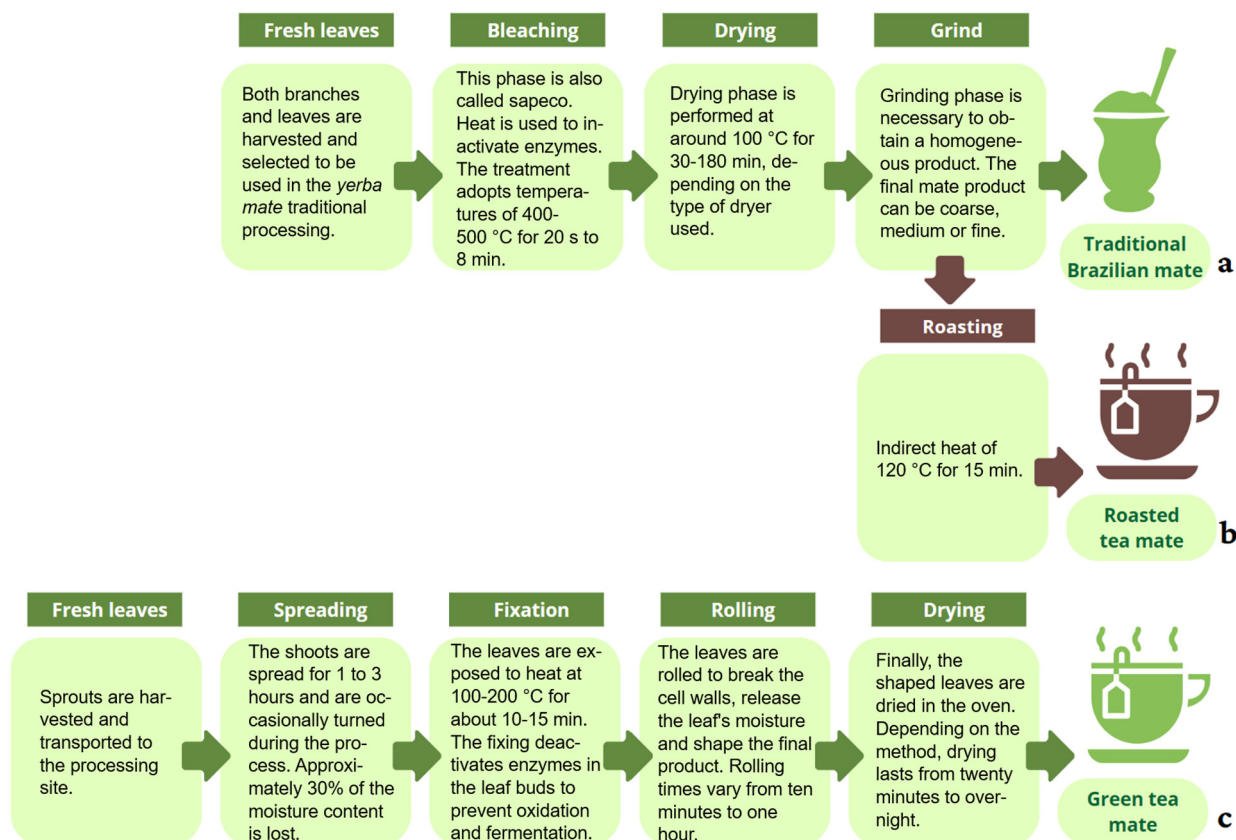


Figure 1. Traditional Brazilian mate (a), roasted tea mate (b), and green tea mate (c) production processes.

2.2. Physicochemical Measurements

2.2.1. Colour Analysis of the Dried Product

The colour was measured through a CR-400 colourimeter (Konica Minolta, Chiyoda, Japan) on the intact mate samples. An amount of product was placed on a plastic plate, creating a layer about 1 cm thick. Measurements were carried out by positioning the instrument directly on the samples. Results were expressed concerning the CIELab tristimulus coordinate system. The coordinates obtained are L^* (lightness), ranging from 0 to 100; a^* (redness), ranging from -128 (green) to $+127$ (red), b^* (yellowness), ranging from -128 (blue) to $+127$ (yellow). Data were further processed, and $^{\circ}h$ (hue angle) and C (chroma) were obtained according to the following formulas [28]:

$$^{\circ}h = \tan^{-1} (b^*/a^*) \text{ when } a^* > 0, b^* > 0, \quad (1)$$

$$^{\circ}h = 180 + \tan^{-1} (b^*/a^*) \text{ when } a^* < 0, b^* > 0 \quad (2)$$

$$C = (a^2 + b^2)^{1/2} \quad (3)$$

2.2.2. Colour Analysis of the Drink

The colour of the drink obtained from the dried samples was again measured with the colourimeter. The mate drink was prepared by adding 1.2 g of *I. paraguariensis* to 100 mL of water heated to 80 °C. The infusion was set for 5 min. The preparation method used was previously tested to guarantee the obtainment of a suitable drink. The infusion proportion was also the one used to prepare green tea mate for consumption, similar to the typical *C. sinensis* tea preparation standard [29]. After the infusion time, the hot-mate beverage was filtered and poured into a small glass bottle. The colour was measured on the bottom of the bottle, opposite of the white bottle cap. The considered parameters were the same as for the dried sample.

2.2.3. Chlorophyll Content Analysis

Total chlorophyll was measured following the protocol adapted from Lichtenthaler (1987) [30]. One gramme of powdered sample was added to 5 mL of acetone (>99.5%) (VWR Chemicals, Milan, Italy) and incubated for 24 h under refrigeration (2 °C). After the incubation time, samples were centrifuged at 3000 rpm for 5 min. The supernatant was collected and filtered with a 0.45 µm syringe filter (VWR International, Milan, Italy). A preliminary dilution (1:3) with acetone was performed, and then absorbance was recorded for each sample at 645 nm and 662 nm with an 1800 UV/VIS spectrophotometer (Shimadzu, Japan). The chlorophyll content calculation was conducted as follows:

$$\text{Chlorophyll a + b (ppm)} = 7.05 \times (A_{662} \times d) + 18.09 \times (A_{645} \times d), \quad (4)$$

where *d* is the dilution factor, *A*₆₄₅ stands for the absorbance measured at 645 nm, *A*₆₆₂ stands for the absorbance measured at 662 nm, and 7.05 and 18.09 are the specific absorption coefficients retrieved from the protocol [30]. Further calculations were performed to express the final result as mg/100 g of the dry sample.

2.3. Nutraceutical Analyses

Preliminary aqueous extracts were prepared because water is normally at the base of plant-based drinks. 10 mg of powdered plant material was added to 2 mL of MilliQ[®] water (concentration: 5 mg/L) from Sartorius Stedim Biotech (Arium, Gottingen, Germany). The material was mixed with vortex Genie 2[®] (Scientific Industries, New York, NY, USA) for 30 s, and the extraction was carried out in a temperature-controlled bath at 60 °C. Every 10 min, the samples were vortexed for 10 s and then replaced in the bath. The total extraction time was 60 min. The extracts were then filtered with a 0.45 µm syringe filter and stored at 4 °C and 95% relative humidity until analysis.

2.3.1. Total Phenolic Content (TPC)

Total phenolic content (TPC) was determined according to the method described by Pantelidis et al. [31] with modifications, using the Folin-Ciocalteu reagent (Sigma-Aldrich, Darmstadt, Germany). 40 µL of the mate extract previously prepared was diluted with MilliQ[®] water (1:4 *v/v*). To the mixture, 1 mL of Folin-Ciocalteu reagent water solution (1:9 *v/v*) and 800 µL of 7.5% Na₂CO₃ water solution were added. Samples were left in the dark for 30 min, then absorbance was measured at 760 nm with a spectrophotometer. Results were expressed in mg gallic acid equivalent (GAE) per gramme of sample (mg GAE/g) on a dry sample basis.

2.3.2. Antioxidant Capacity

Antioxidant capacity was assessed via the FRAP (Ferric Reducing Antioxidant Power) test method following the Benzie and Strain (1996) protocol [32], subsequently modified by Pellegrini et al. [33]. In brief, 900 µL FRAP reagent (0.3 M pH 3.6 acetate buffer, 10 mM 2,4,6-tripiridil-s-triazin in 40 mM HCl, FeCl₃ × 6 H₂O with ratio 10:1:1 *v/v/v*; incubated for 15 min at 37 °C water bath prior use) was added to 90 µL H₂O. Next were added 30 µL of MilliQ[®] water-diluted sample (1:2 *v/v*). Samples were incubated for 30 min at 37 °C in a water bath. Absorbance was measured at 595 nm. Results were expressed as mmol of ferrous ion per kg of sample (mmol Fe²⁺/kg) on a dry sample basis.

2.4. Chromatographic Analysis of Caffeine and Theobromine

Chromatographic analysis of caffeine and theobromine was performed by an Agilent (Agilent Technologies, Santa Clara, CA, USA) 1200 High-Performance Liquid Chromatograph (HPLC) coupled to a diode array detector (DAD). Caffeine and theobromine were identified and quantified on a Kinetex C18 column (4.6 × 150 mm, 5 mm, Phenomenex, Torrance, CA, USA). The mobile phase was composed of H₂O:acetic acid (99.9:0.1 *v/v*) solution and acetonitrile (100%), with a flow rate of 0.6 mL/min. The gradient elution (followed by 3 min of conditioning

time) was programmed. The detailed programme for the gradient elution was reported in Figure S1 in the Supplementary Material. UV-vis spectra were recorded at 280 nm.

Caffeine and theobromine were identified in samples (same extracts of nutraceutical analyses) by comparison and combination of their retention time and UV spectrum with an authentic external standard (concentration range for caffeine: 62.5–1000 ppm; concentration range for theobromine: 15.6–3.9 ppm) analysed under the same chromatographic conditions. The calibration curves for quantification were defined as follows: (i) caffeine: $y = 87.011x + 2197.6$ ($R^2 = 0.9988$) with 3 injections for each calibration point (five points in total); (ii) theobromine: $y = 576.29x + 260.52$ ($R^2 = 0.9919$) with 3 injections for each calibration point (3 points in total).

The mass correction factor used to express results from chemical analyses on the dry matter of the product was calculated by relating the weight of the sample after 48 h in the oven at 105 °C to the mate initial weight.

2.5. Consumer Survey

An online survey was created about knowledge and use of mate to evaluate possible relations between consumer choices and the visual features of the product. It was developed in the original language of the country, Italian, and distributed as a link through WhatsApp and email. Moreover, it was advertised as a QRcode in the Department of Agricultural, Forest, and Food Sciences (DISAFA) of the University of Turin, Grugliasco, Italy. The data were collected anonymously from volunteers (age > 17 years old) previously informed about the purpose of the study. The respondent filled out a consent form before starting. The targeted audience was composed of people living (resident or domiciled) in Piedmont when completing the form. The participants were recruited from an online panel and were not paid for completion. The survey remained available from 1 December 2023 to 1 January 2024. During this period, 453 answers were collected and analysed. 262 (57.8%) were females, 184 (40.6%) were males, and 7 (1.6%) indicated 'Other' as gender, with a mean age of 37.2 years old. More specifically, 50.99% of the interviewees had an age < 30 years old, 22.96% were between 31 and 50 years old, and 26.05% had an age > 50 years old. People answering the survey came from diverse backgrounds and had different lifestyles. This contributed to the collection of data, returning general and complete information.

The questionnaire was structured into four sections: (i) individual socio-demographic characteristics; (ii) knowledge of mate; (iii) mate consumption; and (iv) product liking. The product liking section asked respondents to select attributes and express preferences, showing two pictures: one displaying the Brazilian commercial product (sample "1") and the second presenting the green tea mate (sample "2") (Figure 2). People did not know what the pictures were corresponding to, as the ultimate aim was to evaluate the visual perception of yerba mate; additional information could have influenced the answers.



Figure 2. Picture from the consumer survey. Sample "1" (left) is the Brazilian commercial mate; sample "2" (right) is the innovative green tea mate.

2.6. Preliminary Internal Sensory Evaluation

The green tea mate product was subjected to a preliminary sensory analysis. The aim was to create a new vocabulary for the innovative tea mate, starting from previous elaborations, one relative to mate sensory analysis [34] and one relative to green tea [35]. This methodology does not follow specific sensory test regulations or a recognised methodology; thus, the results should be interpreted as preliminary qualitative feedback to describe the new product. The methodology used does not follow the required number of people to carry out a consumer test and, at the same time, follows an approach normally used for trained panels undergoing descriptive analysis. This fact is a limitation to the interpretation of the results; thus, we need to consider it as a tentative approach to better understand consumers' perceptions of the samples. The sensory analysis carried out for the green tea mate product involved 41 untrained panellists—20 men and 21 women consumers of infuse and tea products—who were tasting the green tea mate for the first time. On average, people were 34.0 years old (30.0–38.0 95% confidence interval) and, in particular, 23 were aged between 18 and 30, 12 were between 31 and 50, and 6 were over 50. In particular, the panel was asked to quantify the perception of 20 attributes retrieved from the references: fermented, bitter, astringent, sweet, acid, green/herbaceous, green herb-life (e.g., basil, laurel), hay, caramel/burnt, toasted, smokey, brown spices (e.g., cinnamon, nutmeg), nutty, tobacco, citrus, floral, fruity, fresh mint, chemical/artificial/medicinal, paper. Each attribute was coupled to an evaluation scale (1 = absent; 2 = slightly present; 3 = quite present; 4 = highly present; 5 = extremely present); all the values were presented, people should mark the number corresponding to the sensation perceived. Green tea mate was served at 80 °C, prepared as an infusion with 12 g of product in 1 L of water, the same proportion used in the preparation of the drink samples to evaluate colour. This protocol is not officially recognised but is the one suggested by the producing company “Mon Julli”, the supplier of green tea mate samples. The session was carried out by untrained panellists (tea and infusion consumers) from the DISAFA and Department of Neurosciences in Grugliasco, Italy.

2.7. Statistical Analyses

As concerns experimental data, mean values \pm standard deviation are reported in figures and tables. A one-way ANOVA was used to detect significant statistical differences ($p < 0.05$), followed by Tukey's post-hoc test. The conditional tests for the analysis were previously verified.

A Principal Component Analysis (PCA) was carried out to evaluate the set of nutraceutical, phytochemical, and physico-chemical data and identify the most discriminant parameters for describing the data structure. PCA allowed for detecting the relationships among all the samples (CI, CB, L1, L2, L3, L) depending on the different production processes by a correlation matrix (Varimax rotation), which included 18 samples (6 samples with 3 analytical repetitions) and 11 variables (TPC, antioxidant capacity, the three CIELab parameters for drink and dried product, and the content of chlorophyll, caffeine, and theobromine). The Bartlett's test of sphericity (BTS) and Kaiser-Meyer-Olkin index (KMO) were performed on the matrix. It was centred column-wise and then scaled, and the relative values were transformed into Z-scores. The original variables were recombined based on their level of correlation into principal components corresponding to an eigenvalue greater than or equal to 1 and capable of explaining at least 70% of the total variance. The original variables were correlated with the PCs by the evaluation of the loadings of each considered parameter in the plane PCs.

Regarding the consumer survey, descriptive statistics were conducted to describe the sample. All the results are reported as frequencies and percentages for categorical variables and as the mean and standard deviation for continuous ones. Comparisons between sexes, colours, and purchasing preferences were performed using a chi-squared test for categorical variables and a *t*-test for continuous ones and shown with their *p*-values. The agreement between the preferred and the purchased image was determined by Cohen's kappa [36]. A

sub-analysis was performed on the mate-consumers. Principal Component Analysis (PCA) on the Likert values was carried out using PROC FACTOR of SAS[®] Statistics Software v.9.4. Moreover, a radar graph was built based on the scores of the questionnaire, assessing the overall liking profile.

An alpha error of 0.05 was considered. All the statistics were performed by SPSS software v.28 by IBM[®] (analysis on experimental data) and SAS[®] Statistics Software v.9.4 (survey data).

3. Results and Discussion

3.1. Physicochemical Measurements

3.1.1. Colour Analysis of the Dried Product

The colour of the dry mate sample is an important parameter to be considered for consumer product acceptance [37]. Results are reported in Table 1. The sample showing a higher brightness was the traditional mate sample purchased in Brazil (CB). In general, the traditional commercial mate samples (CB, CI) reported a higher brightness value than the green tea mate samples (L1, L2, L3), which are grouped in the same statistical class ($p > 0.05$). Molin et al. [38] reported, in agreement with our findings, that, after processing, the younger leaves appear darker than the mature ones. Moreover, the green tea treatment involves two different high-temperature steps that are demonstrated to affect the colour of the final product [38]. This could also be easily verified visually. The roasted tea mate (L) presented a dark brownish colour, due to the Maillard reaction and enzymatic browning processes due to the oven-drying phase [39]. This visual assessment is also proved by the °h value, which is the lowest among the samples (1.23) ($p < 0.05$). Analogously, the brighter colour of traditional Brazilian mate samples may be related to their manufacture. In addition to the biological nature of the samples, the different geographical locations, cultivation, genetic origin, and variability that this implies [22,40,41]. Another difference between the CB and CI samples is the place of purchase of the two samples. CB was purchased directly from the producer in Brazil, while CI was purchased at an import shop in Milan and produced. The CI sample is thus characterised by much more complex supply chain logistics and significantly longer times between the time of production and final consumption.

Table 1. Colour parameters of the dried product and drink of the mate samples.

	Sample	L*	a*	b*	°h	C
Dried product	CI ¹	34.51 ± 1.85 ^β	−8.00 ± 0.17 ^γ	31.99 ± 0.54 ^α	178.67 ± 0.004 ^β	32.96 ± 0.56 ^β
	CB ²	38.78 ± 2.19 ^α	−11.68 ± 0.35 ^δ	34.07 ± 0.62 ^α	178.76 ± 0.01 ^β	36.02 ± 0.68 ^α
	L1 ³	24.27 ± 2.42 ^γ	−0.49 ± 0.42 ^β	14.50 ± 2.45 ^β	178.78 ± 0.98 ^β	14.51 ± 2.46 ^{γδ}
	L2 ⁴	22.27 ± 1.82 ^γ	−0.02 ± 0.27 ^β	11.97 ± 2.27 ^β	180.32 ± 1.60 ^α	11.97 ± 2.27 ^γ
	L3 ⁵	23.93 ± 2.73 ^γ	−0.34 ± 0.40 ^β	15.34 ± 2.70 ^β	179.39 ± 1.50 ^{αβ}	15.35 ± 2.70 ^γ
	L ⁶	24.64 ± 2.92 ^γ	5.27 ± 0.54 ^α	14.99 ± 1.74 ^β	1.23 ± 0.02 ^γ	15.89 ± 1.79 ^γ
Drink	CI ¹	36.14 ± 3.27 ^α	−4.94 ± 1.26 ^β	25.62 ± 3.03 ^α	178.62 ± 0.03 ^α	26.10 ± 3.20 ^α
	CB ²	34.18 ± 2.38 ^α	−4.48 ± 0.99 ^β	22.75 ± 1.01 ^α	178.62 ± 0.05 ^α	23.20 ± 0.81 ^α
	L1 ³	34.29 ± 0.83 ^α	−5.57 ± 2.05 ^β	22.03 ± 2.00 ^α	178.67 ± 0.07 ^α	22.76 ± 2.43 ^α
	L2 ⁴	34.83 ± 1.04 ^α	−4.46 ± 0.93 ^β	24.91 ± 1.08 ^α	178.59 ± 0.04 ^α	25.27 ± 1.09 ^α
	L3 ⁵	34.49 ± 3.07 ^α	−3.39 ± 2.21 ^β	21.99 ± 3.90 ^α	178.60 ± 0.06 ^α	22.36 ± 4.25 ^α
	L ⁶	13.18 ± 0.96 ^β	0.62 ± 0.01 ^α	3.22 ± 0.07 ^β	1.38 ± 0.00 ^β	3.28 ± 0.07 ^β

¹ Commercial mate from Italy; ² Commercial mate from Brazil; ³ Green tea mate (batch 1); ⁴ Green tea mate (batch 2); ⁵ Green tea mate (batch 3); ⁶ Commercial roasted mate. Lightness (L*), redness index (a*), yellowness index (b*), hue angle (°h), and chroma (C). Data are reported as mean value ± standard deviation. Statistical analysis was carried out separately for dried products and drinks for each parameter. Statistically significant differences are marked with different Greek letters ($p < 0.05$).

Concerning the values of a*, CB and CI tend more towards green, with values of −11.68 and −8.00, respectively. Again, samples L1, L2, and L3 are grouped in the same statistical class ($p > 0.05$), with values slightly negative, nevertheless tending towards green.

Considering the yellowness index (b^*), all samples tend towards yellow compared to blue. CB and CI are grouped in the same statistical class ($p > 0.05$) and have the highest values of 34.07 and 31.99, respectively, while the green tea mate batches are statistically comparable ($p > 0.05$), showing values of 14.50 (L1), 11.97 (L2), and 15.34 (L3).

3.1.2. Colour Analysis of the Drink

In the evaluation of the drink colour, only the roasted mate (L) differed significantly from the other samples ($p < 0.05$). As the colour of the dried sample is almost brown due to the production technique, the herbal tea colour is darker (L^* value 13.18), nearer to red and blue rather than green or yellow (a^* value 0.62 and b^* value 3.22). These data lead to lower $^{\circ}h$ (1.38) and C (3.28) values ($p < 0.05$).

Observing the infusions obtained, the one from the traditional samples (CI and CB) was less clear than that obtained from L1, L2, and L3, probably as a function of the powdery consistency of the dry product. As the same plant is in the matrix, similarities in the colour should be justified. Indeed, chlorophyll is not a water-soluble pigment, so changes in its concentration may not be related to the colour of the drink [42].

3.1.3. Chlorophyll Content Analysis

The pigment giving the green colour to the leaves of *yerba mate* is chlorophyll. The chlorophylls of higher plants, and so of *yerba mate*, consist of chlorophyll a as the main pigment and chlorophyll b as an accessory pigment. Both chlorophylls are fundamental components of photosynthetic membranes [30].

Commercial samples for traditional Brazilian mate contain the highest amount of pigment, reaching 1.38 mg/g dry product (CB) and 1.21 mg/g dry product (CI) ($p < 0.05$) (Figure 3). Lower values were obtained from the quantification of the green tea mate (L1 0.25 mg/g dry product, L2 0.39 mg/g dry product, and L3 0.38 mg/g dry product) and the roasted mate sample (0.16 mg/g dry product) ($p < 0.05$) (Figure 3). These findings can be associated with the outcome of the colorimetric analysis, which already pointed out a completely different hue among the teas, as the production process involves a roasting phase with an effect of browning because of the caramelisation of the leaf sugars [43]. It may be considered that chlorophyll content was reported to decrease over time [44], thus justifying the statistical differences between CI and CB, but also between L1 and the other two green mate samples ($p < 0.05$). In the first case, the logistics involved long times that could affect the product preservation; in the second case, as a still rudimental manufacturing process in a pilot set-up was employed, the dried mate tea from L1 could be left in air condition for a longer time with respect to batches L2 and L3.

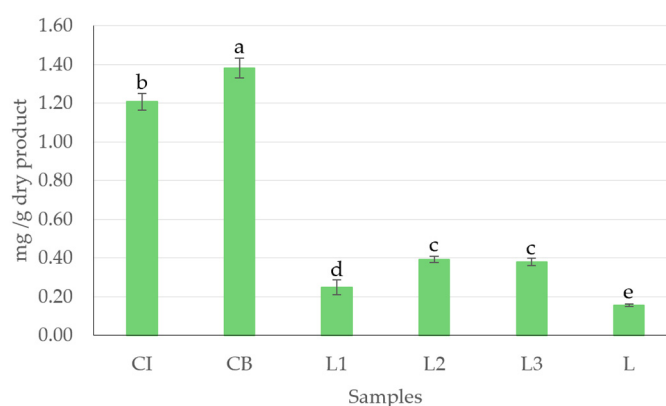


Figure 3. Chlorophyll a + b content of mate samples. Data are reported as mean value \pm standard deviation. Statistically significant differences are marked with different letters ($p < 0.05$).

3.2. Nutraceutical Analyses: TPC and Antioxidant Capacity

Polyphenols have been reported to provide body tissues with protection from oxidative stress that causes ageing, cancer, cardiovascular disease, and inflammation [45,46].

Compared to green tea from *C. sinensis* (148.77 mg gallic acid equivalent (GAE)/g dry leaves), the TPC of mate is lower (94.91 mg GAE/g dry leaves), but the composition in terms of polyphenol characterisation is different [47]. Nevertheless, the overall amount of phenolics is comparable between the two species [16].

The samples can be distinguished into three distinct statistical classes. The green tea mate batches (L1, L2, and L3) do not show significant differences from each other ($p > 0.05$), as they all have the highest TPC compared to the other samples ($p < 0.05$). The CI has a statistically higher phenolic content than L ($p < 0.05$), while CB does not differ in a statistically significant way from both the CI and L ($p > 0.05$). More precisely, results show that the green tea mate samples (L1, L2, and L3) have a higher phenolic content of 117.14 mg GAE/g dry product, 128.10 mg GAE/g dry product, and 126.21 mg GAE/g dry product, respectively ($p < 0.05$) (Figure 4A). The TPC in the CI and CB samples is, instead, respectively, 71.91 mg GAE/g dry product and 54.23 mg GAE/g dry product (Figure 4A). The roasted mate (L) sample has the lowest polyphenol content, with a value of 34.16 mg GAE/g dry product ($p < 0.05$) (Figure 4A). This result is in line with the findings of Kaezer et al. [26], due to the additional roasting process (160 °C for approximately 12 min), which may alter its composition [16].

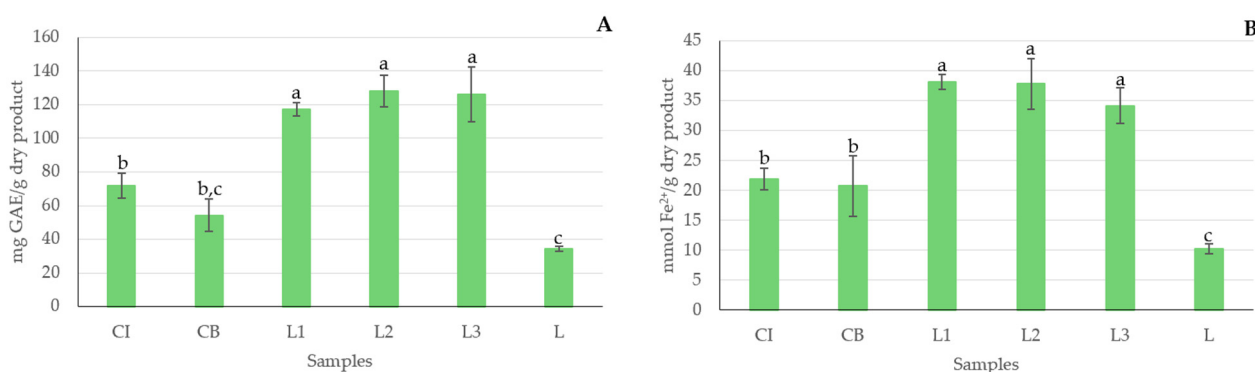


Figure 4. Total phenolic content (TPC) (A) and antioxidant capacity (B) of mate samples. Data are reported as mean value \pm standard deviation. Statistically significant differences are marked with different letters ($p < 0.05$).

Samples with high TPC also present a high antioxidant content measured through the FRAP assay. Indeed, L1, L2, and L3 measured, respectively, 38.11 mmolFe²⁺/g dry product, 37.83 mmolFe²⁺/g dry product, and 34.18 mmolFe²⁺/g dry product, statistically higher than CI (21.93 mmolFe²⁺/g dry product) and CB (20.75 mmolFe²⁺/g dry product) ($p < 0.05$), as almost exclusively composed of *yerba mate* sprouts (Figure 4B) [48]. The roasted mate (L) shows a low level of antioxidants, barely achieving 10.29 mmolFe²⁺/g dry product. This may be due to the degradation process of antioxidants caused by temperature [49].

The variability observed among the various samples of the study can be justified considering that, in addition to being biological matrix samples, they are produced in different regions with different agricultural practices, genetics, climate, soil, harvest time, plant and leaf age, and post-harvest processes [50]. The present study does not aim to define the single influences of each parameter; however, further study might investigate how these affect the characteristics of the final product. Moreover, the degree of grinding can influence the concentration of extracted polyphenols [51]. Lorini et al. [52] show that mate leaves contain a higher concentration of bioactive compounds, including polyphenols, than branches, a result expected since leaves and sprouts are composed of photosynthetic cells with high metabolic activity with a synthesis and accumulation function [53].

Mate samples with high polyphenolic content (L1, L2, and L3) were also found to be the lowest in chlorophyll ($p < 0.05$) [54]. Despite the similarities between the two plants, the relationship may also exist for *yerba mate*. CI, CB, and L are made of both mature leaves and branches, the whole *I. paraguayensis* plant, while L1, L2, and L3 only derive from the plant sprouts. Thus, differences may be attributed to both processing and composition.

3.3. Chromatographic Analysis of Caffeine and Theobromine

Caffeine (1,3,7-trimethylxanthine), together with theobromine (3,7-dimethylxanthine) and theophylline (1,3-dimethylxanthine), is the main purine alkaloid found in many plants from at least 100 species [16,55,56]. These compounds have biological relevance as the main components of nucleoproteins, which compose most of the cell nucleus. Among the purine alkaloids, caffeine is the most abundant in *yerba mate*, while the presence of theophylline in this matrix is still a controversial issue because it is not always detected [57,58].

The caffeine content in the different samples shows some variability depending on the type of processing (Table 2). The traditional mate samples CB and CI show a concentration of 1.57 and 5.13 mg/g dry product, respectively, the lowest values reported in the study ($p < 0.05$). These values are statistically comparable ($p > 0.05$) to the L sample, with 4.46 mg/g dry product of caffeine. On the other hand, green tea mate samples show a significantly higher caffeine content than the traditionally processed ones ($p < 0.05$). L1, L2, and L3 samples reported values of 21.72, 10.53, and 20.63 mg/g, respectively. The difference among the three green tea mate samples, in particular about the caffeine content shown by sample L2 ($p < 0.05$), may derive from climatic and genetic contributions and the fact that the quantity may vary depending on different factors, such as cultivar, growing soil [22,40], and different genotypes [41]. Moreover, the harvesting period could also influence the product traits. For example, a study by Schubert et al. [57] reported a reduced methylxanthine concentration in mate harvested in autumn and winter in relation to materials collected in spring and summer.

Table 2. Caffeine and theobromine content of the mate samples.

Sample	Caffeine (mg/g Dry Product)	Theobromine (mg/g Dry Product)
CI ¹	5.13 ± 1.64 ^c	2.16 ± 0.33 ^b
CB ²	1.57 ± 1.24 ^d	1.53 ± 0.33 ^c
L1 ³	21.72 ± 3.68 ^a	1.51 ± 0.15 ^c
L2 ⁴	10.53 ± 0.48 ^b	1.91 ± 0.14 ^b
L3 ⁵	20.63 ± 3.58 ^a	2.74 ± 0.40 ^a
L ⁶	4.46 ± 1.78 ^{cd}	0.41 ± 0.06 ^d

¹ Commercial mate from Italy; ² Commercial mate from Brazil; ³ Green tea mate (batch 1); ⁴ Green tea mate (batch 2); ⁵ Green tea mate (batch 3); ⁶ Commercial roasted mate. Data are reported as mean value ± standard deviation. Statistical analysis was carried out separately for each parameter. Statistically significant differences are marked with different letters ($p < 0.05$).

The same influences, including cultivar differences, may contribute to the theobromine content [41]. (Table 2). The statistical analysis identified four different classes. The caffeine content in L1, L2, and L3 (21.72 mg/g dry product, 10.53 mg/g dry product, and 20.63 mg/g dry product, respectively) is statistically higher compared to both commercial samples and roasted mate ($p < 0.05$). L (0.41 mg/g dry product) is not statistically diverse from CI and CB ($p > 0.05$). In this case, the processing may have an effect, as has been experienced with other mate-derived beers and soft drinks [59].

The higher content of caffeine with respect to theobromine is validated by literature studies [60,61].

All the analytical results (L*, a*, and b* colour parameters of dried mate and drink, chlorophyll content, TPC, antioxidant capacity, caffeine, and theobromine content) were evaluated through a multivariate Principal Component Analysis (PCA) to observe if products would be clustered according to their commercial indication (Figure 5).

The KMO index showed a level of 0.68. Bartlett's test of sphericity ($p < 0.05$) showed significant statistical collinearity among variables. The variance among samples could be well described with the first two components. The two PCs accounted for 91.1% of the total variance (47.1% explained by PC1 and 44.0% by PC2). The loading plot (Figure 5B) shows that the first component, reported on the x-axis, is mainly defined by the contributions of bioactive molecules (e.g., theobromine), a* (both dried product and drink), and L* and b*

of the drink. The second component, on the y-axis, distinguishes the products according to other bioactive components (e.g., caffeine, polyphenolic compounds), antioxidant capacity, and the L^* and b^* parameters of the dried mate. PCA confirmed the correlation between TPC and antioxidant capacity, as already reported in other studies [62,63]. Regarding the CIELab parameters, PCA showed that L^* and b^* were strongly correlated (both for drink and dried product), while a^* was separated from them [64].

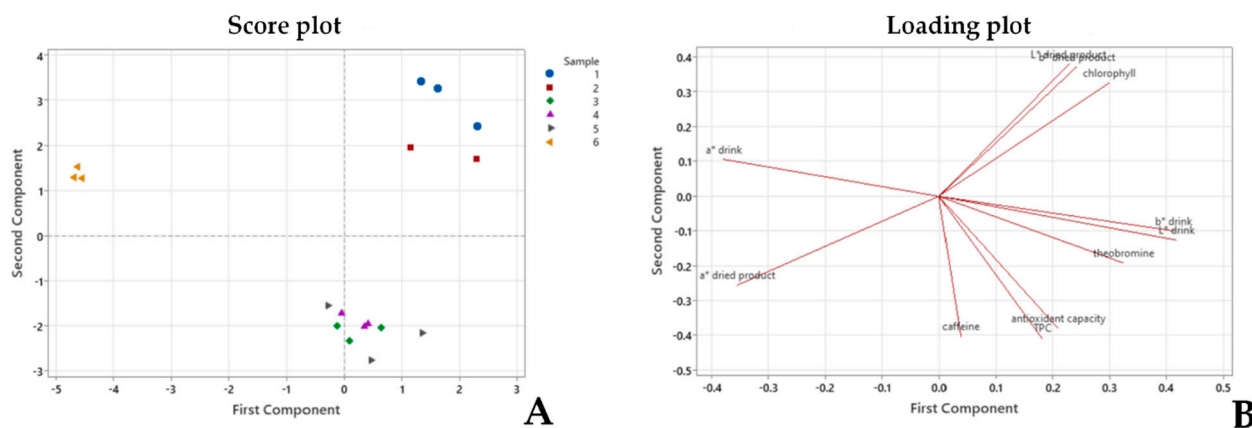


Figure 5. Score plot (A) and loading plot (B) resulting from the Principal Component Analysis (PCA) clustering of the mate samples. 1 = CB; 2 = CI; 3 = L1; 4 = L2; 5 = L3; 6 = L. In the loading plot, in the top right part, “ L^* dried product” and “ b^* dried product” are overlapping.

This study showed that the nutraceutical, phytochemical, and physico-chemical selected parameters well distinguished the different samples. These variables may be useful traits to be applied as traceability markers. Indeed, the resulting score plot (Figure 5A) clearly defines three different clusters. CI and CB are grouped together (traditional Brazilian mate) and separated in a bilaterally balanced manner along the first and second components; the green tea mate samples are clustered mainly along the second component; and the L sample (roasted mate) is completely dislodged from the other mate types, presenting only similarity in the drink colour. Caffeine, TPC, and antioxidant capacity well characterised the “L” group, while commercial mate samples were described by a high content of chlorophyll.

3.4. Consumer Survey

The importance of the visual aspect of a product lies in the fact that during the purchase, the sense most involved is sight, on the basis of which the choice is made [65]. Thus, the visual influence of the product characteristics on the consumer has been investigated.

All the descriptive statistics deriving from the 453 answers received to the survey are reported in Table S1.

It can be observed that 305 people (67.8%) know *yerba mate*, but only 88 (19.5%) consume it. This result suggests that the limited geographical distribution influenced the outcome. Indeed, people had almost theoretical knowledge about mate; they learned about it, but the consumption is still linked to the traditional culture.

Interestingly, 97 people (21.5%) are curious about mate and want to try it. They are probably keen to know the novelty in taste compared to other tea-like beverages [66]. Considering only people who already consume mate, the majority (71, 82.5%) prefer it without other spices.

The majority of respondents (197, 70.6%) know a mate from friends or relatives, followed by travel (40, 14.3%), commercials, advertisements, or exhibitions (28, 10%), because they saw it on social media (7, 2.5%) or used by celebrities (7, 2.5%).

Respondents looked at mate as a product with antioxidant (56.5%) and energising (58.5%) properties. People do not think it is rich in vitamins and minerals (37.3%), diuretic (49.2%), relaxing (27.1%), digestive (38.6%), satiating (20%), and sleep regulator (16.3%).

Anyway, literature findings underline that drinking mate may help, not only by supplying antioxidants and energy but also by enriching the organism with vitamins and minerals, leading to diuretic, digestive, and satiating effects [67]. These results may be the starting point for an information campaign making the consumer aware of the real possible effects derived from mate consumption, as it is rather unknown on the Italian market [68].

As concerns preferences, there is not a preferred colour. 217 (48.1%) prefer the traditional Brazilian mate, sample “1” (Figure 2), while 234 (51.9%) prefer the green tea mate, sample “2” (Figure 2). There are no differences between sexes ($p > 0.05$), while there is a significant difference ($p < 0.05$) between non-consumers (50.7% prefer “1”, 49.3% prefer “2”) and consumers (37.5% prefer “1”, 62.5% prefer “2”). It is realistic to consider this significant difference as an acknowledgement of the different mate-derived products available on the market. The traditional Brazilian mate considered in this research study is different from the Argentinian one, which is darker, has a different shape, and has a distinctive aroma, more similar to sample “2” displayed in Figure 2. Whereas a similar aspect is identified, different processing is involved to obtain the final product. Anyway, this result obtained from the questionnaire clearly defines the fact that, limited to the visual consideration of the product, consumers will compare the innovative green tea mate with the Argentinian one. In addition to this, the geographical zone of distribution of the survey had an impact, as in Italian supermarkets and hypermarkets, the Argentinian *yerba mate* can be found, while the Brazilian one is not easily retrievable, thus probably less known. It is interesting to note that a change between colour and purchasing preferences is present. In particular, people prefer to purchase sample “2” (286, 64.5%) instead of sample “1”. However, there is a discrete concordance ($\kappa = 0.58, 0.51\text{--}0.65, 95\%$ confidence interval) between the two preferences. The mate consumers present the same preference distribution ($p > 0.05$). Preference for sample “1” derives from the colour (34, 21.6%) and the appearance (25, 15.9%) of the product, while sample “2” conveys the idea of natural (89, 30.9%) and is recognised as other already consumed teas (34, 11.8%).

In general, Sample “1” is seen as richer in chlorophyll (384, 84.9%), fresh (320, 70.6%), not natural (303, 66.9%), not genuine (281, 62.0%), and not aromatic (281, 62.0%). Sample “2”, instead, is perceived as natural (365, 80.6%), genuine (347, 76.6%), and aromatic (329, 72.6%). It is interesting to highlight again that every perception is derived uniquely from the observation of the picture given. These uneven perceptions are, by the way, really important from a commercial point of view, as consumer choices are mainly driven by what can be directly sensed during the purchase; thus, every attribute that can be seen has a role in the choice [65].

Finally, 237 people (53.0%) would like to try *cuia* and *bombilla* to assume mate, as the traditional way of consumption arouses curiosity and interest among consumers and may be a positive driver to enhance mate consumption [68].

3.5. Preliminary Internal Sensory Evaluation

This preliminary sensory approach aimed to investigate, even if not following the standard rules and methodologies for a sensory analysis, the general perception and appreciation of the products from the consumers’ point of view. The answers obtained are summarised in Table S2.

From the answers collected emerges that the green tea mate is mainly associated with the “vegetal/grassy” (43.90% for “4” and 39.02% for “5”) and “hay” (21.95% for “4” and 24.39% for “5”) flavour sensations [34,69].

“Citrus” and “chemical/artificial/medicine” flavours were considered absent in the majority of the answers (70.73%). Considering the “astringent” attribute, the perception was mainly absent or with slight and medium intensity present (35.00% for “1”, 27.50% for “2”, and 27.50% for “3”), qualitatively accountable to the absence of fastidious mouth sensations for the untrained panellists.

Distinctions between men and women were not recorded.

Correlations between various attributes were evaluated, highlighting many pairings. This result could be expected as the mate tasting was performed only once and without a previous training. Even though a specific sensory methodology was not followed, this analysis was regarded as a preliminary suggestion for estimating the market's potential interest in the newly introduced product. Hence, the result is an unclear attribution of taste and flavour to the attribute. For example, "caramel" is strongly correlated to "toasted", "smokey", "brown spices", "nutty", and "chemical/artificial/medicine", while being slightly related to tobacco and citrus. It is quite explicit the confusion derived from the presence of a number of attributes referring to the brown, toasted, and nutty sphere; this is a limiting aspect of the analysis; thus, a possible solution should involve the sensory evaluation performed by trained panellists, as untrained people usually distinguish attributes according to their familiarity with the product and their subjective point of view [70].

On the other hand, a strong correlation between "vegetal/grassy" and "hay" corroborates the main result of the analysis.

A curiously strong correlation between bitterness and acidity was defined; nevertheless, the evaluation of concordance did not highlight a significant relationship. It is possible to render this aspect a consequence of the extremely subjective perception of bitter taste, perceived as milder or stronger and causing a more or less rejecting sensation [71]. Mate drink is bitter due to the presence of caffeine or tannins, which are secondary metabolites produced by *I. paraguariensis* in relation to plant age, cultivation place, and harvesting time [72,73].

A PCA analysis carried out among the resulting attribute scores did not show clear clustering, thus it was not possible to refer the green tea mate to a specific product, green tea from *C. sinensis* [35] or traditional mate [34]. For this reason, this preliminary sensory analysis should be implemented and improved, possibly with the introduction of a trained panel and a formal investigation method. Nevertheless, a sensory profile was generated from the collected interviews (Figure 6), which highlights the disproportion of the herbaceous flavours with respect to the other attributes.

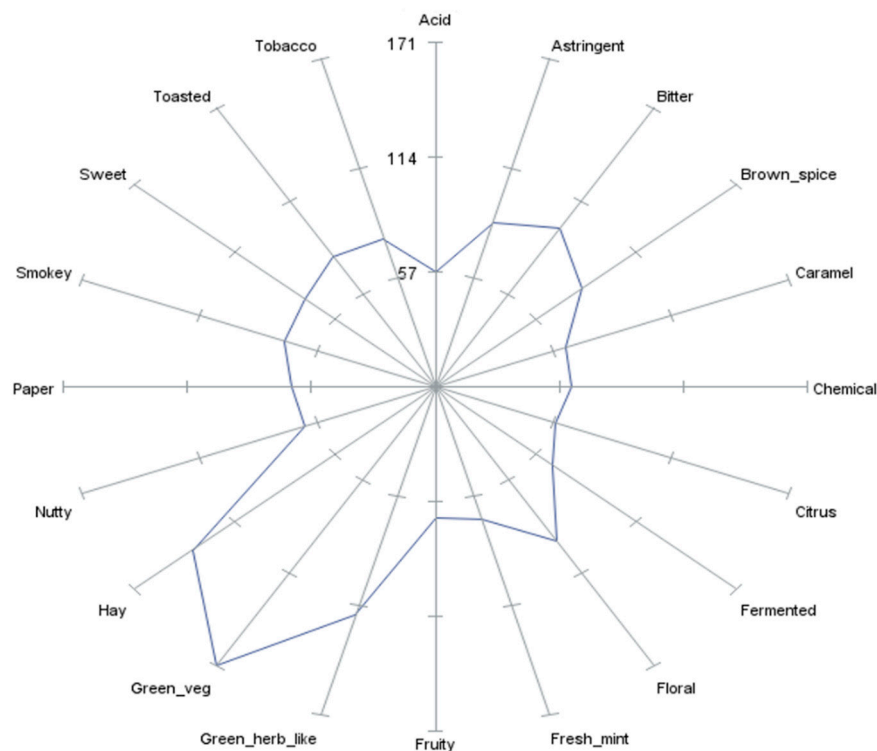


Figure 6. Sensory profile of green tea mate.

4. Conclusions

The present study demonstrated that green tea mate shows enhanced properties in terms of TPC, antioxidants, and alkaloids when compared to traditional mate and roasted mate. As discussed, the composition of young sprouts is the main justification for the results. Thus, the difference among the sample categories is also verified through the clusters identified from PCA.

For further characterisation and to understand the competitiveness of the new product on the European export market, it would be appropriate to compare the green tea mate with other tea-like beverages (green tea, black tea, white tea, and herb infusions). In addition, the aspect of consumption habits may play a role in the diffusion of the innovative product.

As reported in the literature, the consumption of *I. paraguariensis*-derived products might have a role in health promotion and providing energy. Although, as also confirmed by the results of the consumer survey, this plant is not widely known in the Italian context, in fact, its consumption is strictly linked to people who are directly linked to South America for family origins. A widespread and relevant communication should be brought about to favour mate consumption. With this objective, the innovative product may have the right formula to attract the Italian consumer, as the dark green colour reminds me of the leaves of other teas and infusions. The consumer survey may provide more scientific evidence if extended to a wider geographical area, for example, the overall Italian peninsula, so that specific consumption patterns could be highlighted. On the other hand, the bitter taste may result in an obstacle, as the perception of this taste is usually subjective and could create a strong segmenting effect on the market. For this reason, we recognise that the sensory analysis provided limited information. Hence, we propose that the topic be broadened and that a quantitative descriptive analysis be performed with a trained panel. This analysis should be objective and should define the product characteristics, thanks to the informed use of scales. Another limitation that may affect the objectivity of the consumer survey consists in the presentation of Figure 2 on devices with different display quality: the quality of the picture could influence the colour. Generally, we have to underline that the investigation of consumers' perceptions was an attempt made without following standard methods, and, so, results should be interpreted only in relation to this context without drawing wide conclusions.

Cultural exchanges and travels have been, up to now, the major means to get in with the mate culture. Specific market studies should be carried out to flank the export steps to more precisely understand which market segment or niche would be interested in the innovative green tea mate product.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/beverages10030060/s1>. Figure S1: Selected programme for the gradient elution for HPLC analysis, Table S1: Descriptive analyses obtained from the answers to the online survey; Table S2: Descriptive analyses obtained from the answers to sensory sheet.

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