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Research Article

Nutritional and Phytochemical Study of *Ilex paraguariensis* Fruits

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Yerba mate is defined as the product constituted by the dried, slightly roasted, and milled leaves of *Ilex paraguariensis*. However, the fruits of this species are often found in the commercial product. Nowadays the fruits are considered a byproduct. The objective of this work was to obtain the preliminary data of minerals, lipids, methylxanthines and polyphenols in the ripe fruits of *I. paraguariensis*. The results showed a considerable amount of total dietary fiber (42.0 ± 1.6 g/100 g) and nutritionally valuable minerals: potassium (1324 ± 15 mg/100 g), iron (6.4 ± 0.5 mg/100 g), magnesium (168 ± 15 mg/100 g), calcium (150 ± 12 mg/100 g), copper (1.1 ± 0.1 mg/100 g), zinc (2.3 ± 0.3 mg/100 g), and sodium (1.3 ± 0.1 mg/100 g). The lipid content was 4.5 g/100 g. Oleic acid was the predominant unsaturated fatty acid (38.74 ± 0.75 g/100 g). Linoleic acid (1.83 ± 0, 01 g/100 g) was also present. Methylxanthines were quantified: caffeine (0.118 ± 0.001) and theobromine (0.0125 ± 0.0002) g/100 g. The total polyphenol content was 0.717 ± 0.001 g/100 g. The results obtained in this work suggest the potential value of the fruits of *I. paraguariensis* for the development of novel products in the food and pharmaceutical industries. This paper aims to contribute to the scientific knowledge of a natural by-product from industry regarding the need of foods and medicines for the new millennium.

1. Introduction

Ilex paraguariensis St. Hilaire (Aquifoliaceae) is a native tree from Northeastern Argentina, Southern Brazil, and Eastern Paraguay, where it is also cultivated. It is one of the most known and used species in South America since the product obtained from its industrialization, yerba mate, is used to prepare a tea-like beverage (infusions or decoctions) that is appreciated for its peculiar flavour and stimulating, antioxidant, choleretic, and nutritional properties [1, 2]. The leaves are also used in folk medicine to treat arthritis, headache, constipation, rheumatism, obesity, fatigue, fluid retention, and liver disorders. This species is exported to Europe, US, Syria, and Japan where it is marketed as a milled plant or extracts used in herbal formulations and functional food products with stimulating, diuretic, antioxidant, and weight-reducing properties. Yerba mate is included in Codex Alimentarius, Argentine Food Code, Latin-American Food Code, and the main scientific acknowledgment Pharmacopoeias (Martindale, British Herbal Pharmacopoeia, German Commission E Monographs) [3]. Nowadays it is also considered a functional food [4]. According to the Argentine Food Code (CAA), yerba mate is defined as the product constituted exclusively by the dried, slightly roasted, and milled leaves of *I. paraguariensis* which can contain fragments of young branches, pedicles, and floral peduncles. However and as a result of the elaboration process, the fruits of this species may be present in the final product which has been consumed over centuries. The fruit is in a nucule and can reach a diameter of approximately 7 mm; there are four or five single-seed pyrenes (propagules). Mate flowers from October to November and fruiting occurs from December to May. During the ripening process, the fruit color changes from green to white, reddish-brown, and finally black when it is fully ripe. There is a rudimentary embryo in many externally ripe seeds which causes a long period of germination.

The maximum allowed content of fruit or organic material in the final product is 1 g/100 g [5]. The great quantity of fruits remaining after the yerba mate processing is discarded.

Argentina is the main yerba mate producer country, with nearly 280000 tons per year followed by Brazil and Paraguay. The worldwide production of yerba mate has ascended to 874678 tons in 2002 [6]. Nonofficial data suggest that Argentina could generate about 560 tons per year of fruits, and the global annual production could exceed 1700 tons per year. Nowadays the fruits have no economic value and they are treated as waste and used as fertilizer.

The objective of this work was to assess the preliminary data on the nutritional valuable elements, the methylxanthines and total polyphenol content of the ripe fruits of *I. paraguariensis* in order to study their potential value as a source of ingredients to be used in the food and pharmaceutical industries.

2. Materials and methods

2.1. Plant Material. Ripe fruits of *I. paraguariensis* were provided by a "yerba mate" factory located in Gobernador Valentín Virasoro in the province of Corrientes, Argentina. The production process of yerba mate involves the harvest of the green leaves and small stems of this species which contain fruits. They are cut manually, put into 100 kg sacks, and then carried to the factory. After harvesting, they were submitted to a roasting process named "sapecado" (exposition to direct fire at temperatures between 250°C and 550°C during 2–4 min) and then to a drying process (exposition to a current of hot air until a 3-4% of moisture is reached).

The fruits analyzed in this work were harvested in April 2009. They were dark reddish-brown in color, with a diameter of 4 to 6 mm. They were identified by PhD. Gustavo Gibeti, botanical specialist in *Ilex* spp. Comparison with voucher specimens of herbarium standards was done. A sample was deposited in the Herbarium Botany Unit of the Faculty of Pharmacy and Biochemistry of the University of Buenos Aires under number BACP: BAF 2 (series 2010). In our laboratory, the fruits were dried in a stove with hot air circulation and thermostatized at 40°C (until 2% of moisture was reached) and then milled to fine powder with a cutter mill with 1 mm pore mesh.

2.2. Standards and Reagents. Standard solutions CertiPUR Merck Chemicals International were employed for the determination of the mineral content. A mixture of fatty acid methyl esters standards from SUPELCO FAME Mix NHI-C and FAME Mix C4–C24 were purchased from Sigma-Aldrich, Buenos Aires, Argentina. Standards of caffeine and theobromine were purchased from Sigma-Aldrich, Argentina. All solvents and reagents used in the experimental work were analytical-grade chemicals except those used in the high-resolution analytical methods which were HPLC grade and purchased from Merck Chemicals Argentina.

2.3. Proximate Analysis. The recommended methods of the Association of Official Analytical Chemists [8] were adopted to determine the levels of moisture, ash, protein, crude fat, and total dietary fiber. The moisture content was determined by heating 5.0 g of each sample to a constant weight in a crucible placed in an oven maintained at 70°C under pressure (≤50 mm Hg). Ash was determined by calcinations of 3.0 g of each sample placed in muffle furnace maintained at 550°C until constant weight. Protein (% total nitrogen × 6.25) was determined by the Kjeldahl method using 1.0 g samples. Fat content was determined gravimetrically after petroleum ether extraction (boiling point range 35–65°C) of 5.0 g samples.

Total dietary fiber (TDF) was determined by the enzymatic-gravimetric method [9]. The total carbohydrate content was defined as the residue, excluding protein, lipid, TDF, and ash, and was calculated as follows:

$$\text{Total carbohydrate} = 100 - (\%\ \text{moisture} + \%\ \text{ash} + \%\ \text{Protein} + \%\ \text{lipid} + \%\ \text{TDF}). \quad (1)$$

2.4. Mineral Content. Test samples (0.5 g) were digested in 15 mL $HNO_3/HCLO_4$ (2 : 1, v/v) according to AOAC [8], and sodium, potassium, calcium, magnesium, copper, iron, and zinc were determined by atomic absorption spectrophotometry (Perkin–Elmer model AA400).

2.5. Fatty Acids Extraction. Milled fruits (aprox. 9.0 g) were homogenized and lipids were extracted with n-hexane by lixiviation until total extraction [10]. The organic solvent was evaporated under vacuum and the oil obtained was esterified. Methyl esters were prepared by transmethylation according to the procedure of the International Organization for Standardization (ISO) [11] and analyzed by gas chromatography (GC).

2.6. Gas-Liquid Chromatography. The fatty acid methyl esters were analyzed using a Clarus 500 Perkin Elmer gas chromatography device equipped with a flame ionization detector (FID) and the TotalChrom software. A fused silica capillary column SP 2560 (Supelco Park, Bellefonte, PA, USA) (100 m × 0.25 mm and 0.20 μm) was employed. The column temperature was programmed as follows: 150°C (1 min), a gradient ranging from 150°C to 210°C for 20 min at a rate of 5°C/min. The injection port and detector were maintained at 240°C and 280°C, respectively. As carrier gas nitrogen was employed at a gas linear speed of 1.3 mL min^{-1}. The individual fatty acids were identified by comparison of retention times and peak areas with those of known mixtures of fatty acid methyl esters (FAMEs) standards.

2.7. Methylxanthines Extraction. Briefly, 500 mg of each sample were placed in a 250 mL round-bottom flask containing 50 mL of methanol and extracted during 30 minutes under a reflux condenser and then filtered. The residue was subjected to the same procedure twice. The filtrates were then combined and dried in a rotary evaporator [12]. The extract obtained was solubilized in 20 mL of water: acetic acid (98 : 2) and transferred to a 25 mL flask. Methanol was added to reach a

final volume. A 45 μm filter (Millipore) was used to filter the extract before HPLC analysis.

2.8. High Performance Liquid Chromatography. A Varian series 9000 equipment with a Varian 9012 binary pump was used. Quantitation of methylxanthine was done using validated HPLC external standard methods [13]. A reverse-phase IB-SIL RP 18 (5 μm, 250 × 4.6 mm I.D.) Phenomenex column and an elution gradient consisting of solvent A: water : acetic acid (98 : 2) and solvent B: methanol : acetic acid (98 : 2) were used. The elution gradient was: from 17% B to 20% B, 10 min; 20% B (isocratic), 5 min; 20% B to 23% B, 10 min; 23% B to 100% B, 5 min with a flow rate of 1.0 mL·min^{-1}. Identification and quantitation were carried out by simultaneous detection with an UV Varian 9050 UV detector and a Varian 9065 photodiode array detector operating at 273 nm. Samples were injected with a Rheodyne injector fitted with a 100 μL loop.

2.9. Total Polyphenol Determination. The total polyphenol content was determined by spectrophotometry according to the Folin-Ciocalteu method [14] using gallic acid as standard. Exactly around 1.0 mg of methanolic extract was weighted and dissolved in 10 mL of deionized distilled water. Briefly, 1.0 mL of this sample extract was transferred in duplicate to separate tubes containing 7.0 mL distilled water, 0.5 mL of Folin-Ciocalteu's reagent, and 1.5 mL of a 20% sodium carbonate anhydrous solution (added 2 min after the Folin–Ciocalteu's reagent). The tubes were then allowed to stand at room temperature for 60 min and then the absorbance at 765 nm was measured by employing a UV-Vis spectrophotometer (Shimadzu UV 2101). The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 10 to 50 μg/mL (Pearson's correlation coefficient: $r^2 = 0,9996$).

2.10. Statistical Analysis. Data were expressed as means ± standard error of the mean of three independent experiments of the same batch carried out by triplicate.

3. Results and Discussion

3.1. Proximate Analysis. The results of moisture content, ash, lipids, proteins, carbohydrates, and fiber are presented in Table 1. The fruits presented considerable amounts of total dietary fiber (TDF) (42.0 g/100 g) and carbohydrates (38.3 g/100 g).

Since the mid- 1970s, the role of dietary fibers in health and nutrition has received considerable attention [15]. The consumption of dietary and functional fibers has many potential health benefits, namely, the ability to lower the incidence of constipation [16] and irritable bowel syndrome [17], to lower cholesterol levels and diminish the incidence of coronary and cardiovascular heart diseases [18, 19] to prevent obesity [20] and the development of diabetes [21], to avoid colon cancer [22], and to increase survival of patients with breast cancer [23].

Table 1: Proximate composition of the fruits of *Ilex paraguariensis*.

Composition	g/100 g raw fruit, dry weight
Moisture	5.9 ± 0.1
Ash	3.8 ± 0.2
Protein (N× 6.25)	5.5 ± 0.1
Lipid yield	4.5 ± 0.3
Total dietary fiber (TDF)	42.0 ± 1.6
Insoluble dietary fiber (IDF)	37.6 ± 1.3
Soluble dietary fiber (SDF)	4.4 ± 0.3
Carbohydrate	38.3 ± 1.2

Results are expressed as the Means ± SEM of three experiments performed in triplicates. The fiber content has been corrected for protein and ash. The carbohydrate was defined as the residue, excluding protein, lipid, TDF, and ash, and was calculated by difference as follows:
Carbohydrate content = 100 − (% moisture + % ash + % Protein + % lipid + % TDF).

Table 2: Mineral composition of the fruits of *Ilex paraguariensis*.

Element	mg/100 g of dry matter	RDA[(1)]	% RDA[(2)]
Sodium	1.3 ± 0.1	500	0
Potassium	1324 ± 15	2000	66
Iron	6.4 ± 0.5	10	64
Copper	1.1 ± 0.1	0.9	122
Zinc	2.3 ± 0.3	15	14
Calcium	150 ± 12	800	19
Magnesium	168 ± 15	350	48

Results are expressed as the Means ± SEM of three experiments performed in triplicates.
[(1)]RDA (NAS/NRC) [7] based on the recommended daily allowances for adults in the 25–50 age range.
[(2)]% RDA (Mean contribution for mineral requirements in terms of RDA (NAS/NRC) [7].

Dietary fiber-rich products have gained popularity as food ingredients to obtain health benefits and have encouraged food scientists to search for new fiber sources as well as to develop high-fiber products [24].

In the agricultural byproducts of some fruits and greens (apple pomace, citrus fruits, olive cake and oat, among others), TDF content ranges from 10.2 to 87.9 g/100 g [25, 26]. In this work, the insoluble dietary fiber (IDF) (37.6 g/100 g) was the predominant fiber fraction (89.5% of TDF). Similar results were reported for pear pomace (IDF: 82.7% of TDF) and apple pomace (IDF: 77.3% of TDF) [26].

The crude protein content was found to be 5.5 g/100 g and the crude lipids 4.5 g/100 g dry matter. The lipid content is similar to that reported for the aerial parts of legumes, which is about 4-5 g/100 g dry matter [27].

3.2. Mineral Content. The mineral content (sodium, potassium, iron, copper, zinc, calcium, and magnesium) was determined. *I. paraguariensis* fruits contain significant amounts of essential minerals that are associated with improved health status when consumed at doses beyond those necessary for preventing a deficiency state. The results obtained in this work are presented in Table 2. As it is shown, the

Table 3: Fatty acid composition[(1)] of the fruits of *Ilex paraguariensis*.

Fatty acid	%[(1)]
C 6 : 0	2.12 ± 0.16
C 8 : 0	0.68 ± 0.07
C 14 : 0	0.14 ± 0.01
C 16 : 0	30.57 ± 0.79
C 16 : 1	0.38 ± 0.01
C 17 : 0	0.99 ± 0.15
C 18 : 0	12.28 ± 0.19
C 18 : 1 *trans*	0.73 ± 0.23
C 18 : 1	38.74 ± 0.75
C 18 : 1 *cis*	0.65 ± 0.03
C 18 : 2 *trans*	2.21 ± 0.11
C 18 : 2	1.83 ± 0.01
C 20 : 0	0.76 ± 0.01
C 20 : 1	0.24 ± 0.01
C 22 : 0	0.17 ± 0.01
C 24 : 0	0.15 ± 0.03
Total saturated	47.86
Total monounsaturated	40.01
Total polyunsaturated	1.83
Trans fatty acids	2.94
Nonidentified minor components	7.36

[(1)] Percent by weight of total fatty acids identified by GC as fatty acids methyl esters (FAME).
Results are expressed as the means ± SEM of three experiments performed in triplicates.

Table 4: Methylxanthine content in the fruits of *Ilex paraguariensis*.

Methylxanthine	g/100 g raw fruit, dry weight
Caffeine	0.118 ± 0.001
Theobromine	0.0125 ± 0.0002

Data are expressed as the means ± SEM of three independent experiments carried out in triplicates.
The values were obtained by HPLC with DAD. Theophylline was not detected. Detection limit: 1 ppm.

most abundant mineral elements were potassium, iron, and magnesium which represent the 66%, 64%, and 48% of the daily allowances recommended for adults in the 25–50 year age range [7] (Table 2). The high quantity of these elements together with the quantity of calcium and the content of the essential elements zinc and copper allow the fruits to be considered as excellent sources of bioelements [28].

3.3. Fatty Acid Analysis. The yield of fatty acids obtained from the fruits was 4.5 ± 0, 3 g/100 g, where oleic acid as the predominant unsaturated fatty acid reaching a 38.74 g/100 g. Linoleic acid, one of the most important polyunsaturated fatty acids in human food and was also present (1.83 ± 0, 01%). Linoleic acid prevents cardiovascular disorders high blood pressure and is part of the structural components of the plasma membrane [29]. Palmitic and stearic acids were also found at high levels in the lipidic fraction (Table 3).

3.4. Methylxanthine Content. Caffeine and theobromine were identified and quantified. Theophylline was not detected. Results are presented in Table 4. The presence of caffeine in the unripe fruits of this plant has been reported previously and was found to be 0.04 g/100 g [30]. The caffeine and theobromine content (0.118 ± 0.001%) and (0.0125 ± 0.0002) found in this work were higher than those reported by other authors. For example, Schubert et al. [31], who also investigated the unripe fruits, reported an amount of 1.16 ± 0.06 mg/g of total methylxanthines, a value which is also lower than our results. These discrepancies could be due to the fact that our material consisted in the ripe fruits of *I. paraguariensis* and the results previously reported were obtained from the unripe ones. This is the first study on the methylxanthines content in the ripe fruits of *I. paraguariensis*.

3.5. Total Polyphenol Determination. The total polyphenol content found in this work was 0.717 ± 0.001 g/100 g gallic acid equivalents, dry wet. The amount of polyphenols found here was higher than those reported by other authors who studied the unripe fruits. Schubert et al. [31] found $54.25 \pm 1 \times 10^{-3}$ to $110.36 \pm 4 \times 10^{-4}$ 4 mg/g. Borré et al. [30] found 0.03% of chlorogenic acid. These differences could indicate that the ripe fruits contain higher amounts of polyphenols than the unripe ones. This is the first study on the polyphenol content in the ripe fruits of *I. paraguariensis*.

4. Conclusions

This paper aims to contribute to the scientific knowledge of a natural by-product from industry regarding the need of foods and medicines for the new millennium.

The results obtained in this work suggest the potential value of the fruits as a fiber and valuable mineral source. These fruits could also be a source of bioactive compounds such as methylxanthines and polyphenols. The utilization of this material could become profitable and at the same time help to minimize waste disposal problems. The results obtained in this work suggest the potential value of the fruits of *Ilex paraguariensis* for the development of novel products in the food and pharmaceutical industries.

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