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ORIGINAL ARTICLE

Differences in physicochemical properties of yerba maté (*Ilex paraguariensis*) obtained using traditional and alternative manufacturing methods

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Abstract

The aim of this study was to determine the drying kinetics and physicochemical properties of yerba maté obtained using three different alternative manufacturing methods and to compare these products with those of the yerba maté produced using the traditional process. Additionally, triangle tests were performed to establish whether there were sensory differences between the products studied. The assayed alternative manufacturing methods included the following: (a) zapecado using boiling water (BWZ) and (b) zapecado using steam water (SWZ), both followed by a final hot air drying step, and (c) an integral manufacturing method (zapecado + drying) using high-frequency radiation (HFR). The HFR integral method yielded a product with a higher caffeine content (>30%; $p < .05$) than that of traditional yerba maté. Furthermore, HFR yerba maté showed significantly higher total polyphenol content values than those of BWZ and SWZ yerba maté (>10%; $p < .05$). Likewise, the HFR integral method resulted in a yerba maté that was much more similar in color and with imperceptible sensory differences ($p > .05$) when compared to the traditional product.

Practical applications

During traditional yerba maté manufacturing, freshly harvested branches of *Ilex paraguariensis* come into direct contact with combustion gases from the burning of forest biomass. This contributes to the formation of polycyclic aromatic hydrocarbons and their subsequent deposition in yerba maté leaves and stems. Polycyclic aromatic hydrocarbons have been proved to be carcinogenic, in addition to having other toxic effects on humans.

The elimination of combustion gases as a direct source of heat in the manufacturing process of yerba maté is an emerging need. The development of alternative manufacturing methods that avoid the contact of the raw material with the products of the incomplete combustion of forest biomass burning and the subsequent implementation of these methods in the industry will guarantee a higher quality product from a food safety point of view.

1 | INTRODUCTION

Yerba maté (*Ilex paraguariensis* St. Hil.), a native tree of the subtropical region of South America, is industrialized to obtain the commercial product, ***yerba maté***, which is used to prepare a very popular infusion drink in that region, the *maté* (Heck & De Mejía, 2007).

After harvesting, the fresh yerba maté branches go through an industrial process that begins with a severe heat treatment called *zapecado*, which inactivates the polyphenoloxidases and peroxidases that produce the browning of the yerba maté leaves, followed by a drying stage that reduces the moisture content of the product to approximately 3% and a subsequent grinding step after which the yerba maté is aged in order to acquire its traditional flavor and taste (Schmalko & Alzamora, 2001).

During the first two stages of the manufacturing process (*zapecado* and drying), the branches of *I. paraguariensis* come into direct contact with hot air and combustion gases from the burning of different types of forest biomass. Specifically, during the *zapecado*,

the leaves and stems of yerba maté, in addition to coming into contact with hot gases, also come into direct contact with flames from the forest biomass burner, which is located next to the *zapecado* rotating oven. At present, the most commonly used fuels for yerba maté manufacturing is pine and eucalyptus wood chips. Many pollutants are formed during the incomplete combustion of organic matter used as fuel during food processing, including polycyclic aromatic hydrocarbons (PAHs), which are characterized by their hazardous mutagenic and carcinogenic potential. For this reason, many of these compounds are included in the priority pollutants lists of international organizations to be monitored in the environment, foodstuff and other matrices. According to European Commission Regulation 2015/1933 (European Union [EU], 2015), which sets the maximum levels of PAHs in cocoa fiber, banana chips, food supplements, dried herbs and dried spices, yerba maté must not exceed the maximum concentration of 10.0 μg/kg for benzo[a]pyrene (BaP) and the maximum concentration of 50.0 μg/kg for the sum of benz[a]anthracene (BaA), chrysene (Chry), benzo[b]fluoranthene (BbF) and BaP (PAH_4).

Due to these regulations, the yerba maté industry has worked on the development of alternative manufacturing processes to avoid the contact of the product with combustion gases and, consequently, its contamination with PAHs. It is remarkable that, during the *zapecado*, the fresh branches of *I. paraguariensis* are exposed to temperatures between 400 and 500 °C for a few minutes in a rotating oven, and yet it is very difficult to reach these high temperatures by heating air indirectly. On the other hand, the temperatures for the drying process of yerba maté are usually between 80 and 120 °C, which are easily achieved by using heat exchangers that would avoid product contamination. Therefore, major modifications of the yerba maté manufacturing process are required for the *zapecado* stage.

The *zapecado* process is a kind of blanching method and, as it has been mentioned before, the principle of this thermal process varies widely from the technologies commonly used for blanching in the food industry, such as those based on hot water, steam, microwave and high radiofrequency. Alternative *zapecado* processes that have been tested include exposing the yerba maté branches to (a) boiling water (Galeano, 2010; Galeano, Barrionuevo, & Argüello, 2006; Xander, Acosta, Scipioni, & del Argüello, 2000), (b) steam water (Galeano, 2010; Galeano et al., 2006; Zanoelo, Cardozo-Filho, & Cardozo-Júnior, 2006), (c) high-frequency radiation (HFR; Passardi, Schvezov, Schmalko, & González, 2006; Laube, 2008; Ceni et al., 2009) or (d) *zapecado* in a conveyor belt oven (Nabechima et al., 2014; Provesi, Nabechima, Vieira, & Amante, 2010) and indirectly drying the yerba maté using hot air as the thermal agent.

Modifications to the traditional manufacturing process of yerba maté can result in a final product with distinctive sensory and physicochemical characters. To date, little research has been conducted on the physicochemical and sensory properties of yerba maté obtained using alternative manufacturing methods.

The aim of this study was to evaluate the physicochemical properties of yerba maté obtained by alternative *zapecado* methods using (a) boiling water (BWZ) and (b) steam water (SWZ), both followed by a final hot air drying step, and (c) a third integral alternative manufacturing method (zapecado + drying) using HFR. It was also proposed to determine if there are differences between the physicochemical properties and organoleptic attributes of yerba maté obtained using alternative manufacturing methods and yerba maté obtained using the traditional *zapecado* method and hot air drying. In this sense, the caffeine content, water extract, total ash and acid insoluble ash content, total polyphenol content (TPC), free radical scavenging activity (DPPH• assay), PAH content, and color parameters (*L*, *a*, and *b*) were studied. To assess if the use of alternative manufacturing methods led to perceived differences in yerba maté taste, triangle tests were performed.

2 | MATERIALS AND METHODS

2.1 | Yerba Maté samples

Recently harvested yerba maté branches used in this work were obtained from three different local manufacturers of Misiones (Argentina). To study the physicochemical properties of yerba maté, three alternative manufacturing processes were used, namely: (a) boiling water *zapecado* and hot air drying (BWZ); (b) steam water *zapecado* and indirect drying (SWZ) and (c) a HFR integral method; yerba maté samples were taken during all three processes. In all three cases, a control sample was processed using the traditional *zapecado* method (TZ) and hot air drying. To obtain triplicate samples of BWZ, SWZ and HFR yerba mate, and TZ yerba maté, all the procedures for each stage that are detailed below were conducted three times on the same day.

In the first stage, yerba maté obtained using the BWZ method was analyzed. Specifically, approximately 8 kg of recently harvested yerba maté branches were obtained from the local industry before beginning the manufacturing process. Likewise, approximately 4 kg of yerba maté branches from the same raw material were collected after the traditional *zapecado* stage using a rotating oven with wood chips as fuel (TZ_1). Both fractions were taken to the Laboratory of Yerba Mate of the Facultad de Ciencias Exactas, Químicas y Naturales – Universidad Nacional de Misiones (FCEQyN-UNaM). The BWZ sample was obtained by using industrial equipment developed by J. C. Lorenzo, where the fresh branches of *I. paraguariensis* came into direct contact with boiling water for approximately 30 s.

In the second stage, 8 kg of fresh yerba maté branches obtained from a local manufacturer before entering the industrial process were taken to the Laboratory of Yerba Mate to be subjected to a saturated steam treatment using pilot-scale equipment developed by Barrionuevo (2007). Additionally, 4 kg of yerba maté branches were obtained after the traditional *zapecado* procedure from the industry (TZ_2). In the Yerba Mate Laboratory, the fresh leaves and stems of *I. paraguariensis* were conveyed through a chamber with saturated steam at 1.5 atm of pressure. The residence time of the raw material in the saturated steam chamber was 10 min.

In the third stage, HFR yerba maté was analyzed. Briefly, 8 kg of recently harvested yerba maté branches were obtained from a local manufacturer and were taken to the Yerba Mate Laboratory to be subjected to an integral treatment using HFR. To obtain HFR yerba maté, the branches of *I. paraguariensis* were processed using pilot-scale equipment developed by Laube (2008), operating at 1 GHz of

frequency, for 10 min. A sample of the yerba maté processed using the traditional *zapecado* method (TZ_3) was also taken.

After the *zapecado* using (a) wood chips (TZ_1, TZ_2, and TZ_3), (b) boiling water or (c) steam water, the yerba maté branches were dried with hot air at 90 °C in a pilot-scale cross-flow dryer until the moisture content of the material was approximately 8% of the dry matter. Air flows at speeds up to 1.5 m/s were used through the layer of branches.

The drying kinetics was determined by weighing the samples for 10 min each until a constant weight was reached. Then, the moisture content of the dried samples was determined. The moisture content at different times was determined by using the weight of the sample and the final moisture content (García, Brumovsky, Fretes, & Schmalko, 2010).

Finally, all dried yerba maté samples were ground and aged for 45 days at a controlled temperature (at 60 °C) and relative humidity (60%) in an aging chamber with a capacity of approximately 5 kg that was built for this purpose (Hollowaty et al., 2014). After aging, the yerba maté samples were hermetically packaged and stored at −18 °C until analysis.

2.2 | Mathematical modeling of drying kinetics

Many drying kinetics models have been used to evaluate the drying process of foodstuff. Among the most frequently used, the Lewis (Equation (1)), Page (Equation (2)), and Henderson & Pabis (Equation (3)) models have been fitted to accurately describe the drying processes of several vegetable matrices (Onwude, Hashim, Janius, Nawi, & Abdan, 2016).

$$MR = \frac{X - X_{eq}}{X_0 - X_{eq}} = e^{-kt} \quad (1)$$

$$MR = \frac{X - X_{eq}}{X_0 - X_{eq}} = e^{-kt^N} \quad (2)$$

$$MR = \frac{X - X_{eq}}{X_0 - X_{eq}} = ae^{-kt} \quad (3)$$

where MR is the moisture ratio, X is the moisture at any time during drying, X_0 is the initial moisture content, X_{eq} is the equilibrium moisture content, k is the drying rate constant, and N is the drying constant for the Page model and a is the drying constant for the Henderson-Pabis model.

The goodness of fit and similarities between experimentally determined moisture ratios and model predicted moisture ratios were evaluated using the coefficient of determination (R^2).

2.3 | Physicochemical characterization

2.3.1 | Moisture content

The moisture content was determined by drying the sample in an oven at 103 ± 2 °C until a constant weight was reached (IRAM 20503, Instituto Argentino de Racionalización de Materiales [IRAM], 1995c).

2.3.2 | Total ash content and acid-insoluble ash content

The yerba maté samples (5 g) were incinerated in a muffle furnace. The temperature was gradually increased to 525 ± 25 °C and then maintained for approximately 6 hr. The ash contents were quantified gravimetrically (IRAM 20505, Instituto Argentino de Racionalización de Materiales [IRAM], 1995a).

The acid-insoluble ash content was determined from total ash by treatment with 25 mL of a hydrochloric acid solution, filtration through an ashless filter paper and subsequent incineration in a muffle furnace at 525 ± 25 °C, proceeding as established in IRAM 220507 (Instituto Argentino de Racionalización de Materiales [IRAM], 1995b).

2.3.3 | Water extract

Briefly, 2 g of yerba maté was mixed with 200 mL of distilled water in a balloon flask, which was maintained at 100 °C for 1 hr. The extract was then filtered, transferred to a pre-weighed vessel and placed into an oven at 103 ± 2 °C until constant weight was reached (IRAM 20510, Instituto Argentino de Racionalización de Materiales [IRAM], 1995d).

2.3.4 | Caffeine

Caffeine content was determined using reversed-phase high performance liquid chromatography (RP-HPLC) with UV detection. Caffeine standard (Biopack, Argentina), a mobile phase of acetonitrile: water (30:70, v/v) (MERCK, Germany) and a C_{18} column (4.6 × 250 mm, 5 μm) (Zorbax ODS, Agilent Technologies, Lake Forest, CA) were used. HPLC was carried out according to the method described in IRAM 20512 (Instituto Argentino de Racionalización de Materiales [IRAM], 1995e).

2.3.5 | Total polyphenol content and free radical-scavenging activity measurement

For the determination of TPC, Folin–Ciocalteu's reagent (Fluka, Argentina), gallic acid (MP Biomedicals, Argentina), methanol (Merck, Argentina) and anhydrous sodium carbonate (Anedra, Argentina) were used. For the determination of the free radical-scavenging activity, 1,1-diphenyl-1-picrylhydrazyl (Sigma, Argentina) and ascorbic acid (Sigma Ultra, Argentina) were used.

The extraction was done according to the method described in ISO 14502-1 (E) (International Organization for Standardization [ISO], 2004a). Briefly, 0.2 g of yerba maté was placed in an extraction tube, and 5 mL of 70% methanol solution was added. The extract was mixed and heated at 70 °C for 10 min. Then, the extract was centrifuged for 10 min at 2,052*g*, and the supernatant was reserved in a graduated tube. The extraction step was repeated twice. The combined supernatant liquors were readjusted to a fixed volume (10 mL) with 70% methanol. One milliliter of the extract was diluted with water to 100 mL.

TPC was determined by spectrophotometry using Folin–Ciocalteu's reagent, and the results were expressed as gallic acid equivalents in mass percentage of dry matter (g GAE % dm) according to the method described in the ISO 14502-1 (E) international standard (International Organization for Standardization [ISO], 2004a). One milliliter of the diluted sample extract was transferred in duplicate to separate tubes containing 5.0 mL of water-diluted Folin–Ciocalteu's reagent (10% v/v). Then, 4.0 mL of a sodium carbonate solution (7.5% w/v) was added. The tubes were then left at room temperature for

60 min before absorbance was measured at 765 nm against distilled water in a spectrophotometer UV/Vis (Spectrum SP-2102). The TPC of the samples was derived from a standard curve of gallic acid ranging in concentration from 0 to 50 μg/mL (R^2 = 0.996).

Free radical scavenging activity was determined by the 1,1-diphenyl-1-picrylhydrazyl (DPPH•) assay (DPPH• SA) using ascorbic acid as standard, and the results were expressed as ascorbic acid equivalents in mass percentage of dry matter (g AAE % dm) (Hartwig, Brumovsky, Fretes, & Sánchez Boado, 2011). One milliliter of the sample extract was diluted with distilled water (1:25). Then, 100 μL of the diluted sample extract was mixed with 3 mL of a 100 μM DPPH• solution in absolute methanol. The mixture was kept for 2 hr at 37 °C in darkness, and then the absorbance was measured at 517 nm against absolute methanol in a UV/Vis spectrophotometer (Spectrum SP-2102). The free radical scavenging activity of the samples was derived from a standard curve of ascorbic acid ranging in concentration from 0 to 100 μg/mL (R^2 = 0.935).

2.3.6 | Polycyclic aromatic hydrocarbons

A method using reversed-phase high-performance liquid chromatography with fluorescence detection (RP-HPLC-FLD) was developed and validated for the simultaneous determination of the four EFSA (European Food Safety Authority) priority PAHs, or PAH4 (benz[a] anthracene (BaA), chrysene (Chry), benz[b]fluoranthene (BbF), benz[a] pyrene (BaP), in the yerba maté samples. The sample preparation included an ultrasound-assisted extraction (UAE) step using cyclohexane (Merck Chemicals, Buenos Aires, Argentina) as the extraction solvent, followed by a clean-up step with silica solid phase extraction (SPE) cartridges (Bond Elut SI, 3 mL, 500 mg) (Agilent Technologies, Lake Forest, CA).

Briefly, in an assay tube, 10 mL of cyclohexane was added to 2 g of yerba maté, and the mixture was mixed in a vortex for 15 s. The yerba maté samples were extracted by ultrasonication for 30 min, the extracts were centrifuged at 2,680*g* for 10 min, and the supernatants were collected. This process was carried out in triplicate. All the supernatants were combined and concentrated in a rotary evaporator to 3 mL at 39.5 °C. The concentrated extract (3 mL) was filtered through a commercial solid phase extraction cartridge. After conditioning the cartridge with 3 mL of cyclohexane, the extract was applied and eluted with 9 mL of additional cyclohexane. Finally, the extract was evaporated to dryness. The residue was dissolved in 3 mL HPLC-grade acetonitrile (Merck Chemicals, Buenos Aires, Argentina) using the ultrasonic bath. This solution was filtered through a 0.22 μm nylon syringe filter (Agilent Technologies, Lake Forest, CA) before being injected into the chromatography system.

HPLC analysis was carried out according to the method described by Thea, Ferreira, Brumovsky, and Schmalko (2016) using a Supelcosil™ LC-PAH column (250 × 4.6 mm, 5 μm) (Supelco, Bellefonte, PA) and a mobile phase consisting of water and acetonitrile in a gradient mixture (Merck Chemicals, Buenos Aires, Argentina). The identification of the PAHs was performed by comparing their retention time (t_R, min) with those of the PAHs contained in the PAH4 standard mixture (Supelco, Bellefonte, PA). An external standard plot method was used for quantification. The developed method was validated for linearity, accuracy (recovery tests), precision, limit of detection and limit of quantification.

2.3.7 | Color parameters

The color parameters were measured using a solid colorimeter (Miniscan EZ HunterLab model MSEZ-4500L, Hunter Associates Laboratory Inc., Virginia). The response parameters on the Hunter Lab scale were as follows: *L* (lightness or black-white axis), *a* (green-red axis) and *b* (blue-yellow axis). Each color value was measured six times.

Additionally, total color difference (ΔE) was calculated as $((L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2)^{1/2}$, were L_0, a_0, and b_0 are the values of the sample processed in the traditional way (reference sample).

2.4 | Sensory difference test

Difference testing is a way to determine if a sensory difference actually exists between the samples. A discrimination method (triangle test) was carried out to compare the yerba maté obtained by the three alternative methods and traditional yerba maté (ISO 4120, ISO - International Organization for Standardization, 2004b). Three independent pair tests were performed: (a) traditional yerba maté vs. BWZ yerba maté, (b) traditional yerba maté vs. SWZ yerba maté, and (c) traditional yerba maté vs. HFR yerba maté.

For testing, infusions prepared using aged yerba maté obtained by the traditional manufacture process and the alternative methods were presented to the panel. The infusions were obtained in the same way as that by which these beverages are usually prepared and consumed, as described by Hartwig, Brumovsky, and Fretes (2012).

Three coded infusion samples were presented to a panel of 33 members between 20 and 50 years old, all of whom were habitual maté consumers. Each panelist performed the triangle test twice in two different days. The panel members were asked to taste the samples and then indicate the odd one.

The statistical results were obtained from a table of data with the number of correct identifications corresponding to the number of judgements at a confidence level of 95%.

2.5 | Statistical analysis

To assess the significance of the differences between yerba maté samples obtained using the different alternative manufacture methods, ANOVA tests were carried out at a significance level of α = 0.05. The experimental data of the drying kinetics of the yerba maté samples were fitted to mathematical models by means of nonlinear regression analysis. The StatGraphics statistical package (Statgraphics, 2009) was used to process the data.

3 | RESULTS AND DISCUSSION

3.1 | Drying kinetics

Figure 1 shows the drying curves of the yerba maté samples processed using the alternative and traditional manufacturing methods. The branches of yerba maté obtained using different *zapecado*

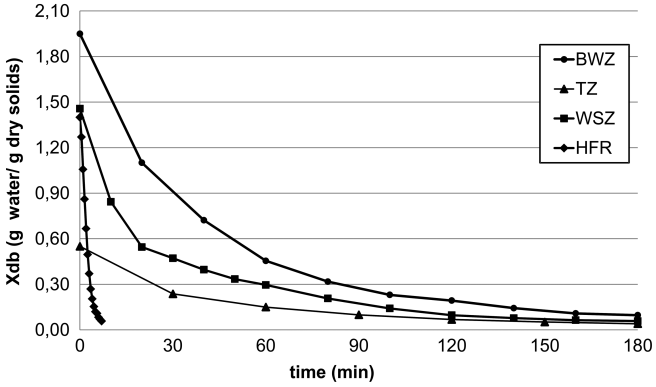

FIGURE 1 Drying kinetics curves of yerba maté obtained using different alternative manufacturing methods. BWZ, Boiling water zapecado and hot air drying; WSZ, Steam water zapecado and hot air drying; HFR, High-frequency radiation integral method; TZ, Traditional zapecado method and hot air drying

methods had different initial moisture contents: 1.76 g of water/g of dry solids for BWZ; 1.45 g of water/g of dry solids for SWZ; 1.40 g of water/g of dry solids for HFR and 0.55 g of water/g of dry solids for TZ.

The drying times to reach the final moisture content were: 100 min for TZ, 140 min for SWZ, and 180 min for the BWZ yerba maté samples. The drying time for the HFR integral method was 9 min. The *zapecado* methods using steam water and boiling water incorporated additional water to the solid material, resulting in longer drying time. The drying times for SWZ and BWZ yerba maté samples were, respectively, 40 and 80% higher than the drying time of yerba maté obtained by traditional processing (TZ). In the integral drying process using HFR, yerba maté reached the final moisture content in a very short time, 90% less than the drying time observed for the conventional manufacturing process.

When the experimental data were fitted to the different thin-layer mathematical models used in this study, the R^2 values varied between 98 and 99% when fitting to Lewis and Page equations and varied between 95 and 99% when fitting to the Henderson and Pabis model.

The Lewis model was selected to compare the drying curves of the different methods since this kinetic model has a single empirical constant, *k*. As shown in Table 1, the values of *k* were not significantly different between the TZ, BWZ, and SWZ drying methods. Nevertheless, the value of *k* for the HFR integral method was significantly higher.

The drying rate constant *k* is related to the diffusion coefficient of water, and the similar values found in the TZ, BWZ, and SWZ methods indicate a similar mechanism of moisture movement during drying. The significantly higher *k* constant observed in HFR integral treatment could be due to the higher temperature reached during this drying process.

3.2 | Physicochemical properties

In this research, all yerba maté samples were obtained from the same raw material and processed using both novel manufacturing methods (BWZ, SWZ, and HFR) and the traditional manufacturing process (TZ). The novel and traditional manufacturing methods were carried out simultaneously. To compare the final products obtained using the alternative methods and the yerba maté obtained using the traditional method, the results of the physicochemical parameters are reported as percent difference between the novel and the conventional methods. If a value of zero is included within the confidence limits of the reported percent difference for each physicochemical property studied, this parameter is not affected by the novel process.

3.2.1 | Total ash content and acid-insoluble ash content

Total ash content and acid-insoluble ash content are two parameters included in the quality control of yerba maté. Both parameters are relevant indicators of the quality, purity and even genuineness of the plant materials used in the food and medicinal industries. Total ash content includes mineral content derived from the plant tissue itself and minerals from the environment, such as sand and soil. The contamination of plant material with soil, sand, heavy metals, and others correlates directly with the acid insoluble ash content.

The total ash content of yerba maté obtained using the different manufacturing methods varied between 5.75 and 6.27 g/100 g of dry matter (Table 2). When comparing the total ash content of yerba maté obtained using the alternative methods with that obtained using the traditional manufacturing process, no significant difference was observed. It is noteworthy that there was no statistically significant difference between the total ash contents of the yerba maté samples obtained using the different alternative manufacturing methods (Figure 2).

Acid-insoluble ash is a parameter directly related to the presence of soil particles added to the vegetable material during cultivation and harvesting. The acid-insoluble ash content of the yerba maté samples varied between 0.34 and 0.46 g/100 g of dry matter (Table 2).

As shown in Figure 2, there were significant differences in the acid-insoluble ash content of the yerba maté samples obtained using the conventional method compared to that of the yerba maté processed by SWZ or the HFR integral method.

Acid-insoluble ash content of HFR yerba maté was the highest, while that of yerba maté obtained using steam water for *zapecado* was the lowest. Traditional *zapecado* is carried out in a rotary dryer, and soil particles adhered to the leaves and stems of yerba maté can break off from the material due to bed agitation. On the other hand,

TABLE 1 Values of the constant *k* (min^{-1}) of the Lewis model (Equation (1)) and their confidential limits

Manufacturing method			
TZ_1	BWZ	WSZ	HFR
0.0168 ± 0.0022 (a)	0.0188 ± 0.0016 (a)	0.0211 ± 0.0024(a)	0.4639 ± 0.0157(b)

Different letters indicate significant differences by Tukey's test at $p < .05$.
TZ, traditional zapecado; BWZ, boiling water zapecado; WSZ, steam water zapecado; HFR, high-frequency radiation.

TABLE 2 Physicochemical properties (mean ± standard deviation) of yerba maté obtained using the different manufacturing methods

Physicochemical parameter		Manufacturing method					
		BWZ	TZ_1	WSZ	TZ_2	HFR	TZ_3
Total ash content (g/100 g of dry matter)		6.27 ± 0.26	6.49 ± 0.26	5.81 ± 0.16	5.70 ± 0.20	5.75 ± 0.18	5.96 ± 0.35
Acid insoluble ash content (g/100 g of dry matter)		0.46 ± 0.07	0.42 ± 0.06	0.34 ± 0.05	0.38 ± 0.07	0.42 ± 0.08	0.13 ± 0.05
Water extract (g/100 g of dry matter)		38.93 ± 1.34	40.74 ± 0.94	37.52 ± 1.66	40.47 ± 0.47	41.06 ± 1.79	40.79 ± 1.49
Caffeine (g/100 g of dry matter)		0.90 ± 0.03	0.90 ± 0.07	1.15 ± 0.04	0.93 ± 0.06	1.23 ± 0.10	0.96 ± 0.05
Total polyphenol content (g GAE/100 g of dry matter)		8.85 ± 0.48	10.14 ± 0.42	7.75 ± 0.88	10.05 ± 1.46	8.33 ± 1.40	8.25 ± 0.64
DPPH• scavenging activity (g AAE/100 g of dry matter)		18.82 ± 0.79	17.20 ± 1.23	20.31 ± 1.22	19.91 ± 1.40	20.06 ± 1.19	18.88 ± 1.48
Color parameters	***L***	37.29 ± 1.67	37.82 ± 1.84	35.42 ± 1.67	38.20 ± 0.95	36.21 ± 0.87	36.08 ± 1.18
	a	−1.47 ± 0.41	−1.62 ± 0.34	−0.17 ± 0.11	−2.06 ± 0.19	−1.92 ± 0.39	−2.43 ± 0.28
	b	12.33 ± 0.77	13.84 ± 0.71	13.06 ± 0.60	14.55 ± 0.33	14.10 ± 0.40	13.22 ± 0.70

in the HFR integral method, the material is placed in a tray and is at rest during the entire treatment. This fact explains the higher content of acid-insoluble ashes in yerba mate obtained by the HFR method. When processing the branches of yerba maté using SWZ, the material is treated with saturated steam water which, on contact with the solids, condenses and creates a washing effect. The insoluble ash concentration for the SWZ method was significantly less than that for the traditional process.

In the BWZ method, the material is dipped in boiling water, and the bath is not removed during the day. In this case, no difference was found between the novel process and the traditional one.

3.2.2 | Water extract

The water extract of yerba maté obtained using different manufacturing methods varied between 41.06 g/100 g of dry matter and 37.52 g/100 g of dry matter (Table 2). As shown in Figure 2, there was no difference between the water extract of HFR yerba maté and that of the yerba maté obtained using the traditional method. Nevertheless, in the BWZ and SWZ yerba maté samples, negative percent differences were found. The lower water extract values of yerba maté obtained by these two alternative methods can be explained by the losses of soluble components in the hot water and condensed steam involved in the process.

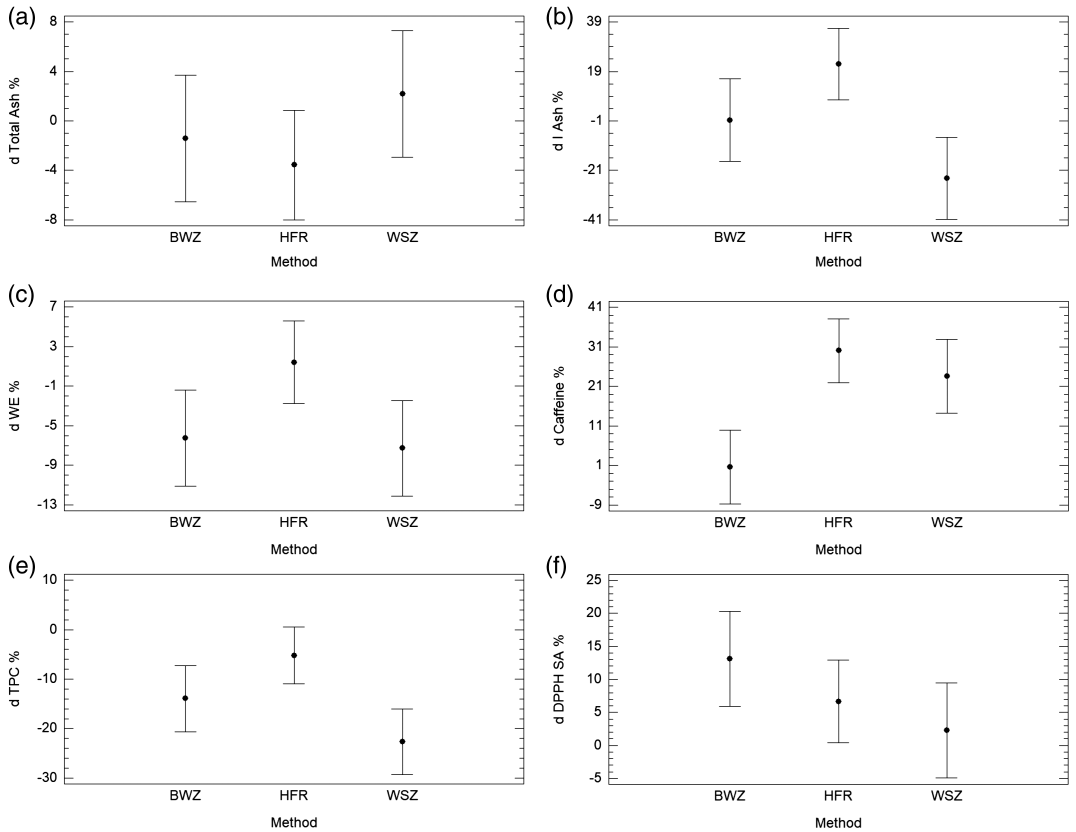

FIGURE 2 Percent differences in total ash content of yerba mate samples obtained using the alternative manufacturing methods: (a) Total ash content, (b) acid-insoluble ash content, (c) water extract, (d) caffeine, (f) total polyphenol content (TPC), (g) DPPH• scavenging activity (DPPH SA). BWZ, Boiling water zapecado; SWZ, Steam water zapecado; HFR, High-frequency radiation. Note that when the zero value is included in the confidence limits, it indicates that there is no significant difference between yerba mate obtained by the alternative method and traditional yerba mate

3.2.3 | Caffeine

The caffeine content of yerba maté obtained using the different manufacturing methods varied between 0.90 and 1.23 g/100 g of dry matter (Table 2).

As shown in Figure 2, there was no significant difference between the caffeine content of yerba maté obtained by the BWZ method and that of traditional yerba maté. However, the yerba maté obtained using the HFR integral treatment and the SWZ method showed higher values of caffeine.

It is well known that caffeine losses occur during the traditional manufacturing process of yerba maté. Schmalko and Alzamora (2001) reported losses of caffeine of about a 30% during manufacture. They observed that these losses take place mainly in the drying step, since during the zapecado the losses only reached 8%. The fall in caffeine content is caused by sublimation, which becomes relevant as the solid dries.

Due to its high water solubility, caffeine leaches out from the vegetable tissue to the treatment medium during the BWZ treatment. Thermal treatments using steam water and HFR prevent the leaching of soluble compounds and, consequently, the loss of caffeine, and other substances (Xiao et al., 2017).

3.2.4 | Total polyphenol content and free radical-scavenging activity

TPC of the yerba maté samples obtained by the different manufacturing processes varied between 7.75 g and 9.02 g GAE/100 g of dry matter (Table 2). Yerba maté obtained using the HFR integral method showed TPC values similar to those obtained using the traditional zapecado process, while the BWZ and SWZ samples had significantly lower TPC values than those of TZ yerba maté (Figure 2).

Zanoelo et al. (2006) reported an increase in phenolic content when drying yerba maté with superheated steam (at 120 °C), compared with drying yerba maté with hot air (120 and 140 °C). In contrast, in this work, minor values of TPC were found in materials treated with steam compared with those from traditional zapecado (Figure 2).

Not only the temperature but also the duration of thermal treatment affects polyphenol degradation. Although steam blanching usually requires less time than hot water or hot air treatments, when it is carried out at low temperature and flow rate (as in this case), it needs more time than hot water or hot air blanching to achieve the desirable degree of enzyme inactivation (Xiao et al., 2017). Additionally, it has been demonstrated that boiling water treatments significantly reduce the TPC and antioxidant levels in hot water-processed vegetables mainly by leaching (Xiao et al., 2017). In addition, when referring to the *zapecado* processes where boiling water or steam is used, longer drying time is needed to reach the final moisture content. All these factors could cause the observed differences in the TPC of yerba maté samples obtained using the different manufacturing methods.

The free radical-scavenging activity of the yerba maté samples varied between 18.32 g EAA/100 g and 20.31 g EAA/100 g of dry matter (Table 2). The free radical-scavenging capacities of fruits and vegetables generally correlate positively with their total phenolic contents (Velioglu et al., 1998). In this research, no correlation between free radical-scavenging activity and TPC was found. Yerba maté obtained by BWZ and the HFR integral method showed higher DPPH• scavenging activities than yerba maté obtained using the traditional manufacturing process. No significant difference was observed in the free radical-scavenging capacities of yerba maté obtained using the SWZ method and the traditional process (Figure 2).

It is likely that microwave treatment not only inactivates enzymes and dries vegetable materials but also induces the formation of the derivatives of phenolics, which enhances the scavenging capacity of the products after the treatment (Dorantes-Alvarez, Jaramillo-Flores, González, Martinez, & Parada, 2011). This could explain the higher free radical-scavenging capacity of yerba maté obtained using the HFR integral method in comparison with the traditional product. Referring exclusively to the BWZ method, the leaching of soluble components in boiling water during the thermal treatment can be positively influenced by the solid content of the water used; therefore, recycled water, which has a high solid content, will lead to less loss of soluble phenolics or other antioxidant components (Xiao et al., 2017). For the manufacture of BWZ yerba maté, recycled water was used, and this could be the reason for its higher DPPH• scavenging activity when compared with conventionally obtained yerba maté.

It is noteworthy to mention that there was no significant difference between the free radical-scavenging capacities of yerba maté obtained using the three alternative manufacturing methods.

3.2.5 | Polycyclic aromatic hydrocarbons

Table 3 presents the PAH contents of the yerba maté samples obtained using the different manufacturing methods. The PAH_4 and BaP contents of traditional yerba maté were 63.3 and 17.4 μg/kg of

TABLE 3 PAH contents (μg/kg of dry matter) (mean ± standard deviation) of yerba maté obtained using the different manufacturing methods

PAH	Manufacturing method					
	BWZ	TZ_1	WSZ	TZ_2	HFR	TZ_3
BaA	ND (0.18[b])	12.4 ± 0.60	ND (0.18[b])	2.40 ± 0.43	ND (0.18[b])	13.42 ± 1.75
Chry	ND (0.35[b])	16.5 ± 0.60	ND (0.35[b])	2.01 ± 0.08	ND (0.35[b])	23.90 ± 2.43
BbF	< LOQ (0.41[a])	17.3 ± 0.05	<LOQ (0.41[a])	2.51 ± 0.47	ND (0.12[b])	22.75 ± 2.93
BaP	ND (0.06[b])	17.4 ± 0.42	<LOQ (0.20[a])	2.77 ± 0.20	ND(0.06[b])	37.10 ± 5.47
PAH_4	NQ	63.6	NQ	9.69	ND	97.17

PAH_4(BaA + Chry+BbF + BaP).
<LOQ, Limit of Quantification; ND, not detected NQ, not quantifiable.
[a] Limit of quantification.
[b] Limit of detection.

dry matter, respectively, exceeding the maximum values allowed by the European Community for these parameters in yerba maté (European Union [EU], 2015). During traditional *zapecado*, freshly harvested branches of yerba maté come into direct contact with hot air, combustion gases, and even flames from the burning of wood, wood chips and/or sawdust, contributing significantly to the contamination of the final product with PAHs (Vieira et al., 2010; Ziegenhals et al., 2008).

As shown in Table 3, most of the PAHs studied were not detected in the yerba maté samples obtained by BWZ, SWZ, and HFR alternative manufacturing methods. However, in the yerba maté samples obtained using boiling water and steam water, some PAHs were identified but could not be quantified because their concentrations were below the limit of quantification of the analytical method. The presence of PAHs in yerba maté obtained using alternative manufacturing methods could be caused mainly by the deposition of polluted air particulates on the plant leaves' waxy surface in the yerba maté plantation, or during the transport and handling of the raw material after the harvest prior to industrial processing. The contamination of plant organisms with PAHs from the atmosphere has been widely reported in the literature (Srogi, 2007).

These results demonstrate that the implementation of novel procedures in yerba maté manufacturing results in a higher quality product when referring to food safety.

3.2.6 | Color parameters

The color parameters (*L*, *a*, and *b*) of yerba maté obtained using the three novel manufacturing methods were determined. The results are presented in Table 2.

The color of yerba maté is a remarkable organoleptic attribute for consumers (Cruz, Garitta, & Hough, 2002, Grigioni et al., 2004) and is also an important indicator of product quality (Schmalko & Alzamora, 2001; Holowaty, Trela, Thea, Scipioni, & Schmalko, 2016; Zaions, Picolo, Gonçalves, Borges, & Valduga, 2014). Regarding this, it is important to mention that each market demands a product with certain color characteristics. Thus, the Brazilian market is highlighted by the preference of a yerba maté of a bright green color, while consumers in Argentina, Paraguay, Uruguay, and even Chile prefer a darker color product, such as olive green with yellow undertones (Zaions et al., 2014). For this reason, when testing novel manufacturing processes, it is very important to obtain a final product that maintains the color characteristics accepted by the habitual yerba maté consumers of each market.

The BWZ and SWZ methods produced a darker, less green and less yellow product when compared with the product from the traditional process, while the HFR integral method resulted in a yerba maté that is much more similar in color to the traditional product (Figure 3).

The effect of microwave treatment on the color parameters of yerba maté was previously studied by Ceni et al. (2009). They found an increase in *L*, *a*, and *b* color parameters when increasing the treatment time (from 0 to 220 s), which implies in a simple way that after applying a microwave energy treatment, the yerba maté leaves were visually lighter green than the raw material. In this research, an HFR treatment of 600 s was applied to the yerba maté. When comparing the color parameters reported by Ceniet al. (2009) with those obtained in our experience, it was observed that HFR yerba maté had similar values of *L* and higher *a* and lower *b* values. The differences observed in the results of both experiments are probably due to the different microwave treatment times, considering several studies reporting an increase in the green color of vegetables during the initial part of heating treatments (Lau, Tang, & Swanson, 2000; Severini, Giuliani, Filippis, Derossi, & Pilli, 2016; Tijskens, Schijvens, & Biekman, 2001). Furthermore, the observed differences between the color of the yerba maté obtained using the HFR integral method and that of the product obtained by Ceni et al. (2009) using microwave energy could be attributed to the different frequencies used in both studies: 1 GHz and 2.4 GHz, respectively.

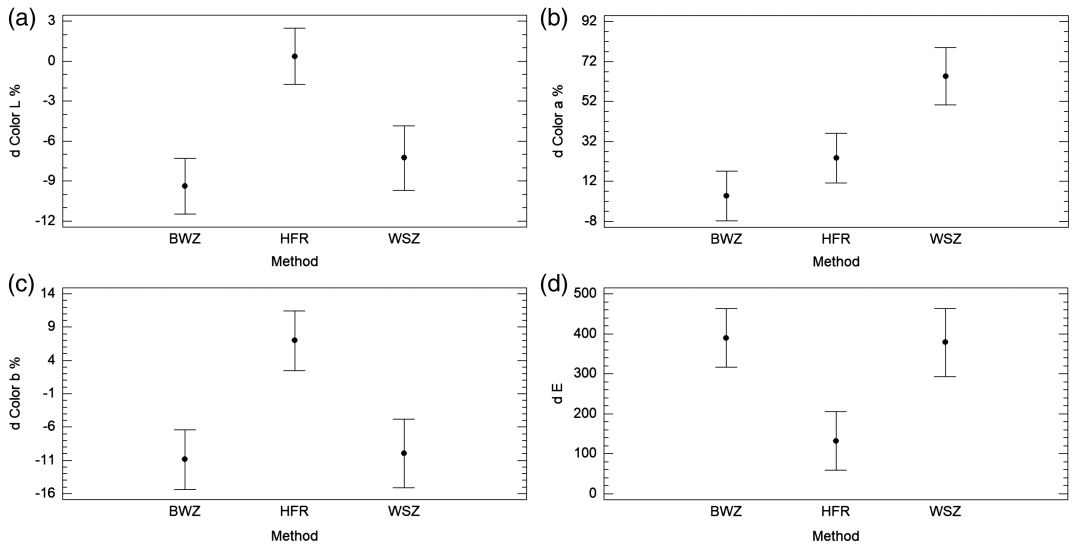

FIGURE 3 Percent differences in the color parameters (*L*, *a*, and *b*) and total difference in the color value (E) of yerba maté samples obtained using the alternative manufacturing methods: (a) color parameter *L*, (b) color parameter *a*, (c) color parameter *b*, and (d) *E* value. BWZ, Boiling water zapecado; SWZ, Water stream zapecado; HFR, High-frequency radiation. Note that when the zero value is included in the confidence limits, it indicates that there is no significant difference between yerba maté obtained by the alternative method and traditional yerba maté

3.3 | Sensory difference test

No less important than the physicochemical quality of yerba maté are its organoleptic characters. Yerba maté consumers frequently make comments on the appearance and the flavor of the product, specially its subtle smoke flavor.

The triangle test methodology has been used by other authors to assess if the concentration of certain soluble compounds or the aging degree of the yerba maté results in perceptible sensory differences in the final product (Holowaty et al., 2016; Surkan, Albani, & Ramallo, 2009).

Three trials were conducted between the yerba maté samples obtained using the novel manufacturing processes (BWZ, SWZ, and HFR) and the traditional yerba maté (TZ).

Perceptible differences were found in the BWZ and SWZ yerba maté samples. The percentage of the population that perceived difference between the traditional yerba maté and those produced using novel procedures were 59% for BWZ ($p < .001$) and 49% for SWZ ($p < .0314$). However, only 38% of the panelists ($p < .4010$) were able to correctly discriminate between HFR yerba maté and the product obtained by conventional procedures.

4 | CONCLUSIONS

There were no differences between the total ash content, water extract and free radical-scavenging activity of the yerba maté obtained using any of the three novel manufacturing methods studied and those of traditional yerba maté. The caffeine contents were higher in the yerba maté obtained using the HFR integral method and the steam water zapecado method (SWZ). TPC was similar in the yerba maté obtained using the traditional manufacturing process (TZ) compared to that of the HFR product. Lower TPC values were found in the yerba maté obtained by the boiling water zapecado method (BWZ) and that obtained using the SWZ method. Additionally, when evaluating the PAH content of the yerba maté samples obtained by using the three alternative methods, it was verified that contamination of the product during processing was null.

In reference to the color parameters, the BWZ and SWZ methods produced a darker, less green and less yellow product compared with the traditional manufacturing process. The HFR integral method resulted in a yerba maté that was much more similar in color to the traditional product.

The panelists could perceive sensory differences between the BWS and SWZ yerba maté samples and the traditional product, but no significantly perceptible difference was found between traditional yerba maté and the product obtained using the HFR integral method.

The HFR integral method yields a product of equivalent physicochemical quality and without perceptible sensory difference when compared to the traditional yerba maté manufacturing process. In terms of food safety, the product obtained using the HFR integral method had a superior quality.

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