

Nutritional parameters of "mate cocido" with milk prepared from two species of *Ilex* and their commercial mixture for school age population

Parámetros nutricionales del "mate cocido" con leche preparado a partir de dos especies de *Ilex* y su mezcla comercial, para la población en edad escolar

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ABSTRACT

The infusion at 1.5% of yerba mate (*Ilex paraguariensis* A.St.-Hil.) (Ip) corresponds to "cooked mate" (MC), whose combination with milk (50:50 v/v) (M) and refined sugar (S) is the "mate cocido with milk", a popular food in southern South America offered to preschool and primary education students (3-13 years old) in Argentina. Another species of the genus, *I. dumosa* Reissek (Id), has low caffeine; appears in the market as an Id:Ip (70:30 w/w) mixture. The substitution of Ip by the mentioned mixture maintains analogous nutritional values and significantly lowers the caffeine levels ($\alpha=0.05$); this allows for a higher consumption of the food during the day, without reaching the xanthine daily limits (40-100 mg day⁻¹) for the age range, favoring the students intellectual and physical performance without adverse effects. The daily intake of a double serving (breakfast and afternoon snack) of MC+M+S provides high energy and is a source of carbohydrates, proteins, vitamins A, B₁, B₂, B₅, B₆, and B₁₂, biotin and essential minerals (Mn, P, Zn, Cu, Ca, Mg, Na, Cl, K, Fe and Al), with low cholesterol. The nutritional parameters are within international limits. Heavy metals do not make it to the infusion or they remain well below harmful values.

Keywords

"Chá mate" • *Ilex dumosa* • *Ilex paraguariensis* • "Mate cocido" • "Mate cocido" with milk • nutritional value • schoolchildren diet

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RESUMEN

La infusión al 1,5% de "yerba mate" (*Ilex paraguariensis* A.St. -Hil.) (Ip) constituye el "mate cocido" (MC), que con leche (50:50 v/v) (M) y azúcar refinada (S) es el "mate cocido con leche", alimento popular en el Cono Sur de América ofrecido en la escuela a niños de educación inicial y primaria (3-13 años) en Argentina. Otra especie del género, *I. dumosa* Reissek (Id), tiene baja cafeína; se presenta en el mercado en mezcla con Ip (Id:Ip 70:30 p/p). Sustituyendo Ip por la mezcla, el MC+M+S mantiene valores nutricionales análogos con niveles significativamente bajos de cafeína ($\alpha=0.05$); aún con consumos diarios altos de este alimento, no se alcanza los límites de xantinas establecidos para el rango etario (40-100 mg día⁻¹), favoreciendo el desempeño intelectual y físico sin efectos adversos. La ingesta diaria de una doble porción (desayuno y merienda) de MC+M+S aporta altos valores energéticos y es fuente de carbohidratos, proteínas, vitaminas A, B₁, B₂, B₅, B₆ y B₁₂, biotina y minerales esenciales (Mn, P, Zn, Cu, Ca, Mg, Na, Cl, K, Fe y Al), con bajo colesterol. Los parámetros nutricionales están comprendidos dentro de límites internacionales. Los metales pesados no pasan a la infusión, o están muy por debajo de los valores peligrosos.

Palabras clave

"chá mate" • *Ilex dumosa* • *Ilex paraguariensis* • "mate cocido" • "mate cocido" con leche • valor nutricional • dieta escolar

INTRODUCTION

"Yerba mate" or "erva mate" (hereinafter, YM) is a plant food product made from shoots and leaves of *Ilex paraguariensis* A.St.-Hil. (Fam. Aquifoliaceae) once they are toasted, dried, milled and conditioned (6, 26). It is wanted for its nutritional value, highlighting its content in xanthines, some vitamins and minerals, and chlorogenic acids. Its infusion is the most traditional non-alcoholic beverage in the southern Cone of Latin America (Argentina, southern Brazil, Paraguay and Uruguay, and partially Chile), and is regulated by its respective Food Codes and specific provisions (3, 6, 7, 26, 37, 38, 39, 48).

The first three countries are the only producers of YM in the world, and half of this production takes place in Argentina, on a surface of 165,000 ha, with an annual yield of 820,000 t of "green leaves",

from which 37,000 t of produced YM are exported annually (23).

The most frequent forms of YM consumption are brewed mate (in a hollowed pumpkin or another container), "tereré" (a cold maceration), and the tea-like infusion known as "mate cocido" (in Spanish) or "chá mate" (in Portuguese), which has recently gained wide dissemination among the rural and urban population in the region.

"Mate cocido" (hereinafter, MC) is obtained by adding approximately 1 L of recently boiled water to 20-30 g of YM; the resulting infusion is then filtered and usually sweetened with cane sugar or honey. "MC in a cup" has recently gained wide acceptance due to its easy preparation using a porous paper "sack" or "bag" (39) containing approximately 3 g of

YM, over which around 200 mL of recently boiled water are poured, and then usually sweetened. In both cases, MC with milk is prepared by adding cow milk in variable proportions, significantly increasing the infusion nutritional value. This product is a food supplement offered more frequently in schools to students between 3 and 13 years old, in the preschool and primary education sector in Argentina. With its administration, it is intended to mitigate factors that determine nutritional deficiencies and low-quality diet of children in Argentina, as response to the food insecurity of disadvantaged sectors of the population (4, 5).

A related species, *Ilex dumosa* Reissek, commonly known as "dumosa", "caá mirí" or "yerba señorita", has been included in the Argentine Food Code in the group of "herbs for infusions" (CAA 2017: Cap. XV, Art. 1192). It is of interest due to its low xanthine content, so that its cultivation has been promoted in the recent years (42, 43). It has been demonstrated that a mixture in a 70:30 w/w proportion of *I. dumosa* and *I. paraguariensis* shows a great part of the organoleptic characteristics of YM made from *I. paraguariensis*, with a slightly lower caffeine proportion due to the predominance of *I. dumosa*; currently, it is a commercial product that satisfies a wide range of consumers (29).

The goal of the present study is to assess the comparative nutritional value of MC with milk obtained from the *Ilex* species mentioned above, proposing as alternative the use of the 70:30 w/w mixture of *I. dumosa*:*I. paraguariensis* to obtain a low-caffeine food product with good nutritional quality.

MATERIALS AND METHODS

Materials

The following samples, from the Argentine market, were used: a) commercial YM "sacks" from 6 different brands (3 from the province of Misiones and 3 from the Province of Corrientes), acquired in shops from San Luis and Corrientes, with around 3 g each, made from *I. paraguariensis* A. St.-Hil. (hereinafter, Ip); other similar samples were prepared in the laboratory from *I. dumosa* Reissek (hereinafter, Id), and separately from a commercial 70:30 w/w mixture of *I. dumosa* + *I. paraguariensis* (hereinafter, Id+Ip mix), whose crude drug was provided by the Establishment "Las Marías" (Gobernador Virasoro, Province of Corrientes); b) commercial samples of whole cow milk, sterilized by ultra-high temperature, from the dairy basin of the Argentine center-east (hereinafter, M); and c) commercial refined sugar (hereinafter, S) from the province of Jujuy.

Samples mentioned in b) and c) were acquired in the local market of San Luis. All samples were included within the framework of the Argentine Food Code requirements (6), and the reactants used were of analytical grade.

Extracts

The aqueous extracts were prepared according to the procedure described in Maiocchi *et al.* (2016), which represents the common conditions of use and the manufactures recommendations, by pouring 200 mL of recently boiled deionized water (ca. 84° C) over a sack of 3 g YM of each species and their mixture, leaving them in contact with the water for 5 min. The resulting infusions, at 1.5%, were filtered through Whatman n° 5 filter paper. The MC with milk was prepared by

mixing the 1.5% infusions of Id, Ip and Id+Ip mix with whole milk (M) in a 50:50 (v/v) proportion, and adding 10 g of refined sugar (S), equivalent to two teaspoons.

Analytical methods

The 1.5% infusions (MC) and the MC+M+S mixtures were mostly analyzed using the A.O.A.C. methods (21) for hygroscopic moisture, lipids, ashes, crude protein and chlorogenic acids; total dietary fiber was evaluated by the N^o 20511 IRAM Norm (25); and total nitrogen was assessed by the Kjeldahl method.

Total carbohydrates (non-nitrogenous extractive substances) (17), in grams per serving, were calculated by the difference with respect to the real extract. Calories per portion (in kcal and kJ) were calculated using the Atwater conversion factors (33). Caffeine was determined by UV spectroscopy (method of reference in Argentina) (27) as well as by HPLC (24).

Fat-soluble vitamins (A, D, E, K and provitamin A) and some water-soluble vitamins of the B complex (B₁, B₂) were determined by high performance liquid chromatography with a HPLC-UV/DAD UltiMate[®] 3.000 chromatograph (Dionex), Gemini C18 column (Phenomenex[®]), mobile phase acetonitrile:phosphoric acid 0.05% (20:80), flow of 0.8 mL min⁻¹, at a 330 nm wavelength; the UV spectra were collected within the 200-367 nm interval, comparing with Sigma[®] standard patterns and processing the data with the software associated to the instrument (Chromleon[®]); while vitamins B₅, B₆ and B₁₂, biotin and niacin were evaluated by microbiological methods (44).

The elemental composition of the infusions and mixture was established by the samples mineralization as described by Abou-Arab & Abou-Donia (2000), with some modifications (31); the concentration of 32 selected chemical elements and an internal standard (Indium) was

determined by ICP-OES (inductively coupled radial spectrometer, Varian[®]-Pro Ser. EL-05083717), with a Czerny-Turner[®] monochromator, a holographic diffraction grid and a VistaChip CCD (charge coupled device) array detector, calibrated quarterly, and using a Kretz[®] 5530 precision calibrated scale. Shown in table 1 (page 207), are the wavelengths used and the limits of detection for each analyzed element.

Serving size and daily average intake

The serving size consisted in a medium cup of 200 mL capacity (30), both for the 1.5% infusion (MC) as well as for the MC+M+S combination. The daily intake was set at 2 servings per person per day, that is, 400 mL of MC with milk and sugar, corresponding to breakfast and afternoon snack, just like it is commonly provided to children at home and/or schools.

Nutritional value

It was calculated based on the requirements of children between 3 and 13 years old, according to the most accepted international parameters, such as the Dietary Reference Intakes (DRIs), Recommended Dietary Allowance (RDA), Adequate Intake (AI) and Tolerable Upper Intake Level (UL) (6, 11, 15, 16), in addition to the Estimated Dietary Intakes (8) and the Typical Daily Nutritional Intake (36), taking into account the levels of nonobservance of adverse effects (NOEA in Spanish), that is, "the maximum intake of a determined nutrient not associated with adverse effects" (6).

Statistical analysis

Experimental design had 5 repetitions. The data of samples and formulas were analyzed using the *Infostat* program (10), by means of one-way ANOVA, followed by a comparison of averages (LSD test, $\alpha=0.05$); in all cases the p-value was lower than 0.0001.

Table 1. Wavelength (λ) and limit of detection for each element.

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

| Element | Ag | Al | As | B | Ba | Ca | Cd | Co |
|--|---------|---------|---------|---------|---------|---------|---------|---------|
| Wavelength (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 |
|--|---------|---------|---------|---------|---------|---------|---------|---------|
| Wavelength (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 |
|--|---------|---------|---------|---------|---------|---------|---------|---------|
| Wavelength (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 |
|--|---------|---------|---------|---------|---------|---------|---------|---------|
| Wavelength (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 |

RESULTS AND DISCUSSION

Nutritional parameters

The nutritional contributions to the daily reference intake (DRI) of one serving of "mate cocido" with milk and sugar, made from both species of *Ilex* and their mixture, are comparatively shown in table 2 (page 208-209).

None of the three formulations present significant differences for most of the analyzed nutritional parameters, although they do differ significantly in the concentration of caffeine and chlorogenic acids (table 2, page 208-209). In the Id: Ip mixture case, the nutritional values are directly related with the proportion in which both species appear in the commercial product.

Taking into account the energy needs of the age range between 3 and 13 years old (the considered schoolchildren age), between 1050 and 2550 kcal have been established (14); the daily intake of two servings of the studied products provides 8-22% of the required calories, while it also supplies up to 58% of the required

proteins and 22-26% of carbohydrates. Regarding vitamins, such intake doubles or triples the daily requirements (12) of biotin, provides 44-96% of cyanocobalamin, 30-76% of riboflavin, 10-38% of thiamine, 20-32% of pantothenic acid, 14-28% of vitamin A and 9-26% of pyridoxine. Due to the 50:50 v/v dilution, the total fat is half of the supplied by an equal intake of whole milk, with a proportional decrease in the cholesterol value. All these amounts are below the NOEA (levels of nonobservance of adverse effects) limits established as safe, regarding vitamins, by the Argentine Food Code (6).

The same occurs with the other nutritional factors, when compared with the allowed maximum intake levels (15). Finally, it is worth mentioning that these formulas do not provide dietary fiber.

Even though caffeine is not required as a nutritional principle, it is considered here due to the stimulating effect generally caused by xanthines on the CNS.

Table 2. Nutritional value per serving (200 mL) of "mate cocido" with milk and sugar, obtained from *Ilex paraguariensis* (Ip), *I. dumosa* (Id) and the Id:Ip (70:30 w/w) mix.
Tabla 2. Valor nutricional por porción (200 ml) de "mate cocido" con leche y azúcar, obtenido de *Ilex paraguariensis* (Ip), *I. dumosa* (Id) y la mezcla Id:Ip (70:30 p/p).

| Nutritional parameters | Units | DRI | MC _{Ip} +M+S | | MC _{Id} +M+S | | MC _{mix} +M+S | |
|------------------------------------|-------|---------------------|-----------------------|---------|-----------------------|---------|------------------------|--------|
| | | | 200 mL ^(a) | %DV | 200 mL ^(a) | %DV | 200 mL ^(a) | %DV |
| Energetic value | kcal | 1050-2550 | 104.2±8.79 | 4-11 | 103.9±9.69 | 4-11 | 104.0±7.64 | 4-11 |
| | kJ | 4390-10660 | 436.2±36.8 | | 435.2±40.6 | | 435.5±31.9 | |
| Water | mL | 1700-2400 | 189.8±27.6 | 7-13 | 189.7±32.25 | 7-13 | 189.7±29.8 | 7-13 |
| Proteins | g | 15.5-44 | 3.28±1.18 | 5-29 | 3.27±0.235 | 7-23 | 3.28±0.16 | 7-22 |
| Total fat | g | Und. | 3.37±1.21 | und. | 3.37±1.21 | und. | 3.37±1.21 | und. |
| Cholesterol | mg | the lowest possible | 10.31±5.86 | und. | 10.31±6.24 | und. | 10.31±0.60 | und. |
| Carbohydrates | g | 130 | 15.48±0.9 | 11-13 | 15.41±1.41 | 11-13 | 15.43±1.12 | 11-13 |
| Total dietary fiber | g | 19-26 | 0 | 0 | 0 | 0 | 0 | 0 |
| Vitamin A | µg | 400-600 | 50.0±5.6 | 7-14 | 49.6±3.7 | 8-13 | 49.7±4.77 | 7.5-14 |
| Thiamine (B ₁) | mg | 0.6-0.9 | 0.072±0.03 | 5-17 | 0.071±0.042 | 3-19 | 0.071±0.03 | 4.5-17 |
| Riboflavin (B ₂) | mg | 0.6-0.9 | 0.185±0.04 | 16-37 | 0.182±0.045 | 15-38 | 0.183±0.034 | 16-36 |
| Niacin (B ₃) | mg | 8-12 | 0.128±0.02 | 0.9-1.8 | 0.123±0.015 | 0.9-1.7 | 0.125±0.03 | 0.8-2 |
| Pantothenic acid (B ₅) | µg | 3-4 | 0.447±0.035 | 10-16 | 0.450±0.02 | 11-16 | 0.449±0.025 | 11-16 |
| Pyridoxine (B ₆) | mg | 0.6-1 | 0.060±0.015 | 4.5-12 | 0.057±0.01 | 5-12 | 0.058±0.02 | 4-13 |

| | | | | | | | | |
|--|----|----------------------|---------------|---------|---------------|---------|---------------|---------|
| Folic acid (B₉) | µg | 200-300 | 6.34±0.88 | 2-4 | 6.36±1.065 | 2-4 | 6.35±0.915 | 2-4 |
| Cyanocobalamin (B₁₂) | µg | 1.2-1.8 | 0.480±0.06 | 23-45 | 0.483±0.09 | 22-48 | 0.482±0.07 | 23-46 |
| Vitamin C | mg | 25-45 | 1.081±0.18 | 2-5 | 1.083±0.209 | 2-5 | 1.082±0.195 | 2-5 |
| α-tocopherol (vit. E) | mg | 7-11 | 0.075±0.02 | 0.5-1 | 0.075±0.02 | 0.5-1 | 0.075±0.02 | 0.5-1 |
| Vitamin K | µg | 55-60 | 0.316±0.04 | 0.5-0.6 | 0.316±0.04 | 0.5-0.6 | 0.316±0.04 | 0.5-0.6 |
| Vitamin D | µg | 15 | 0.038±0.01 | 0.2-0.3 | 0.038±0.01 | 0.2-0.3 | 0.038±0.009 | 0.2-0.3 |
| Biotin | µg | 12-20 | 20.0±3.20 | 84-193 | 20.0±3.2 | 84-193 | 20.0±3.2 | 84-193 |
| Carotenoids | µg | und. | 18.5±1.6 | und. | 18.5±1.6 | und. | 18.5±1.6 | und. |
| Other physico-chemical parameters | | | | | | | | |
| Dry extract | g | - | 22.87±3.16 | - | 22.83±2.73 | - | 22.84±3.55 | - |
| Total ashes | g | - | 0.805±0.18 | - | 0.793±0.148 | - | 0.796±0.139 | - |
| Total nitrogen | g | - | 0.574±0.149 | - | 0.557±0.176 | - | 0.559±0.166 | - |
| Caffeine | mg | 45-85 ^(b) | 22.4±1.85 (A) | - | 1.94±0.16 (B) | - | 8.04±0.26(C) | - |
| Chlorogenic acids | g | - | 0.14±0.006(A) | - | 0.06±0.003(B) | - | 0.09±0.004(C) | - |

MC_{ip}: "mate cocido" from *Ilex paraguariensis* (Ip); **MC_{id}**: "mate cocido" from *I. dumosa* (Id); **MC_{mix}**: "mate cocido" from 70:30 (w/w) mix of Id+Ip; **M**: sterilized whole milk (UHT); **S**: refined sugar; **DRI**: daily reference intake (covers nutritional requirements of entire population between 3-13 y/o, based on DRIs (Dietary Reference Intakes, www.nap.edu) and others (6, 8, 36)).^(a) **serviing size**: a medium cup (100mL MC+100mL M+10g S = ca. 200mL).^(b) according to Nawrot *et al.* (2003) and Health Canada (2012).^(c) **%DV**: range of the Dietary Value covered by a serving of "mate cocido" +M+S for a daily diet of 1050-2550 kcal or 4390-10660 kJ (14).^(c) **und.**: undetermined.^(c) **(A) (B) (C)**: the means with different letters indicate statistically significant differences (for each parameter) at 0.05 level.

MC_{ip}: "mate cocido" de *Ilex paraguariensis* (Ip); **MC_{id}**: "mate cocido" de *I. dumosa* (Id); **MC_{mix}**: "mate cocido" de la mezcla 70:30 (p/p) de Id+Ip; **M**: leche entera esterilizada (UAT); **S**: azúcar refinado; **DRI**: ingesta diaria de referencia (cubre requerimientos nutricionales de la población entre 3 y 13 años, basada en las DRIs (Dietary Reference Intakes, www.nap.edu) y otras (6, 8, 36)).^(a) **tamaño de porción**: una taza mediana (100ml MC+100ml M+10g S = ca. 200ml).^(b) según Nawrot *et al.* (2003) y Health Canada (2012).^(c) **%DV**: % del Valor Dietario cubierto por una porción de "mate cocido" +M+S para una dieta diaria de 1050-2550 kcal ó 4390-10660 kJ (14).^(c) **und.**: indeterminado; **(A) (B) (C)**: las medias con diferentes letras indican diferencias estadísticas significativas (para cada parámetro) a un nivel de 0,05.

These bioactive principles contribute, in certain extent, to a better school performance, both intellectual as well as physical (2, 19, 22, 28, 45, 47), in particular when they are part of the breakfast provided in schools, since in many cases it is the first food intake of the day (40, 41), and present a strongly dose-dependent pharmacokinetics and psychomotor effects.

However, children compose a group of risk in which high doses of caffeine can provoke adverse effects (45, 46). Although there are no established international limits, it is considered that the daily consumption of caffeine in children and teenagers should not exceed 2.5 mg kg^{-1} of body weight, whereas for adults, it should be below 400 mg day^{-1} (18, 20, 35).

Assuming that only YM provides caffeine to the MC+M+S, the one made from Ip reached 20-25 mg of caffeine per serving. This value is similar to that from diverse sources regarding tea infusions (c. $23 \pm 12 \text{ mg}$), and 2-3 times lower than the indicated for the coffee infusion (c. $57 \pm 20 \text{ mg}$) under the common preparation conditions (20, 22, 32, 34). Regarding the MC+M+S made from Id (with 1.5-2.5 mg of caffeine/100 mL), it had, on average, 11 times less caffeine than Ip, while the Id:Ip (70:30 w/w) commercial mixture (with 7-9 mg/100 mL) had, in average, 2.75 times less caffeine than YM with Ip.

Table 3 and figure 1 (page 211), show the limits of caffeine consumption and of potential servings of MC+M+S per day for the considered age groups, in comparison to those of tea with milk and coffee with milk.

A diet including 2 medium cups (of 200 mL) per day of MC made with *Ilex paraguariensis* with milk (50:50 v/v) and sugar, involves a caffeine intake of c.

$40\text{-}48 \text{ mg day}^{-1}$, similar to the provided by the same amount of tea with milk, and well beyond the limits established for the considered age range ($45\text{-}100 \text{ mg day}^{-1}$) (18, 35). In contrast, the daily intake of the same volume of coffee with milk contributes with 40-85 mg of caffeine, reaching such limits.

If the Id:Ip (70:30 w/w) mixture is used in the making of MC with milk, with a daily intake of 400 mL, the daily caffeine does decrease to 15-17 mg. Thus, the use of such mixture seems to be the best combination to be used in the elaboration of MC with milk, for their safer doses of caffeine which allow greater consumption without increasing the risks.

Mineral content

The ash content of the MC made with the two studied species and their mixture, reached (on average) between 0.054 and 0.066 g/100 mL, while for milk it was an average of 0.7 g/100 mL and for sugar 0.046 g/10 g. Infusions prepared from *Ilex dumosa* and *I. paraguariensis*, as well as from the Id:Ip (70:30 w/w) mixture, presented high extractability of the mineral elements, oscillating between 60 and 70% of the mineral matter detected in the crude drug, in agreement with previous studies (31).

Table 4 (page 212) shows the mineral contribution of a 200 mL serving of MC obtained from *Ilex paraguariensis*, *I. dumosa* and their mixture (Id:Ip, 70:30 w/w), combined with whole milk and sugar.

No significant differences were found in the mineral composition of the 3 studied formulas. Taking into account one serving, some minerals (P, Zn and Cu) reached percentage values of DV enough for the MC+M+S to be considered as a source of those nutrients, since they can exceed 20% of the daily requirement.

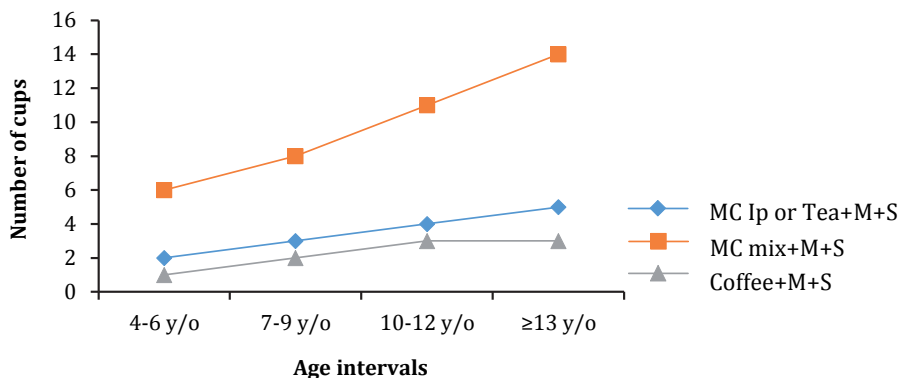
Table 3. Comparative number of cups to reach the caffeine limit of consumption of MC, Tea and Coffee (with milk and sugar) in 4-13 y/o children.

Tabla 3. Número comparativo de tazas para alcanzar el límite máximo de cafeína por consumo de MC, Té y café (con leche y azúcar) en escolares entre 4 y 13 años de edad.

| Childrens age group | Daily caffeine intake limit ^(c) | N° of cups per day to reach the limit (a cup = 200 mL) ⁽¹⁾ | | | |
|--|--|---|--|---|--|
| | | MC _{Ip} +M+S 22±1.8 mg caffeine/cup ⁽²⁾ | MC _{mix} +M+S 8±0.3 mg caffeine/cup ⁽²⁾ | Tea+M+S 23±12 mg caffeine/cup ⁽³⁾ | Coffee+M+S 57±20 mg caffeine/cup ⁽³⁾ |
| 4-6 y/o | ≤45 mg | 2 | 6 | 2 | 1 |
| 7-9 y/o | ≤62.5 mg | 3 | 8 | 3 | ≤2 |
| 10-12 y/o | ≤85 mg | 4 | 11 | 4 | ≤3 |
| Adolescents ≥ 13 y/o (40-50kg bw) ^(d) | ≤ 2.5 mg kg ⁻¹ bw (100-125 mg) | (4) 5 (6) | (12) 14.5 (16) | ≤5 | ≤3 |

As in table 2 (page 208-209), (including serving size), plus: **y/o**: years old.- **bw**: body weight.- ^(c) according to Nawrot *et al.* (2003) and Health Canada (2012).- ^(d) statistical estimates of the "Looking at us: IMC Brigade" Project on the local school population from Educational Center N° 2, San Luis, Argentina (M.G. Del Vitto, pers. comm., August 2017).- ⁽¹⁾ in round numbers.- ⁽²⁾ in this work.- ⁽³⁾ according to Heckman *et al.* (2010), IFIC (2008) and CSPI (2014).

Como en tabla 2 (pág. 208-209), (incluyendo tamaño de porción), más: **y/o**: edad.- **bw**: peso corporal.- ^(c) según Nawrot *et al.* (2003) y Health Canada (2012).- ^(d) estimaciones estadísticas del Proyecto "Mirándonos: Brigada IMC" sobre la población escolar del Centro Educativo N° 2, San Luis, Argentina (M.G. Del Vitto, com. pers., agosto 2017).- ⁽¹⁾ en números redondos.- ⁽²⁾ en este trabajo.- ⁽³⁾ según Heckman *et al.* (2010), IFIC (2008) y CSPI (2014).



MC: "mate cocido".- **Ip:** *Ilex paraguariensis*.- **Id:** *I. dumosa*.- **mix:** Id+Ip 70:30 (w/w).- **M:** milk; **S:** sugar; **y/o:** years old.

MC: mate cocido.- **Ip:** *Ilex paraguariensis*.- **Id:** *I. dumosa*.- **mix:** mezcla de Id+Ip 70:30 (p/p).- **M:** leche; **S:** azúcar; **y/o:** años de edad.

Figure 1. Number of cups to reach the daily caffeine limit of consumption for MC, Tea and Coffee (with milk and sugar).

Figura 1. Número de tazas de MC, té y café (con leche y azúcar) para alcanzar el límite diario de cafeína.

Table 4. Mean contribution to the mineral daily intake per serving of "mate cocido" with milk and sugar, obtained from *Ilex paraguariensis*, *I. dumosa* and their mixture (1d:1p 70:30 w/w).

Table 4. Contribución promedio a la ingesta mineral diaria por porción de "mate cocido" con leche y azúcar, obtenida a partir de *Ilex paraguariensis*, *I. dumosa* y su mezcla (1d:1p 70:30 p/p).

| Element | DRI mg day ⁻¹ | MCIp+M+S | | MCId+M+S | | MCmix+M+S | |
|---|-----------------------------|-------------|--------------------|-------------|--------------------|-------------|--------------------|
| | | mg/200 mL | %DV ⁽²⁾ | mg/200 mL | %DV ⁽²⁾ | mg/200 mL | %DV ⁽²⁾ |
| Quantitative essential elements (required >100 mg day ⁻¹) | | | | | | | |
| K | 3800-4500 | 157.8±26.2 | 4.08-4.84 | 153.7±22.4 | 2.9-4.63 | 155.2±18.2 | 3.04-4.56 |
| Ca | 1000-1300 | 121.69±19.8 | 7.84-14.15 | 122.1±23.2 | 7.6-14.5 | 121.85±23.6 | 7.56-14.54 |
| Mg | 130-240 | 15.40±2.3 | 5.45-13.61 | 15.55±3.5 | 5.02-14.6 | 15.45±1.9 | 5.64-13.34 |
| P | 500-1250 | 94.18±7.6 | 6.93-20.36 | 93.23±8.4 | 6.79-20.33 | 93.43±8.1 | 6.83-20.31 |
| Na | 1200-1500 | 49.28±7.2 | 2.8-4.71 | 48.97±6.8 | 2.81-4.64 | 49.07±7.8 | 2.75-4.74 |
| Cl | 1900-2300 | 142.4±25.3 | 5.09-8.83 | 140.74±21.7 | 5.17-8.54 | 141.24±22.7 | 5.15-8.63 |
| Trace essential elements (required <100 mg day ⁻¹) | | | | | | | |
| Mn | 1.5-1.9 | 1.38±0.32 | 55.8-113.3 | 1.613±0.24 | 72.3-123.5 | 1.603±0.26 | 70.68-124.2 |
| Fe | 8-10 | 0.305±0.05 | 2.55-4.44 | 0.295±0.06 | 2.35-4.43 | 0.296±0.08 | 2.16-4.7 |
| Zn | 5-8 | 0.82±0.22 | 7.5-20.8 | 0.84±0.18 | 8.25-20.4 | 0.828±0.14 | 8.6-19.36 |
| Cu | 0.44-0.7 | 0.08±0.01 | 10.0-20.45 | 0.085±0.01 | 10.7-21.6 | 0.083±0.02 | 9-23.41 |
| Ultra-trace elements and others | | | | | | | |
| Al | 2-10 | 0.07±0.02 | 0.5-4.5 | 0.07±0.01 | 0.6-4 | 0.07±0.01 | 0.6-4 |
| Si | und. | 1.65±0.54 | und. | 1.24±0.36 | und. | 1.363±0.42 | und. |
| Ba | 8-11 | 0.04±0.01 | 0.27-0.62 | 0.035±0.01 | 0.23-0.56 | 0.036±0.007 | 0.26-0.54 |
| Sr | und. | 0.12±0.02 | und. | 0.115±0.04 | und. | 0.117±0.03 | und. |
| B | 20 | 0.03±0.01 | 0.1-0.2 | 0.04±0.01 | 0.15-0.25 | 0.03±0.01 | 0.1-0.2 |

As in table 2 (page 208-209) and table 3 (page 211), (including serving size). **Note:** Ag, As, Cd, Co, Cr, Hg, Li, Mo, Ni, Pb, Sb, Se, Sn, Th, Ti, Tl, U and V were not detected or they were found below the instrumental limit of detection. DRIs according to FNB (2004).

Como en tabla 2 (pág. 208-209) y tabla 3 (pág. 211), (incluyendo tamaño de porción). - **Nota:** Ag, As, Cd, Co, Cr, Hg, Li, Mo, Ni, Pb, Sb, Se, Sn, Th, Ti, U y V no fueron detectadas o se hallaron por debajo del límite instrumental de detección. DRIs según FNB (2004).

In the case of Mn, it exceeds 50% of the DV and reaches up to 1.24 times the daily requirement, which is attributable to the contribution of both species of *Ilex*, in agreement with previous results (31).

For their part, a double daily portion of MC+M+S provides up to 40% of the DRIs of P, Zn and Cu, up to 29% of Ca, 27% of Mg, and 15% of Na and Cl, in addition to almost 10% of K, Fe and Al. It is noteworthy the contribution of Mn, since it far exceeds the DRI. These values of essential elements demonstrate the importance of this product in the student mineral nutrition (13).

In no case the analyzed elements exceeded the limits established by the international norms; the heavy metals did not make it to the infusion or milk, or were in a proportion well below the harmful values, which makes the product a safe food in terms of mineral elements.

Considering the nutritional goals set by diverse authors for school canteens in Argentina (5), the double serving of MC+M+S would provide approximately 40% of the required Ca, 35% of proteins, 30% of the energy value and vitamin A, 10% of Fe and 8% of vitamin C. These amounts are a bit higher than the calculated in this work, based on the DRIs, in regard to vitamin A, significantly higher in terms of energy value and vitamin C, almost triple in terms of Ca, double in Fe and significantly lower in proteins.

CONCLUSIONS

"Mate cocido" as a 1.5% infusion, does not represent *per se* a relevant food due to its dilution, except for some minerals and vitamins, and its content in chlorogenic acids and xanthines. However, mixed with

whole cow milk in a 50:50 proportion and sweetened with 10 g of refined sugar per cup ("mate cocido with milk"), it can become an important factor in the diet of students between 3 and 13 years old when their diet is supplemented with 1 to 2 daily servings of this food (serving size: a medium cup of 200 mL).

Considering a daily intake of 2 servings, except for niacin, folic acid and vitamins C, E and D, the rest of the nutritional parameters of the product exceed (sometimes by far) 20% of the DV, so that this food has to be considered as source of the following nutritional parameters and principles: energy value, proteins, carbohydrates, vitamins A, B₁, B₅ and B₆ ($\geq 20\%$ of the DRI); vitamins B₂ and B₁₂ ($\geq 50\%$ of the DRI); biotin ($\geq 100\%$ de la DRI), in addition to a low contribution of cholesterol. Regarding minerals, it has interesting contributions of P, Zn and Cu ($\leq 40\%$ of the DRI), Ca and Mg ($\leq 30\%$), Na and Cl ($\leq 15\%$) and K, Fe and Al (8-10%); with a noteworthy contribution of Mn, which doubles the DRI but still without reaching toxic values. In no case the heavy metals make it to the infusion or are well below the values considered as harmful.

The use of *Ilex paraguariensis* (Ip), *I. dumosa* (Id) or their commercial mixture (Id:Ip, 70:30 w/w) does not involve significant differences in nutritional parameters, both in the resulting infusions as well as in "MC with milk", except for the values of caffeine and chlorogenic acids. With Ip, "MC with milk" is close in caffeine values to "tea with milk", and contains from 1/2 to 1/3 of the caffeine found in "coffee with milk". "MC with milk" obtained from Id, contains 11 times less caffeine than "MC with milk" from Ip.

The mixture ensures the persistence of favorable organoleptic characters of *I. paraguayensis*, while the greater proportion of *I. dumosa* significantly lower the caffeine content in the final product (7-9 mg, that is, 2.75 times lower than the obtained from Ip and tea, and 10-20 times lower than in coffee), that is, within the recommended safety limits (40-100 mg day⁻¹) for the age range between 3-13 years.

The use of this mixture (Id:Ip, 70:30 w/w) in the preparation of "mate cocido" seems to be the best combination, since it maintains the product nutritional quality and palatability for most of the consumer

population, contributing to a better intellectual and physical performance of the students, without detrimental effects due to its lower doses of caffeine making it safer against the possible intake of multiple servings per day.

The studied food provides interesting nutritional values for some of the goals established as "reasonable" for some school canteens in Argentina (5), in particular in regard to energy, proteins, some vitamins (A and C), and minerals (Ca and Fe).

Finally, YM manufactured in "bags" of 3 g each, allows to properly dose the crude drug for consumption.

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