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Multielemental composition and nutritional value of "dumosa" (*Ilex dumosa*), "yerba mate" (*I. paraguariensis*) and their commercial mixture in different forms of use

Composición multielemental y valor nutricional de "dumosa" (*Ilex dumosa*), "yerba mate" (*I. paraguariensis*) y su mezcla comercial en diferentes formas de uso

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Abstract

The multielement composition and nutritional value of "dumosa" (*Ilex dumosa*) (*ID*), "yerba mate" (*I. paraguariensis*) (*IP*) and their commercial 70:30 mixture (*ID*:*IP*, w/w) in crude drug, brewed mate and tea-bag-like infusion were studied by ICP-OES. This method allowed the detection of an unprecedented number of elements for these species. The order was K>Ca>Mg>P>Mn>Al>Na>Si>Fe in crude drug. *IP* showed higher concentration of minerals than *ID*, but most extractability in the latter largely offset its shortcomings. Brewed mate had higher nutritional value than mate tea because their higher solute/solvent ratio. A portion of brewed mate of both species exceeded the daily recommended intake (DRI) of Mn, and providing Mg, Cu, Fe, Zn and K, too. The portions of mate tea not contribute significantly to the DRI. Some essential trace elements and many ultra-traces ones that exist in crude drug were not detected in aqueous extracts. Most of the heavy metals were not leached into extracts, and in any case never exceeded international safety limits. Therefore, the aqueous extracts of *ID* and *IP* and its mixture are interesting from the nutritional point of view in minerales, and safe in terms of health.

Keywords

Ilex dumosa • *Ilex paraguariensis* • mate • multielemental composition • nutritional value

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Resumen

La composición multielemental y el valor nutricional de "dumosa" (*Ilex dumosa*) (*ID*), "yerba mate" (*I. paraguariensis*) (*IP*) y su mezcla comercial 70:30 (*ID*:*IP*, p/p) fue estudiada por ICP-OES en droga cruda y dos formas de consumo: "cebadura" y "mate cocido". Este método permitió detectar un número sin precedentes de elementos en estas especies. En droga cruda, el orden resultó K>Ca>Mg>P>Mn>Al>Na>Si>Fe. *IP* mostró mayor concentración de minerales que *ID*, pero la mayor extractabilidad en esta última compensó en gran medida sus deficiencias. La cebadura mostró mayor valor nutricional que la infusión, a causa de su mayor relación soluto/disolvente. La porción de cebadura de ambas especies superó la ingesta diaria recomendada (IDR) de Mn, aportando también Mg, Cu, Fe, Zn y K. La porción de mate cocido no contribuyó significativamente a la IDR. Algunos elementos traza esenciales y muchos ultra-trazas presentes en droga cruda no fueron detectados en los extractos acuosos. La mayoría de los metales pesados no fueron lixiviados hacia los extractos, y en todo caso nunca excedieron los límites de seguridad internacionales. Por ello, los extractos acuosos de *ID* e *IP* y sus mezclas son interesantes desde el punto de vista nutricional y son seguros en cuanto a la salud.

Palabras clave

Ilex dumosa • *Ilex paraguariensis* • yerba mate • composición multielemental • valor nutricional

Introduction

According to food laws in Argentina and neighboring countries, "yerba mate" consists of leaves and young stems of *Ilex paraguariensis* A. St.-Hil. (*IP*), undergoing to toasted, drying, milled and aging (7).

The nonalcoholic beverage made from this herb, the brewed mate, is the most popular in Southern South America (Argentina, Brazil, Uruguay, and Paraguay), having extended its use to other countries in America and even the Middle East. Although the processes are generally the same, some particular conditions determine various flavors and types of "yerba mate" to satisfy the different consumers taste (5).

On the other hand, Chile accepts too as "yerba mate" the species *I. brasiliensis* (Spreng.) Loes (11).

In the last years, *I. dumosa* Reissek (*ID*) has been adapted to farming and industrial processes typical of the "yerba mate" (27, 30, 31, 32, 33, 35, 36, 37, 43, 44). This plant, commonly called "yerba señorita" (Spanish), "caa-mirí" (Guarani) or "congonha" (Portuguese), has been considered since colonial times alternatively as a substitute or adulterant of the "yerba mate" (19).

I. dumosa has been formally incorporated into the Argentinean Food Code under the name "dumosa" (8), among the "herbs for infusions". It is particularly interesting because their low xanthine content (0.1-0.2% in dry weight *vs.* 1-2% in *IP*) and resistance against some diseases (44). Also, it can be grown in large lowland areas of Corrientes province, in Northeastern Argentina (43). It has been recognized two varieties of *I. dumosa*: *I. d.* var. *dumosa* (the typical one), *I. d.* var. *guaranina* Loes. Technically, the first is the most suitable for cultivation, and some of its cultivars have been selected and identified (43). Although its infusion has some differences in taste with the "yerba mate", it is palatable at least for some consumers. It is present in the Argentinean market since 2007, especially in a mixture with *IP*, in the form labeled "light-relax", prepared by a ratio of 70% of *ID* and 30% of *IP*, suitable for infusions, with low xanthine and similar taste to traditional "mate" (31). Meanwhile, in the wild "yerbales" (*Ilex* forests) in the Brazilian states of Santa Catarina and Paraná, *ID* is commercially exploited together with *IP*.

The aim of this paper was to report the results of multielemental analysis, determine the nutritional mineral value and heavy metals content in both the crude material and in two different forms of consumption of *I. dumosa*: brewed "mate" in a gourd ("cebadura" or "chimarrão") and tea-bag-like infusion in a cup ("mate cocido" or "chá mate", 1.5% infusion), compared to *IP* and the mixture 70:30 of both species, as currently occurs in the market.

Material and methods

Plant material

Leaves and young branches of *Ilex dumosa* and *I. paraguariensis* were harvested separately from standardized commercial crops located at the province of Corrientes, Argentina. The voucher specimens are as follows:

Ilex dumosa Reissek var. *dumosa*

Argentina, Prov. Corrientes, Dpto. Santo Tomé, Gob. Virasoro, Establecimiento "Las Marías", "dumosa" de campo (cultivo), -28° 09' 21.73", -56° 00' 17.34". M.G. Maiocchi w/n., a. 2003 (UNSL #531b).

Ilex paraguariensis A. St.-Hil.

Argentina, Prov. Corrientes, Dpto. Santo Tomé, Gob. Virasoro, Establecimiento "Las Marías", "yerba mate" de campo (cultivo), -28° 09' 21.73", -56° 01' 01.59". M.G. Maiocchi w/n., a. 2003 (UNSL #532).

All material was treated according to the modern techniques of "yerba mate" industry, which comprise blanching, drying, and aging. The blanching was made by flash heating over open flame, (400-550°C for 2 minutes). After cooled it was crushed and dried in a rotary kiln with airflow (110°C during 4 h), up to 2-6% remaining moisture. Then, the leaves were separated from the stems by a grid (1x20cm), and stored (50-60°C for 60 days); after which they were milled and passed through a series of standard sieves (ASTME-11/95 n°100 and n°40) to achieved commercial size (0.150mm up to 0.425mm). Moreover, a mixture 70:30 w/w (*ID*:*IP*) was especially prepared to simulate the commercial product recently admitted to the market.

The material to tea-bag-like infusion ("mate cocido") was prepared as above, but using the fraction obtained by milling and sieving of the leaves into particles of about 0.425mm to 0.500mm (ASTME-11/95 standard n° 40 and n° 35 sieves). This material was used to fill the paper bags of 3g each, which are used for preparing the 1.5% infusion.

Obtaining aqueous extracts

The traditional preparation of mate in a gourd (called "cebadura" in Spanish and "chimarrão" in Portuguese) was simulated in the laboratory by means of a device especially developed for this purpose by Maiocchi & Avanza (1998) using a separating funnel with Whatman n°5 paper and vacuum connection (figure 1), from 50g sample and 500mL of pure water at 84°C, divided into 10 treatments of 50mL each (as done in the normal mate brewed procedure), and placed in contact with the material for 1 minute, finally obtaining 10 extracts. These values obey the usual conditions of use of a dry gourd to brew the mate and a metallic straw ("bombilla") to suck the infusion.

The tea-bag-like 1.5% infusion (called "mate cocido" or "chá mate") was prepared by adding 200mL of recently boiled (*c.* 84°C) pure water upon 3g of each product, allowing to stand for 5 minutes, according to the most common market presentation (bags of 3g each) and time recommended in various commercial packaging.

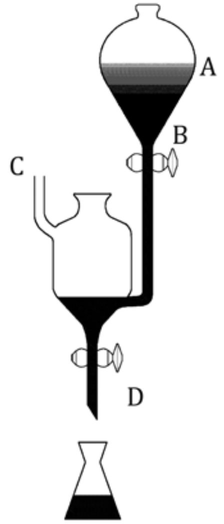

Figure 1. Device for brewed "mate" simulation. A: separatory funnel, B: filter media, C: vacuum connection, D: out.

Figura 1. Dispositivo para la simulación de la "cebadura". A: embudo de decantación, B: medio filtrante, C: conexión a vacío, D: salida.

Then it was filtered through a mesh (33) and through Whatman N° 5 paper in order to retain the particles of material that had passed through the mesh.

These conditions determined both the serving size of brewed mate (50g:500mL) and tea-bag-like infusion (3g:200mL) (33, 37).

Mineral composition analysis and nutritional evaluation

Crude drug

It was applied the method described by Abou-Arab & Abou Donia (2000) with some modifications to adapt it to our working conditions: 5g of crude drug of each powdered sample were dried in an oven (102-105°C during 48 h) until constant weight. This mixture was transferred to a porcelain crucible, and then 5mL of Indium 500mg/L (used as internal standard to evaluate the degree of recovery) was added. Then cover it with a cap and reduced to ash in a muffle (650°C during 5 h).

After cooled, it was dissolved with 10mL of concentrated HNO_3, transferred to a 50mL volumetric flask and filled to the mark with deonised water.

The concentrations of 32 selected elements as well as the internal standard (In) were determined by ICP-OES, using an inductively coupled radial spectrometer Varian-PRO (Series EL 05083717), with a Czerny-Turner monocromator, holographic diffraction grid and a VistaChip charge coupled device (CCD) array detector, calibrated quarterly, and used a precision scale Kretz model 5530 adequately calibrated by the Centro Metrológico Cuyo (CEMEC, ISO 9001).

The working wavelength and the detection limit for each element are shown in table 1 (page 149).

Table 1. Wavelenght and detection limit for each element

Tabla 1. Longitud de onda y límite de detección para cada elemento

Element	**Ag**	**Al**	**As**	**B**	**Ba**	**Ca**	**Cd**	**Co**
Wavelenght (nm)	328.068	308.215	193.696	249.773	493.408	317.933	226.502	228.615
Detection limit (mg kg^{-1})	0.021	0.06	0.105	0.009	0.003	0.021	0.003	0.006
Element	**Cr**	**Cu**	**Fe**	**Hg**	**K**	**Li**	**Mg**	**Mn**
Wavelenght (nm)	267.716	324.754	259.940	194.164	766.491	670.783	279.800	257.610
Detection limit (mg kg^{-1})	0.012	0.009	0.012	0.03	0.45	0.006	0.06	0.003
Element	**Mo**	**Na**	**Ni**	**P**	**Pb**	**Sb**	**Se**	**Si**
Wavelenght (nm)	202.032	588.995	231.604	213.618	220.353	206.834	196.026	251.611
Detection limit (mg kg^{-1})	0.015	0.06	0.03	0.15	0.084	0.063	0.15	0.06
Element	**Sn**	**Sr**	**Th**	**Ti**	**Tl**	**U**	**V**	**Zn**
Wavelenght (nm)	189.927	421.552	401.913	334.947	377.572	409.013	292.401	213.857
Detection limit (mg kg^{-1})	0.051	0.001	0.09	0.006	0.081	0.45	0.009	0.006

Calibration curves were established for each element using five concentrations, and the regression coefficient (r^2) values of such curves ranged from 0.994 to 0.999.

The validation of the method was verified by mean of the addition of a standard (22, 25, 45), in this case Indium, for the evaluation of the recovery percentage as well as eventual losses during the ashing step (26).

The recovery value, calculated by equation [(found - initial) / added] x 100, varies from 95.2% to 101.8%, with a mean of 98.5 ±3.3% (n = 32). This result is considered acceptable taking in mind both the instrumental error and detection limit as well as the results reported in the literature (1). All reagents used were of analytical grade (Merck, Sigma).

The results were expressed in mg kg^{-1} of dry matter.

Aqueous extracts

For its part, the aqueous extracts (brewed mate and tea-bag-like infusion) were dried and the residues charred; then were treated with the same procedure as in the case of the crude drug.

Daily consumption was established on an average of 1L of brewed mate per person per day and 600mL of tea-bag-like infusion per person per day, *i. e.*, 3 cups of 200mL each (37).

The appraisal of nutritional value and/or potential hazard to health of mineral content was made on the basis of the requirements of male or female between 19-50 years old, according to international parameters, especially the dietary reference intakes (DRIs) (17, 49, 50, 51, 52), and in case of some elements, the recommended dietary allowance (RDA), the adequate intake (AI) and the tolerable upper intake level (UL, from www.nap.edu), even the estimated dietary intakes (12) as well as the typical daily nutritional intake (42).

Statistical analysis

Data collected from ten batches of each species and its mixture were subjected to statistical analysis, by means of the Software *Infostat* (14). One-way ANOVA (α=0.005) and LSD (Fisher's Least Significance Difference) were applied. The values are represented by their arithmetic mean and corresponding standard deviation.

Results and discussion

Ashes

The ashes content in crude drug and aqueous extracts are shown in table 2.

Ilex paraguariensis contains ashes in a significantly ($p<0.05$) higher proportion than *I. dumosa*, both in crude drug (*c.* 13% more), brewed mate (*c.* 20%) and tea-bag-like infusion (*c.* 27%).

The deashing profiles behave similarly in both species throughout the extraction process, which increases to "mate" #3 and then gradually decreases (table 3, page 151).

Similar values were reported especially for *I. paraguariensis* (3, 15, 28, 46), that fall within the provisions of the Argentinean Food Code (7, 37) that sets a maximum of 9 g% on dry weight.

Samples analytical data on 32 elements

Analytical data on 32 elements from samples of crude drugs and their aqueous extracts were obtained. Essentials quantitative (required >100 mg per day), essentials trace (<100 mg per day) and ultra-trace elements that are useful in the nutrition and maintenance of human health are listed in table 4 (page 152), table 5 (page 153) and table 6 (page 154), along with some elements whose importance is still being investigated (6) or that have proven harmful properties.

Table 2 shows that element content of crude drug of *IP* was higher or in some cases very similar than those of *ID*, except for Zn, Cu, Ba, Sr and Th. It highlights the high Mn content and the low Na in both species.

The mineral content of the mixture was always related directly to the 70:30 proportion.

Among the major essential elements, crude drug contain valuable amounts of K>Ca>Mg>P, in the same sequence in both plant species, but a higher concentration in *IP* than in *ID* (2 times for P, 1.4 times for K, 1.3 times for Ca and Mg).

In terms of essential trace elements, no significant differences were found at species level about content of Mn, Fe, and Se, what did happen in the case of Zn, Li, Cu, Ni, Co, Cr and V of both plant species.

The most abundant essential trace element is Mn, at a similar level in both species (*c.* 600-700 mg kg^{-1}).

Finally, Mo was not detected at all in *ID* and the mixture, while in *IP* was present but in amounts below the instrumental limit.

Table 2. Total ashes in crude drug and aqueous extracts.

Tabla 2. Cenizas totales en droga cruda y extractos acuosos.

Total ashes	***Ilex dumosa*** (n=10)	***I. paraguariensis*** (n=10)	**Mixture 70:30** (n=10)
Crude drug (g% DW)	4.7 ± 0.6	5.3* ± 0.7	4.5 ± 0.9
Brewed mate ("Cebadura") (mg per serving)	1294 ± 68	1631* ± 82	1419 ± 75
Tea-bag-like 1.5% infusion ("mate cocido") (mg per serving)	77.3 ± 3.7	106* ± 2	86.9 ± 4.2

DW= dry weight of crude drug; brewed mate serving size= 50g: 500mL; tea-bag-like infusion serving size= 3g: 200mL.

DW= peso seco de droga cruda; porción de cabadura= 50g: 500mL; porción de infusión de saquitos = 3g: 200mL.

Table 3. Brewed mate: ashes per serving size (mg).

Tabla 3. Cebadura: cenizas por porción (mg).

Mate n°	***Ilex paraguariensis***		***Ilex dumosa***	
	mg ashes/kg DW	Standard error	mg ashes/kg DW	Standard error
1	71	6.5	82	2.0
2	255	5	196	6.5
3	285	6.5	209	7.5
4	249	6.5	172	6.5
5	215	8.5	150	6.5
6	168	6.5	126	3
7	132	5.5	109	4
8	106	5.5	91	5.5
9	83	3	85	5.5
10	67	2.5	74	5.5

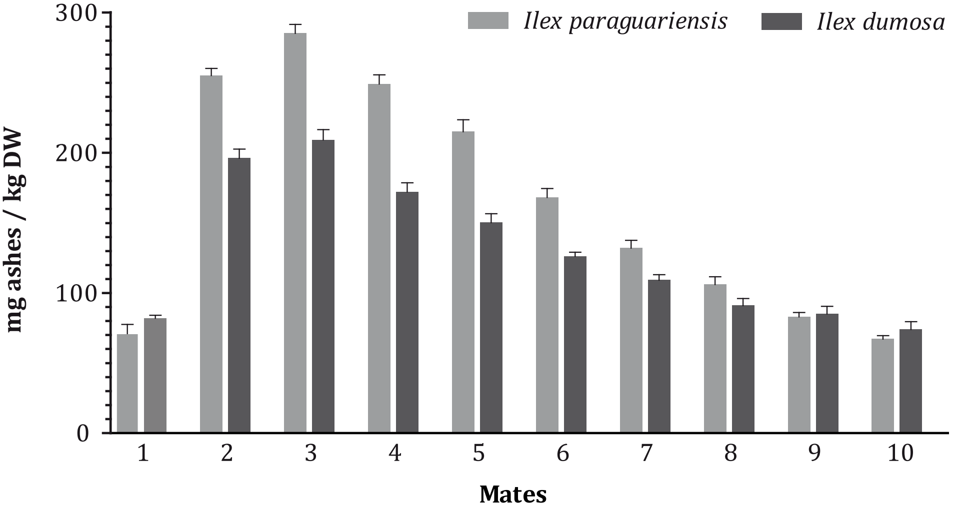

Table 4. Elemental content in crude drug (DW).

Tabla 4. Contenido elemental en droga cruda (DW).

Element	lod (mg kg^{-1} ashes)	***Ilex dumosa*** (mg kg^{-1} DW) (n=10)	***I. paraguariensis*** (mg kg^{-1} DW) (n=10)	**Mixture 70:30** (mg kg^{-1} DW) (n=10)	**Minimum-maximum** values from others sources and methods (mg kg^{-1} DW)	
					I. dumosa*	***I. paraguariensis*****
Quantitative Essentials Elements (required >100 mg day^{-1})						
K	0.45	7958 ± 616	11352 ± 1271	8254 ± 957	2400-8400	8000-20800
Ca	0.021	6266 ± 558	7688 ± 1256	6857 ± 1270	6100-8800	3500-14500
Mg	0.06	5246 ± 629	6988 ± 1340	5959 ± 869	4000-4450	2800-9100
P	0.15	641 ± 41	1371 ± 259	823 ± 98	500	670-2100
Na	0.06	148 ± 11	372 ± 55	231 ± 28	42	15-1706
Essential Trace Elements (required <100 mg day^{-1})						
Mn	0.003	631 ± 80	685 ± 148	648 ± 177	540-840	580-2330
Fe	0.012	104 ± 13	124 ± 31	109 ± 9	50-85	69-290
Zn	0.006	90 ± 10	38 ± 7	77 ± 22	34-500	10-77
Li	0.006	7.8 ± 1.5	23 ± 5	13 ± 2	No data	No data
Cu	0.009	15.4 ± 3.3	8.7 ± 1.9	13 ± 3	8.5-10	4.7-18
Ni	0.03	2.7 ± 0.4	4.9 ± 1.5	3.4 ± 0.6	No data	0.01-3.5
Co	0.006	0.31 ± 0.03	0.63 ± 0.18	0.40 ± 0.08	No data	No data
Cr	0.012	0.39 ± 0.06	0.99 ± 0.26	0.60 ± 0.16	No data	1.2-2
Se	0.15	0.64 ± 0.11	0.79 ± 0.09	0.70 ± 0.24	No data	No data
V	0.009	0.19 ± 0.09	0.53 ± 0.11	0.27 ± 0.08	No data	No data
Mo	0.015	nd	<ld	nd	No data	No data
Ultra-trace Elements						
Al	0.06	296 ± 43	643 ± 96	412.8 ± 92.4	No data	120-700
Si	0.06	138 ± 15	333 ± 42	189.1 ± 42.3	No data	515-580
Ba	0.003	97 ± 16	85 ± 20	95.24 ± 18.67	No data	No data
Sr	0.001	88 ± 14	46 ± 10	76.06 ± 9.88	No data	No data
B	0.009	60 ± 13	92 ± 16	72.74 ± 15.97	No data	15-70
Ti	0.006	3.5 ± 0.9	7.6 ± 1.9	4.89 ± 0.76	No data	27-33
Th	0.09	3.4 ± 0.6	2.5 ± 0.7	3.12 ± 1.06	No data	No data
Pb	0.084	0.83 ± 0.27	2.9 ± 0.6	1.26 ± 0.35	No data	0.03-0.18
Sb	0.063	0.35 ± 0.11	0.61 ± 0.09	0.477 ± 0.114	No data	No data

DW: dry weight of crude drug; **lod**: limit of detection; **nd**: not detected; ***** according to 34, 53 (reference); ****** according to 9, 10, 20, 21, 24, 34, 38, 47, 53, 55 (reference).- **Note:** Ag, As, Cd, Hg, Sn, Tl and U were detected in amounts below the instrumental limit

DW: peso seco de droga cruda; **lod**: límite de detección; **nd**: no detectado; ***** según 34, 53 (bibliografía); ****** según 9, 10, 20, 21, 24, 34, 38, 47, 53, 55 (bibliografía).- **Nota:** Ag, As, Cd, Hg, Sn, Tl y U fueron detectados en cantidades inferiores al límite instrumental.

Table 5. Brewed mate: elemental content, nutritional value and/or toxicity level.

Tabla 5. Cebadura: contenido elemental, valor nutricional y/o nivel de toxicidad.

Element	DRIs and/or ULs [1] (mg day^{-1})	***Ilex dumosa*** (n=10)		***I. paraguariensis*** (n=10)		**Mixture 70:30, *ID:IP*** (n=10)	
		mg per serving[2]	Input to DRI/UL (%)	mg per serving[2]	Input to DRI/UL (%)	mg per serving[2]	Input to DRI/UL (%)
Quantitative Essentials Elements (required >100 mg day^{-1})							
K	4700[a]	354 ± 78	7.5[a]	477 ± 85	10.1[a]	393 ± 69	8.4[a]
Ca	1000-1200[a]; 2500[b]	26 ± 3	2.1-2.6[a]	7.7 ± 1.4	0.64-0.77[a]	20 ± 4	1.6-2[a]
Mg	310-320[c]; 350[b]	136 ± 30	42-44[c]	129 ± 42	40-42[c]	141 ± 36	44-45[c]
P	700[c]; 4,000[b]	20 ± 6	2.9[c]	38 ± 5	5.4[c]	24 ± 5	3.4[c]
Na	1500[a]; 2300[b]	6.2 ± 0.9	0.4[a]	16 ± 1.4	1.1[a]	8.5 ± 1.2	0.60[a]
Essential Trace Elements (required <100 mg day^{-1})							
Mn	1.8-2.3[a]; 11[b]	24 ± 3	1026-1312[a]	16 ± 4	684-875[a]	21 ± 5	916-1171[a]
Fe	8-18[c]; 45[b]	3.0 ± 0.4	16-38[c]	2.7 ± 0.7	15-34.1[c]	2.9 ± 0.5	16-37[c]
Zn	8-11[c]; 40[b]	1.1 ± 0.1	9.8-13[c]	0.65 ± 0.13	4.8-8.1[c]	0.99 ± 0.27	9-12[c]
Li	0.2-0.6[d]	0.01 ± 0.003	1.3-4[d]	0.03 ± 0.01	3.3-10[d]	0.02 ± 0.01	1.7-5[d]
Cu	0.9[c]; 10[b]	0.30 ± 0.07	32[c]	0.33 ± 0.10	35[c]	0.26 ± 0.06	29[c]
Ni	1[b]	0.07 ± 0.02	7[b]	0.10 ± 0.03	10[b]	0.08 ± 0.03	8[b]
Co	undetermined	<lod	-	0.011 ± 0.003	-	0.008 ± 0.002	-
Ultra-trace Elements							
Al	2-10[d]	0.27 ± 0.05	2.7-13[d]	0.65 ± 0.12	6.4-32[d]	0.37 ± 0.08	3.6-18[d]
Si	Not determined	2.3 ± 0.4	-	5.0 ± 1.2	-	3.2 ± 0.8	-
Ba	8-11[c]; 40[b]	0.20 ± 0.05	1.7-2.4[c]	0.17 ± 0.04	1.5-2.1[c]	0.18 ± 0.24	1.6-2.2[c]
Sr	Not determined	0.15 ± 0.02	-	0.21 ± 0.04	-	0.17 ± 0.05	-
B	20[b]	0.27 ± 0.08	1.3[b]	0.15 ± 0.03	0.70[b]	0.22 ± 0.05	1.0[b]

Ref.: **lod**= limit of detection; **[1]** calculated on the basis of RDAs (recommended dietary allowances) and/or ULs (tolerable upper intake level), based on the needs of men and women aged 19-50 years, where **[a]** is the Adequate intake (AI, www.nap.edu); **[b]** Tolerable upper intake level (UL, FNB 1998); **[c]** Recommended dietary allowance (RDA, www.nap.edu); **[d]** Typical daily intake (Nielsen, 2003); [e] Estimated dietary intake (COT, 2003).- **[2]** Serving size: 50g of crude drug in 500mL water recently boiled (84°C).- **Note**: essential trace elements **Cr**, **Se**, **V**, and **Mo** and ultra-trace **Ti**, **Th**, **Pb**, **Sb**, **Ag**, **As**, **Cd**, **Hg**, **Sn**, **Tl**, and **U** were not detected in the brewed mate.

Ref.: **lod**= límite de detección; **[1]** calculado en base a las RDAs (consumo de referencia alimenticio) y/o ULs (ingesta máxima admisible), fundadas en las necesidades nutricionales de hombres y mujeres entre 19-50 años, donde **[a]** es la Ingesta Adecuada (AI, www.nap.edu); **[b]** el nivel de ingesta máxima admisible (UL, FNB 1998); **[c]** el consumo de referencia alimenticio (RDA, www.nap.edu); **[d]** la ingesta diaria típica (Nielsen, 2003); [e] la ingesta dietaria estimada (COT, 2003).- **[2]** tamaño de porción: 50 g de droga cruda en 500 ml de agua recientemente hervida (84°C).- **Nota**: los elementos esenciales traza **Cr**, **Se**, **V** y **Mo** y los ultra-traza **Ti**, **Th**, **Pb**, **Sb**, **Ag**, **As**, **Cd**, **Hg**, **Sn**, **Tl** y **U** no fueron detectados en la cebadura.

Table 6. Tea-bag-like infusion: elemental content, nutritional value and/or toxicity level.

Tabla 6. "Mate cocido" (infusión): contenido elemental, valor nutritional y/o nivel de toxicidad.

Element	**DRIs and/or ULs** [(1)] (mg day^{-1})	***Ilex dumosa*** (n=10)		***I. paraguariensis*** (n=10)		**Mixture 70:30, *ID*:*IP*** (n=10)	
		mg per serving[(2)]	Input to DRI/ UL (%)	mg per serving[(2)]	Input to DRI/ UL (%)	mg per serving[(2)]	Input to DRI/ UL (%)
Quantitative Essentials Elements (required >100 mg day^{-1})							
K	4700[a]	22.4 ± 4.0	0.47[a]	32.6 ± 8.0	0.69[a]	23.4 ± 6.0	0.5[a]
Ca	1000-1200[a]; 2500[b]	1.5 ± 0.3	0.13-0.15[a]	0.67 ± 0.10	0.05-0.07[a]	1.0 ± 0.2	0.08-0.10[a]
Mg	310-320[c]; 350[b]	7.9 ± 1.7	2.4-2.5[c]	7.5 ± 1.8	2.3-2.4[c]	7.7 ± 2.1	2.4-2.5[c]
P	700[c]; 4000[b]	1.2 ± 0.4	0.17[c]	3.1 ± 0.8	0.44[c]	1.6 ± 0.4	0.23[c]
Na	1500[a]; 2300[b]	0.35 ± 0.08	0.02[a]	0.96 ± 0.21	0.06[a]	0.53 ± 0.12	0.03[a]
Essential Trace Elements (required <100 mg day^{-1})							
Mn	1.8-2.3[a]; 11[b]	1.4 ± 0.6	61-79[a]	0.94 ± 0.29	40-52 [a]	1.2 ± 0.4	51-65.5[a]
Fe	8-18[c]; 45[b]	0.16 ± 0.03	0.89-2[c]	0.18 ± 0.03	1-2.2[c]	0.16 ± 0.05	0.89-2[c]
Zn	8-11[c]; 40[b]	0.06 ± 0.02	0.54-0.75[c]	0.04 ± 0.01	0.36-0.51[c]	0.06 ±0.01	0.45-0.62[c]
Li	0.2-0.6[d]	<lod	-	<lod	-	<lod	-
Cu	0.9[c]; 10[b]	0,03 ± 0.01	3.1[c]	0.03 ± 0.009	3.0[c]	0.03 ± 0.009	3.1[c]
Ni	1[b]	<lod	-	<lod	-	<lod	-
Co	Not determined	<lod	-	<lod	-	<lod	-
Ultra-trace Elements							
Al	2-10[d]	<lod	-	0.04 ± 0.01	-	<lod	-
Si	Not determined	0.12 ± 0.03	-	0.94 ± 0.13	-	0.31 ± 0.06	-
Ba	8-11[c]; 40[b]	0.01 ± 0.007	0.09-0.12[c]	0.01 ± 0.002	0.08-0.11[c]	0.02 ± 0.004	0.09-0.12[c]
Sr	Not determined	0.01 ± 0.005	-	0.03 ± 0.009	-	0.02 ± 0.004	-
B	20[b]	0.02 ± 0.005	0.1[b]	<lod	-	<lod	-

Ref.: **lod**= limit of detection; **[(1)]** calculated on the basis of RDAs (recommended dietary allowances, www.nap.edu) and/or ULs (tolerable upper intake), based on the needs of men and women aged 19-50 years, where **[(a)]** is the Adequate intake (AI, www.nap.edu); **[(b)]** Tolerable upper intake level (UL, FNB 1998); **[(c)]** Recommended dietary allowance (RDA, www.nap.edu); **[(d)]** Typical daily intake (Nielsen, 2003); and **[(e)]** Estimated dietary intake (COT, 2003).- **[(2)]** Serving size: 3g of crude drug in 200mL boiling water and let stand 5 min.- **Note**: essential trace elements **Cr**, **Se**, **V** and **Mo** and ultra-trace **Ti**, **Th**, **Pb**, **Sb**, **Ag**, **As**, **Cd**, **Hg**, **Sn**, **Tl**, and **U** were not detected in the tea-bag-like infusion.

Ref.: **lod**= límite de detección; **[(1)]** calculado en base a las RDAs (consumo de referencia alimenticio, www.nap.edu) y/o ULs (ingesta máxima admisible), fundadas en las necesidades nutricionales de hombres y mujeres entre 19-50 años, donde **[(a)]** es la Ingesta Adecuada (AI, www.nap.edu); **[(b)]** el nivel de ingesta máxima admisible (UL, FNB 1998); **[(c)]** el consumo de referencia alimenticio (RDA, www.nap.edu); **[(d)]** la ingesta diaria típica (Nielsen, 2003); y [(e)] la ingesta dietaria estimada (COT, 2003).- **[(2)]** Tamaño de porción: 3 g de droga cruda en 200 ml de agua hirviente y dejando reposar 5 min.- **Nota**: los elementos esenciales traza **Cr**, **Se**, **V** y **Mo** y los ultra-traza **Ti**, **Th**, **Pb**, **Sb**, **Ag**, **As**, **Cd**, **Hg**, **Sn**, **Tl** y **U** no fueron detectados en el "mate cocido".

Aluminium was the most abundant ultra-trace element, followed by Si; in both cases the concentration is significantly greatest in *IP*, near doubling that of *ID*. On the contrary, Sr appears more abundant in *ID*, and as for Ba, concentrations in both species are quite similar.

Other elements (Th, Ti, Pb and Sb) are present in low concentration (<4 mg kg^{-1}) in crude drug of both species, and the other ones (Ag, As, Cd, Hg, Sn, Tl and U) were found at concentrations below the instrumental limit. For this reason, it has been developed an analytic method with preconcentration, whereby were detected ultratrace of lead in infusions of yerba mate (7.6-8.9 μg L^{-1}; 38), always well below the admissible limits in foods (16).

The results are comparable to those of other studies carried out on leaves and leaves + stems of both plant species (especially *IP*; table 4, page 152), being included in the dispersion of values found both in Argentinean and Brazilian materials (24, 47, 53), although the methodology used here provides values for an unprecedented number of mineral elements.

Both *ID* and *IP* have shown similar amounts in terms of essential elements to crude drug of other plant species used for nutritional beverages, as coffee, tea, and cocoa (13, 18, 23, 24, 29), while the trace and ultra-trace elements varies from similar amounts to several times in more or less concentration, depending on botanical source, soil, climate and anthropogenic factors (2, 3, 40, 41).

From the nutritional point of view, the brewed mate of these species can be considered a source of Mg, Mn, Fe, Cu, and Al, because a serving provides more than 15% of dietary reference intake (DRI) of these minerals (table 5, page 153). Mn is present in large amounts (between 10-13 times more than daily requirements in the case of *ID* and 7-9 times for *IP*), far exceeding the limit of upper daily intake (11 mg day^{-1}), but it should be noted that because of its low bioavailability (54), this mineral is absorbed by the human body only in 3% of their concentration (48), turning acceptable their high level in this form of consumption.

A serving of brewed mate also covers between 40-45% of daily needs of Mg, 15-38% of Fe requirement, 28-35% of the needs of Cu, and between 7.5-10% of K requirement. Regarding Aluminium, their contribution is higher in the brewed mate of *IP* (between 6-32%), and progressively reduced in the mixture (3-18%) and in *ID* (2-13%). Another important contribution is about Zn (*c.* 5-13%), and to a lesser extent those of Ca, P, Li, Ni and Ba.

The tea-bag-like infusion shows only 3 elements (Mn, Mg, and Cu) in interesting amount (table 6, page 154). A serving of *ID* infusion provides 60-80% of daily requirement of Mn, and 40-50% in the case of *IP*; hence, infusion of both species and its mixtures in any proportion can be considered sources of this element, in despite to their low absorption by the human body (48, 54). Cu and Mg are provided at a rate of 2-5% of the DRI, with minor differences between species.

For its part, K, Ca, P, Na, Fe, Zn, Si, Ba, Sr and B are present in smaller amounts than 1% of the daily requirement, while Li, Ni, Co, and Al appear below the instrumental limit. Finally, some trace essential elements (Cr, Se, V and Mo) and many ultra-trace elements (Ti, Th, Pb, Sb, Ag, As, Cd, Hg, Sn, Tl, and U) were not detected at all in these form of infusion.

Extractability

The two species showed differences in the extractability of minerals. *ID* had a higher extraction capacity for some elements, but the aqueous extracts of *IP* are generally richer in minerals, especially by the original highest concentration in the crude drug, in addition to state and solubility of each mineral, as well as the relations established between them and with organic substances.

The extractability values for K, Mg, P, and Na ranging from 94% (K) to 37% (Mg), with noteworthy interspecific differences. *ID* has the highest extractability of Mg: 50% and 52% in brewed mate and tea-bag-like infusion respectively, compared with 35% and 37% in *IP*. This fact compensates the significantly lowest concentration shown by the crude drug of the former species (5200 *vs.* 7000 mg kg^{-1}).

As expected, Ca has a very low rate of extractability (2-8%), always higher in *ID*. Mn appears in high concentration in crude drug of both species, providing this element in large quantities to both aqueous extracts, although *ID* shows significantly greater extractability (75%) than *IP* (46%). Other elements extracted in a high proportion were Fe (40-60%), Cu (35-80%) and Ni (40-50%), but because the low concentration of the latter in the crude drug, it was not detected in the tea-bag-like infusion.

Both Cu and Zn are extracted in greater proportion from *IP*, which largely offset the lower concentrations of both elements in comparison with *ID*. In contrast, the extractability of Fe, Ni, Co, and Li showed no significant differences between these plant species. The Fe content is comparatively low, with similar values in both species, and polyphenols (that are abundant in these plants) could be responsible for the low dialyzability of this element.

Although Ag, As, Cd, Hg, Mo, Sn, Tl, and U were found in the crude drug, in the aqueous extracts were recorded below the instrumental limit. Cr, Pb, Sb, Se, Th, Ti, and V have exceeded the limit of detection in crude drug, but were not found in the extracts.

Heavy metals

Interpreted in the broadest sense of the word, none of the numerous analyzed heavy metals has been found in high concentration in crude drug of both species (table 4, page 152) except to Mn, whose quite low gastrointestinal absorption (47, 53) determines real values nearest to 0.6-0.8 mg day^{-1} for brewed mate of *ID*, 0.3-0.6 mg day^{-1} for *IP*, and 0.5-0.8 mg day^{-1} for the mixture. Since most of them were not detected in the aqueous extracts (table 5, page 153; table 6, page 154), heavy metals are not a problem in daily intake of brewed mate and tea-bag-like infusion. From this point of view, and in our working conditions, both species constitute safe foods for human consumption.

Conclusions

At least 26 elements occur both in crude drug of *Ilex dumosa* (*ID*), *I. paraguariensis* (*IP*), and their commercial mixture (70:30 w/w). While *IP* shows c. 20% more ash concentration, *ID* reveals major extractability of minerals. The use form brewed mate in a gourd ("cebadura" or "chimarrão") is significantly more valuable from the standpoint of mineral nutrition than tea-bag-like infusion in a cup ("mate cocido" or "chá mate", 1.5% infusion).

The brewed mate is a dietary source of Mn, Mg, Cu and Fe, and contributes with an interesting amount of dietary intake of Zn, K, P, Al, Ni and Li. Instead, the tea-bag -like infusion provides only a low proportion of the daily requirements of Mn, Mg, and Cu. Moreover, both forms of use give a low amount of Na, while heavy metals never exceed the limits of food regulations.

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