

Chemical composition of *Macadamia integrifolia* (Maiden and Betche) nuts from Paraguay

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Abstract

Centesimal composition, fatty acids profile, mineral, total antioxidant capacity (TAC), total phenolic compounds (TPC) and α -tocopherol contents of 3 cultivars of *Macadamia integrifolia* nuts cultivated in Paraguay was evaluated. Its high lipid content (73.5 ± 1.4 g/100g) makes this nut a high energy food (737 ± 18 kcal/100g). High content of monounsaturated fatty acids MUFA (49.4 - 58.7 mg/100g), mainly oleic (34.5 - 47.0 mg/100g) and palmitoleic (7.1 - 12.8 mg/100g) was observed. Minerals Mg, Zn, Cu and Mn reaches good concentration facing nutritional standards. Its antioxidant properties are related to levels of TPC (77.9 - 96.3 mg galic acid equivalent/100g), α -tocopherol (0.2 - 18.4 mg/100g) and TAC (21.0 - 36.8 μ M Trolox equivalent/g). The cultivar HAES 344 showed the highest TPC content and the highest TAC level. San Joaquin cultivar exhibited the highest α -tocopherol content. The high content of MUFA, α -tocopherol, as well as, the level of TPC and TAC is consistent with the claims of antioxidant potential of these nuts.

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Introduction

Macadamia integrifolia is a native tree from the rain forests of Queensland (Australia) and is the botanical source of the appreciated macadamia nuts. Despite its geographic origin, macadamia had the greatest technological development as a crop in Hawaii, where the first studies were carried out early 1900s, and the results published after 1940. The development of the main commercial varieties and clones of macadamia took place in Latin America and the rest of the world (Wall, 2013). Paraguay is a subtropical country with a strong agriculture-based economy. Cultivation of *M. integrifolia* in Paraguay started in early 1990s with the introduction of plants with improved genetic traits (Paraguay, 2010). These nuts, as a part of the human diet, are a source of energy and nutrients, but their high fat content have impacted negatively on consumption, because widespread concern about weight gain associated with its intake (King *et al.*, 2008). Nevertheless, nuts intake was associated with reduced cancer and total mortality. Observational investigations demonstrated the health advantages of nut intake for subjects at risk for CVD or cancer, such as obese subjects, affected by diabetes, metabolic syndrome or are less adherent to the Mediterranean diet (Bonaccio *et al.*, 2015).

The most notable feature of *M. integrifolia* nuts is its high lipid content. However, the oil is rich in

unsaturated fatty acids (FA), mainly monounsaturated MUFA, vitamin E and sterols (Maguire *et al.*, 2004; Wall 2010; Gray, 2013). High concentrations of tocotrienols as α -tocotrienol has been reported by Kaijser *et al.* (2000) in *M. tetraphylla* from Australia. Wall (2010) reported values of 46.5 to 91.6 μ g/g oil of total tocotrienols and their relationship with the oxidative stability in *M. integrifolia* kernels, however, data about their chemical profiles of macro and micro components in South America are limited. Despite being a high-energy food, a study performed with hypercholesterolemic male volunteers have shown health benefits with regular consumption of macadamia nuts demonstrates, that short-term consumption modifies favourably the biomarkers of oxidative stress, thrombosis and inflammation, which are risk factors for coronary artery disease (Garg *et al.*, 2007). A growing interest concerning benefits of natural antioxidants on health is being observed in the world. About this, it has been shown that consumption of macadamia nuts reduces circulating levels of leukotrienes and 8-isoprostane, being the last a reliable *in vivo* marker of oxidative stress (Garg *et al.*, 2007). The composition of both macronutrients and phytochemicals of the fruits are the result of a number of factors, such as genetics and age of trees, and adaptation to climatic conditions, among other factors (Wakeling *et al.*, 2001). The profiles and content of polyphenols are affected also

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by the year of harvest, orchard location, processing steps, and storage, thus the results of TPC analysis of macadamia nuts carried out in different places and at different times, can produce different results (Munro and Garg, 2008; Bolling, McKay and Blumberg, 2010).

Since the cultivation of *M. integrifolia* in Paraguay is relatively recent, data on the composition of the nuts produced in the country are not available, but the health benefits associated to frequent consumption support the interest in descriptive studies of its potential as a valuable source of antioxidants. The aim of the present study is to describe macronutrients, dietary fiber and mineral content, fatty acid profile, α -tocopherol content, total phenolic compounds and the antioxidant activity of three varieties (HAES 344, Cannon and San Joaquín) of *M. integrifolia* nuts harvested in two years from an orchard at the Departamento Cordillera, Eastern Paraguay.

Materials and Methods

Macadamia samples

Samples were collected from healthy mature trees (12 years old) from an orchard placed at Caragatay, Cordillera Department, Paraguay (25°13'S 56°49'W) at 236 m above sea level. The yearly average sunlight in the cultivation place ranges between 6 and 14 h per day. The dry season begins in June and ends in August, and the rainy season begins in November and continues until March. The climate is mild and dry with average annual temperature of 22°C, maximum of 40°C and minimum 0°C. The average annual rainfall is 1550 mm, with a monthly average of 153 mm, except during the months of June and August, in which only reaches 80 mm (DINAC, 2015).

Three different introduced cultivars were included in the study, namely HAES 344, Cannon and San Joaquín. The HAES 344 variety was introduced in 1992 from Espiritu Santo (Brazil). It retains its number of selection in the Hawaii Agricultural Experiment Station which was originally developed for commercial cultivation. The Cannon and San Joaquin cultivars were adapted in California (USA) and introduced to Paraguay in 1999. Nuts were harvested by hand from trees of the different varieties from January to May 2013 and from January to May 2014. They were dehusked and dried by forced air circulation at room temperature (25°C). Dried nuts were mechanically cracked and manually removed from the shell. The kernels were dried in an oven at 40°C for 72h. Representative samples were taken from each cultivar. A food processor from Sensio Inc. (Bella, Montreal, Canadá) was used for

homogenisation of the macadamia nuts, and passed through a 18-mesh sieve, for extraction and further analysis.

Chemicals

Solvents n-hexane, methanol, acetonitrile and water HPLC and MS grade, were purchased from J.T. Baker (Mexico State, Mexico). HPLC-grade acetonitrile for liquid chromatography was purchased from Merck (Darmstadt, Germany). Petroleum ether residue analysis grade 40-60 and ABTS Biochemica reagent was purchased from AppliChem (Darmstadt, Germany). Standards of fatty acids mix 37 FAMES, 10 mg/mL in dichloromethane, α -tocopherol standard (>95%), and Trolox (\pm)-6-Hydroxy-2,5,7,8-tetramethyl-chromane-carboxylic acid 97%, were purchased from SIGMA-ALDRICH (Saint Louis, MO, USA) and Folin-Ciocalteu's phenol reagent and gallic acid monohydrate \leq 98% was from Sigma (Steinheim, Germany).

Multi-element standard solution of Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Tl, Zn, anthrone reagent, D(+)-glucose monohydrate and BIOQUANT® kit for total dietary fiber measurement, were purchased from Merck (Darmstadt, Germany). The boron trifluoride-dimethanol complex 50-52% (Cat. B21357, L15847) was purchased from Alfa Aesar (Lancaster, England). The acetylene 2.8 AA atomical absorption grade, analytical helium 5.0, nitrogen 4.6 FID and argon 5.0, were purchased from White Martins (Sao Paulo, Brazil). Others reagents were of analytical grade, purchased from Merck (Darmstadt, Germany).

Proximate and minerals composition

The proximate composition of macadamia nuts was assessed according to the Association of Official Analytical Chemists (Horwitz, 2000) methods, as follows: water content (934.06), ashes (968.08), fat (948.22), total nitrogen (950.48) using 5,3 as conversion factor of total nitrogen to protein for nuts, phosphate (970.39) and dietary fiber (985.29). Crude proteins were measured using a Kjeldahl apparatus (Gerhardt, Sao Paulo, Brazil). A Soxhlet apparatus (Gerhardt, Sao Paulo, Brazil), vacuum evaporator Q 219, Q.344.2 (Gerhardt, Sao Paulo, Brazil), shaker water bath (Precision Model 25, Cat. 0792M32, Ohio, USA), precision electronic balance (AYD HR 120, Bradford, England), muffle furnace (Naber, Bremen, Germany) and a stove (Tecnal E-394/2, Sao Paulo, Brazil) were also used for the proximate analysis. The contents of total carbohydrates and soluble sugars were analyzed by the anthrone colorimetric method, with and without

prior hydrolysis, respectively (Dreywood, 1946), using an UV-1800 Spectrophotometer (Shimadzu, Kyoto, Japan). The results were expressed in g/100g of nut. The energetic value was calculated using conversion factors of 16.7 kJ/g for proteins and carbohydrates, and 37.6 kJ/g for lipids (Greenfield and Southgate, 2003). All analyses were performed in triplicates. The mineral content and composition of ashes were determined by atomic absorption spectroscopy, according with the AOAC (Horwitz, 2000) recommendations. Measurements were carried out in a AA 6300 spectrophotometer (Shimadzu, Kyoto, Japan). The following elements were analyzed: sodium, magnesium, potassium, calcium, manganese, copper, iron, and zinc. The phosphorus analysis was performed by AOAC spectrophotometric method 970.39 (Horwitz, 2000) measured by an UV-1800 Spectrophotometer (Shimadzu, Kyoto, Japan). The results were expressed in mg/100g of weight and all analyses were performed in triplicate.

Oil analysis

Total lipids extracted from the kernels were used to determine the fatty acid composition according to Savage, Dutta and McNeil (1999) and Wall (2010). The ground nuts were extracted as follows: twenty grams of sample were mixed with 100 mL of hexane/isopropanol (3:2, v/v) during 2 h, under stirring. The lower layer was collected, filtered through quantitative filter paper in a Büchner funnel, and the residues were washed twice with 20 mL of the same solvent, and taken to dryness, under reduced pressure (40°C) and nitrogen flow. Each extraction was repeated three times. The extracted oil was stored at -20°C until analysis.

Fatty acid analysis

Fatty acids were derivatized from the oil extracted into fatty acids methyl esters (FAME) at 80°C by trans-esterification, following the method outlined by Savage *et al.* (1999). FAME were identified by GC-MS, using a GC/MS-QP2010 S gas chromatograph/mass spectrometer SHIMADZU (Tokyo, Japan) equipped with a SPTM-2560 Capillary GC Column (L×I.D. 100 m × 0.25 mm, df 0.20 μm). The separated peaks were identified by comparison of its mass spectra with those included in the NIST 107 library and by comparison of its retention times with the mixture of 37 FAMES standards. The conditions were as follows: interface temperature was 250°C, injector temperature 245°C, ion source temperature 250°C, the temperature program started from 245°C for 1 min, following by a ramp of 8°C/min to 300°C, with He as carrier gas (1ml/min, 30-35

cm/sec), split injection mode (split ratio 1:100). The ionization mode was electron impact at 70 eV and the emission current, 100 μA. Standard curves for fatty acids were built for quantitation with different FAME concentrations within the 8–160 μg/mL range. Results were expressed as mg by 100g of macadamia nuts.

Alpha-tocopherol analysis

For α-tocopherol determination, twenty grams g of the homogenized nuts were extracted with 60 mL of n-hexane, during 2 hours under stirring, filtered and taken to dryness under reduced pressure. Then saponification procedure was carried out with KOH-ethanol and the unsaponifiable matter was extracted with hexane by the method proposed by Ryyänen *et al.* (2004). The upper layer was collected, and taken to dryness under nitrogen flow. The remnant was re-dissolved in 500 μL of mobile phase and 5 μL were injected into the LC-MS/MS.

The LC-MS/MS method was carried out with a Waters ACQUITY Ultra Performance LC[®] chromatograph (Waters Corporation, Milford, MA, USA) coupled to a triple quadrupole (QqQ) mass spectrometer Xevo TQD (Waters Corporation, Manchester, UK), equipped with an ACQUITY UPLC[®] BEH C18 column, 2.1 x 50 mm x 0.57 μm column (Torrance, Milford, MA, USA) was used for LC separation at 15°C and an autosampler (Waters Autosampler manager FTN). The mobile phase was composed by A: water + 0.1 % formic acid and B: methanol, at a flow rate of 0.4 μL/min. Gradient elution was used with the following conditions: 90% of A at 0.5 min, then increase to 100% B in 2 min, maintaining this percentage during 2 min, and then back to 90% A, between 4 to 5 min. The total run time was 5 min and the injection volume was 5 μL.

Mass spectrometer parameters: were set according to Górnaś *et al.* (2014), with modifications. The mode of acquisition was multiple reaction monitoring (MRM). Transition m/z 431→165 was used for quantitation purposes and transition 431→56 for confirmation. The Xevo TQD mass spectrometer was used in positive electrospray mode. The ion source was operated at 150°C with a capillary voltage of 4.0 kV and collision energy was set at 3V. Nitrogen was employed as desolvation gas at 1000 L/h (500°C) and argon as the collision gas at a pressure of 4.0 x 10⁻³ mBar. The interface temperature was set to 150°C. The data were acquired using Waters MassLynxTM software and processed using TargetLynxTM Application Manager. The α-tocopherol quantitation was carried out with an external standard calibration curve, covering the range 0.1-7.0 μg/mL (R²=0.9993).

The experimental values of the limit of detection and quantitation were 0.012 µg/mL and 0.04 µg/mL, respectively. The concentrations are presented as mg/100g nut.

Total phenolics assay

Total phenolics compounds (TPC) were determined by the Folin-Ciocalteu colorimetric method (Singleton *et al.*, 1999). Measurements were carried out by triplicates using a calibration curve obtained with gallic acid (0-120 µg/mL aqueous solution). Briefly, 500 µL of test sample, diluted appropriately with water, or gallic acid standard were mixed with 2500 µL of diluted Folin-Ciocalteu reagent (1 mL 2N Folin reagent with 9 mL water) and 2 mL of 10% sodium carbonate solution. The mixture was stirred and kept for 30 min at room temperature in the dark. The absorbance was measured spectrophotometrically at 765 nm against a blank of reagent. For measurements, a gallic acid standard curve ($y=0.0122x-0.0217$, $R^2=0.9984$, concentration interval 0-120 µg/mL aqueous solution) was plotted at 765 nm in the UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Results were expressed as mg of gallic acid equivalents (GAE) per 100 g of nut (mg of GAE/100 g).

Antioxidant activity assay

The antioxidant activity was determined by the TEAC assay using the radical cation ABTS^{•+} (Re *et al.*, 1999). The ABTS^{•+} stock solution (7 mM) was prepared using ammonium persulphate (NH₄)₂S₂O₈ as the oxidant agent. The working solution of ABTS^{•+} was obtained by diluting the stock solution in ethanol to give an absorption of 0.70± 0.02 at λ=734 nm. The extraction of the antioxidants was performed in triplicates and in two steps, as follows: 2 g of homogenized whole nuts were extracted with 50% aqueous MeOH (10 mL) and then with 70% acetone (10 mL). The two extracts were centrifuged and combined for the assay. The assay was carried out spectrophotometrically at 730 nm. For measurements, a calibration curve ($y=0.4481x+0.1977$, $R^2=0.9997$) with Trolox (0-500 µM aqueous solution) was plotted at 730 nm. The assay was performed as follow: 500 µL of test sample, diluted appropriately with water, or Trolox standard was mixed with 5 mL of ABTS^{•+} solution and incubated at 30°C for 30 min in the dark. Absorbance was read after 60 min. Results were expressed as micromoles of Trolox equivalents (TEAC) per gram of nut.

Statistical analysis

Results were expressed as means ± standard

deviation (SD) from three independent replicates. Data were stored in a spreadsheet (Excel 2007, Microsoft, USA). Dixon Q test for the identification and rejection of outliers in each series of data, with a significance level of 95% was used. When appropriate, to compare cultivars values ANOVA and Tuckey's post-test were used. Comparisons of the same cultivar in different years was performed by Student t- test. Values with $p\leq 0.05$ were considered as statistically significant with the assistance of Graphpad Prism 5.0 software (GraphPad Software, Inc., CA, USA) for the calculations.

Results and Discussion

Chemical composition and energetic value

The major components of the analyzed samples of *M. integrifolia* were, in order of importance, lipids (>70g/100g), total carbohydrates, protein, dietary fiber, moisture and minerals. When comparing the results of each cultivar per year, significant differences were observed between moisture levels in all three cultivars being the highest in all of 2014's samples (Table 1). The amount of total lipids extracted from the kernels ranged from 71.73 - 75.39 g/100 g. No significant differences were observed, except for the San Joaquín variety, when comparing the harvest from both years. A previous study (Wall, 2010), performed with samples of the HAES 344 variety, collected at the experimental station in Hawaii, shows that they presented 70.5 y 69.2 g/100g of total lipids, respectively for two consecutive years of harvest. No significant differences were observed in this study for that period. However, we found that the same variety presented higher levels of total lipids (74.21 and 75.39 g/100g in 2013 and 2014, respectively). Other authors (Maro *et al.*, 2012) studied these varieties harvested in Sao Paulo, Brazil and reported 61.7 y 46.2 g/100g for HAES 344 and Cannon, respectively. These values are lower than those observed in the present study, however, it has been reported up to 71.8 g of lipids/100g of *M. integrifolia* nuts commercialized at supermarkets in Brazil (Rodrigues *et al.*, 2013). The content of the various chemicals and nutrients in macadamia nuts can vary considerably depending on the cultivar, seed maturity, growing locations, and growing conditions (Munro and Garg, 2008). Protein contents vary from 5.19 to 7.56 g/100g. However, a study reported a value of 13.3 g/100g of protein for nuts of the HAES 344 cultivar harvested from 5 year old trees (Maro *et al.*, 2012). The age of the tree could also explain this difference. About carbohydrates, some authors report total carbohydrates based upon the difference.

Table 1. Proximate composition, mineral and α -tocopherol content of Paraguayan macadamia nuts, from 3 cultivars, in two years.

Compound	HAES 344		Cannon		San Joaquin	
	Harvest year					
	2013	2014	2013	2014	2013	2014
Moisture	1.61 ± 0.04 ^a	3.57 ± 0.07 ^b	1.75 ± 0.08 ^a	3.38 ± 0.01 ^b	1.62 ± 0.07 ^a	4.05 ± 0.06 ^b
Total lipids	75.39 ± 0.44 ^a	74.21 ± 0.50 ^a	73.52 ± 0.27 ^a	74.01 ± 3.42 ^a	74.21 ± 0.21 ^a	71.73 ± 0.61 ^b
Crude protein	7.50 ± 0.20 ^a	7.56 ± 0.32 ^a	6.17 ± 0.08 ^b	6.89 ± 0.02 ^b	5.19 ± 0.04 ^c	6.50 ± 0.04 ^b
Total	11.89 ± 0.90 ^b	7.95 ± 0.77 ^a	10.53 ± 0.49 ^b	10.79 ± 0.63 ^b	9.91 ± 0.73 ^b	10.09 ± 0.43 ^b
carbohydrates						
Sugars	9.62 ± 0.08 ^b	6.08 ± 0.30 ^a	9.52 ± 0.39 ^b	9.00 ± 0.88 ^c	8.91 ± 0.32 ^c	9.48 ± 0.03 ^c
Dietary fiber	5.72 ± 0.03 ^a	5.77 ± 0.04 ^a	6.02 ± 0.16 ^b	6.18 ± 0.39 ^b	6.07 ± 0.04 ^b	7.08 ± 0.18 ^c
Ash	1.50 ± 0.02 ^a	1.51 ± 0.01 ^a	1.55 ± 0.03 ^b	1.50 ± 0.04 ^b	1.51 ± 0.05 ^b	1.46 ± 0.07 ^b
Na	17.71 ± 0.34 ^a	19.11 ± 0.23 ^a	21.13 ± 0.86 ^{ab}	23.10 ± 0.89 ^b	23.72 ± 0.25 ^b	22.61 ± 1.1 ^b
K	390.41 ± 6.90 ^{bc}	429.85 ± 9.20 ^a	279.10 ± 2.00 ^b	315.08 ± 8.40 ^{bc}	303.51 ± 8.70 ^b	401.01 ± 5.80 ^c
Ca	58.41 ± 4.03 ^a	45.60 ± 1.19 ^a	76.22 ± 6.31 ^b	69.62 ± 1.76 ^b	83.23 ± 5.25 ^b	68.01 ± 2.19 ^b
Mg	110.42 ± 3.01 ^a	146.00 ± 5.10 ^b	112.21 ± 5.01 ^a	140.24 ± 14.02 ^b	118.11 ± 2.30 ^b	132.04 ± 13.4 ^{ab}
P	239.85 ± 15.04 ^a	175.09 ± 2.00 ^b	161.97 ± 5.11 ^c	154.07 ± 4.09 ^c	176.04 ± 4.05 ^b	181.03 ± 4.02 ^b
Fe	2.93 ± 0.48 ^a	3.11 ± 0.20 ^a	1.83 ± 0.10 ^b	2.23 ± 0.50 ^b	1.64 ± 0.07 ^c	2.08 ± 0.12 ^b
Zn	1.43 ± 0.22 ^a	1.73 ± 0.06 ^a	1.46 ± 0.24 ^a	1.50 ± 0.07 ^a	1.51 ± 0.02 ^a	1.57 ± 0.06 ^a
Cu	0.58 ± 0.03 ^a	0.97 ± 0.05 ^b	0.53 ± 0.02 ^a	1.01 ± 0.11 ^b	0.54 ± 0.05 ^a	0.66 ± 0.08 ^a
Mn	2.08 ± 0.27 ^a	4.86 ± 0.08 ^b	4.08 ± 0.35 ^c	3.27 ± 0.07 ^c	2.88 ± 0.14 ^a	2.47 ± 0.33 ^a
TP	96.31 ± 3.20 ^a	90.34 ± 8.0 ^b	84.19 ± 0.33 ^b	77.90 ± 9.51 ^b	86.20 ± 1.80 ^b	86.96 ± 7.03 ^{bc}
TAC	36.81 ± 2.90 ^a	26.11 ± 1.11 ^{bc}	23.32 ± 2.21 ^{bc}	21.00 ± 1.60 ^c	28.81 ± 0.20 ^b	24.81 ± 0.80 ^{bc}
α -tocopherol	3.32 ± 1.51 ^a	0.77 ± 0.17 ^a	3.24 ± 0.22 ^a	0.17 ± 0.03 ^b	18.40 ± 2.49 ^b	11.59 ± 0.41 ^b

Values are presented as mean \pm SD (n=3). Values in the same row with the same superscript letter are not significantly different ($p > 0.05$) as measured by ANOVA and Tuckey's post test, between cultivars and collection year. Moisture, total lipids, crude proteins, total carbohydrates, soluble sugars, and dietary fiber are presented as g/100g FW. Minerals as Na, K, Ca, Mg, P, Fe, Zn and Cu are presented as mg/100g FW. TP: Total phenolic compounds, are presented as mg GAE/100g FW, GAE: Galic acid equivalents are. TAC: total antioxidant capacity, are presented as μ M TAEC/g FW, TEAC: Trolox equivalents antioxidant capacity. The α -tocopherol content is present as mg/100g kernel.

Rodrigues *et al.* (2013), reports 19.1 g/100g values higher compared to this work (7.95-11.89 g/100g) because they include the dietary fiber. However, an interesting research (Wall and Gentry, 2007) reports that in macadamia nuts from the island of Hawaii, the total carbohydrates contents analyzed by HPLC does not exceed 5.6 g/100g, they do not contain significant quantities of starch. In the present study, dietary fiber levels vary from 5.72-7.08 g/100g, while the dietary fiber reference contents reported for *M. integrifolia* is 8.6 g/100g (USDA, 2015). According to these results, a portion (25 to 30 g) of macadamia nuts could yield between 1.4 and 1.8 g dietary fiber supplying from 5% to 7% of the average of the Recommended Dietary Allowances (RDA) for dietary fiber; 25 g/day (Institute of Medicine of the National Academies/ Food and Nutrition Board, 2002/2005). However, overall crude fiber was reported at levels ranging from 6.98 to 30.1 g/100g, which by definition often exceeds the recommended value of dietary fiber, by including the cellulose content (Silva, 2003; Venkatachalam and Sathe, 2006; Maro *et al.*, 2012).

The results show that total fat (up 75.4g/100g) exceeding 100% of the RDA (FAO/WHO, 2003) one of the highest reported for oleaginous nuts.

Compared to other nuts, low levels of total protein were observed, which cover from 2.06 to 2.52% of the RDA per a portion of 25 g (Table 2). The protein content of nuts is quite variable, but most nuts are considered to be a good source of protein they have between (8-15 g/100 g), but in the cashew, pistachio, almond and peanut the content is higher (18-26 g/100g) (Gray, 2013). Concerning total available carbohydrates (7.95-11.89 g/100g), analyses show that macadamia nuts represent a low contribution of these nutrients covering less than 1% of the RDA per a portion of 25g (Table 2). The energy value accounted for 100g was between 2986 and 3192 kJ. It is important to note that its high lipid content limits consumption of macadamia nuts in people prone to obesity or hypertriglyceridemia. However, a recommended 25 g portion that appears to have no adverse effect and may even have beneficial effects (Mozaffarian *et al.*, 2011), would provide about 790 kJ covering up to 9.3% total energy (Table 2), always based on a diet of 8400 kJ.

Analysis of fatty acids profile

The main fatty acids, identified by GC/MS analysis, were the monounsaturated oleic C18:1 and

Table 2. Percent relative contribution of macronutrients and energetic value based on the daily reference values, for one portion (1 portion: 25g) in two years 2013 y 2014

Compound	RDA ^a	HAES 344		Cannon		San Joaquín	
		2013	2014	2013	2014	2013	2014
Total lipids	55 g/day	34.30	33.70	33.40	33.60	33.70	32.30
Saturated fats	22 g/day	15.60	19.90	16.10	19.50	17.60	18.20
Proteins	75 g/day	2.52	2.50	2.30	2.06	2.17	2.06
Carbohidrates	300 g/day	0.66	0.99	0.90	0.88	0.84	0.83
Soluble sugars	90 g/day	1.69	2.67	2.50	2.64	2.63	2.48
Dietary fiber	25 g/day*	5.77	5.72	6.18	6.02	7.08	6.07
Energetic value	8400 kJ/day	9.26	9.32	9.15	9.16	9.17	8.80

Daily reference value source; FAO/WHO, 2003. Values were based on 25 g macadamia nut serving. ^aRecommended Dietary Allowances (RDA) on 8.400 kJ diet. *Value expressed as recommended intake.

palmitoleic C16:1 acids, and the saturated stearic C18:0 and palmitic C16:0 acids (Table 3). It was generally observed that the main fatty acids are those of C16 to C20 chain, length both saturated (SFA) and monounsaturated (MUFA), among which include palmitic, palmitoleic, stearic, oleic, arachidic and cis-11 eicosenoic acid. Trace amounts of C14:0, 17:0, 24:0, 17:1, 22:1, 18:3, and 20:3 were present in all cultivars; these fatty acids made up <0.5% of the total fatty acids. The main fatty acid C18:1 (approximately 60% of lipids) showed significant differences for each variety, when comparing the results of the two years sampling. In general, higher levels of C18:1 were observed in 2013, whereas in the 2014 harvest these values were about 20% lower in each variety (Table 3). However, no significant differences between varieties were observed, in both year of harvest. These results are consistent with those reported by Silva (2003) and Rodrigues *et al.* (2013), who observed contents of 51.8 to 57.6 g/100g MUFA in *M. integrifolia*, however Chung *et al.* (2013) and the USDA (2016) reported higher values of 81.3 and 77.4 g/100g, respectively. The total polyunsaturated fatty acids (PUFA), ranged from 1.33 to 3.95 g/100g. It was observed that in 2014's harvest (Table 3), the average contents of C18:2, C18:3 and C20:3 were higher for all varieties. These results indicate that in the same year, the studied varieties synthesize about the same amount of oleic acid and PUFAs, which in turn depends on the environmental conditions for the crop development and on the variety. We believe that more research is needed to clarify the effects of this factors on the fatty acids content of macadamia kernels harvested in Paraguay.

The increase in the content of PUFAs, influences the relation $\omega 6/\omega 3$, which was lower in all crop

varieties in 2014 (Table 3). The results of this study show a ratio of $\omega 6/\omega 3$, greater than those reported by Freitas and Naves (2010) with values ranging from 10 to 26. However, when compared to other nuts and seeds, it is a much more desirable relationship than that presented by oats ($\omega 6/\omega 3=74.5$), chestnuts ($\omega 6/\omega 3=232$) or almonds ($\omega 6/\omega 3=238$). As the optimum intake of linoleic acid $\omega 6$ and linolenic $\omega 3$, must be balanced, based on their metabolic functions for the production of eicosanoids, FAO/WHO (2003) recommended that the $\omega 6/\omega 3$ ratio on diet to range from 5:1 to 10:1, and that high intake of polyunsaturated fatty acids associated with low intake of linolenic fatty acid, contributes to the development of cardiovascular diseases (Simopoulos, 2002). In both adults men and women, 2% of the total daily energy intake should come from PUFA $\omega 6$ and 0.5% PUFA $\omega 3$. Meanwhile, FAO/WHO (2003) states that the recommendation is to eat 5-8 g/day of linoleic acid C18:2 and 1-2g of linolenic acid C18:3. According to these recommendations, macadamia nuts could cover from 11.5- 63.0% of the recommended intake of linoleic acid $\omega 6$, considering their content from 0.92-3.16 g linoleic acid per 100g and 2.5-31% of the RDA for linolenic acid. Thus, the macadamia nuts analyzed exhibit a high nutritional value, with a considerable supply of essential fatty acids, especially linoleic acid $\omega 6$.

Mineral analysis

The macadamia nuts analyzed for macrominerals (Na, Mg, P, K and Ca), showed significant content of Mg (110.42-146.00 mg/100g), P (154.07-239.85 mg/100g), K (279.10-429.85 mg/100g) and Ca (45.60-83.23 mg/100g), which are minerals normally found in high concentrations in nuts.

Table 3. Fatty acid composition of Paraguayan macadamia nuts, from 3 cultivars, in two years.

Cultivar	HAES 344		Cannon		San Joaquín	
Harvest year	2013	2014	2013	2014	2013	2014
SFA	13,7	17,5	14,3	17,2	15,5	16,0
C14:0	0.39±0.01 ^a	0.55±0.01 ^b	0.21±0.01 ^c	0.31±0.01 ^d	0.19±0.01 ^c	0.27±0.005 ^d
C16:0	6.07±0.08 ^a	6.03±0.17 ^a	6.30±0.06 ^b	5.58±0.10 ^b	5.45±0.11 ^b	5.63±0.14 ^b
C17:0	Nd	0.40±0.04 ^a	0.02±0.00 ^b	0.07±0.02 ^c	0.02±0.00 ^b	0.26±0.00 ^b
C18:0	3.47±0.03 ^a	4.78±0.18 ^b	4.34±0.11 ^c	5.64±0.04 ^d	5.52±0.04 ^d	4.73±0.04 ^b
C20:0	3.10±0.03 ^a	3.76±0.13 ^b	2.86±0.10 ^a	3.77±0.10 ^b	3.64±0.16 ^b	3.39±0.12 ^{ab}
C22:0	0.71±0.03 ^a	1.33±0.05 ^b	0.55±0.02 ^d	1.20±0.02 ^c	0.71±0.04 ^a	1.11±0.04 ^c
C24:0	Nd	0.63±0.04 ^a	Nd	0.61±0.01 ^a	Nd	0.57±0.02 ^b
MUFA	58.10	51.00	57.10	49.3	50.3	50.8
C16:1	12.00±0.21 ^a	12.80±0.20 ^b	9.19±0.01 ^c	10.40±0.24 ^d	7.01±0.10 ^e	11.10±0.12 ^d
C17:1	0.04±0.00 ^a	0.15±0.01 ^b	0.03±0.00 ^a	0.14±0.01 ^b	0.03±0.00 ^a	0.14±0.00 ^b
C18:1	44.40±0.35 ^a	33.90±1.36 ^b	46.61±0.37 ^a	35.31±0.73 ^b	41.80±0.01 ^a	36.20±0.64 ^b
C20:1	1.71±0.04 ^a	4.03±0.13 ^b	1.27±0.04 ^c	3.34±0.08 ^d	1.48±0.01 ^{bc}	3.30±0.11 ^d
C22:1	0.01±0.00 ^a	0.07±0.01 ^b	0.01±0.00 ^a	0.04±0.00 ^b	0.01±0.00 ^a	0.06±0.01 ^b
PUFA	1.33	3.57	1.56	3.95	1.85	3.90
C18:2 ω6	0.92±0.02 ^a	2.69±0.08 ^b	1.24±0.04 ^c	3.16±0.07 ^d	1.43±0.03 ^e	3.11±0.10 ^d
C18:3 ω3	0.06±0.00 ^a	0.30±0.02 ^b	0.05±0.01 ^a	0.27±0.01 ^b	0.06±0.00 ^a	0.31±0.01 ^b
C20:3 ω6	0.13±0.01 ^a	0.52±0.01 ^b	0.07±0.01 ^d	0.42±0.04 ^c	0.10±0.01 ^a	0.43±0.01 ^c
Total ω6	1.05	3.21	1.31	3.58	1.53	3.54
Total ω3	0.06	0.32	0.05	0.29	0.06	0.34
Ratio ω6/ω3	17.50	10.03	26.20	12.34	25.50	10.41

Values represented as mean ± SD (n=3). Means within rows followed by the same letter are not significantly different ($P > 0.05$), as measured by 1-sided ANOVA and Tuckey's post test. Nd: No detected, SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid. The fatty acids are present as mg/100g kernel. The ratio ω6/ω3 is adimensional.

Regarding trace minerals, high concentrations of Fe (1.64-3.11 mg/100g) and Mn (2.08-4.86 mg/100g) was also observed (Table 1). The observed values for Ca, Mg, Mn and Cu are in the range reported by Silva, (2003) and the USDA (2016), for raw *M. integrifolia* nuts in different samples of commercial macadamias from Brazil and the United States, respectively. Furthermore, Moodley, Kindness and Jonnalagadda (2007) found exceptionally high levels of Ca (337 mg/100g), Mg (495 mg/100g), and Fe (6.8 mg/100g) in commercial samples collected in South Africa. However, the study does not specified the species/cultivars of the nuts analyzed. It is worthy to mention that the levels of ash found in this study were twice as high (4%) as the average observed in our study (1.5%). According to Leterme *et al.* (2006) the mineral composition is related to the fertility conditions of each region, since the minerals are absorbed from the soil. Consuming the recommended daily amount of macadamia (25g) for different cultivars would contribute 22.6-67.5% Mn, 14.4-28.0% Cu, 2.28-9.70% Fe, 5.5-8.58% P, 6.55-10.6% Mg, 1.14-2.08% Ca, 1.49-2.29% K and 0.29-0.40% Na, based on RDA or adequate intake (AI) for adults (USDA, 2016). It is noteworthy to mention the high Mn content observed in the three varieties,

especially HAES 344 and Cannon, with values up to 4.8 and 4.00 mg/100g, respectively. According to this, one single portion could cover up to 50% of the RDA for this mineral. Thereby, under current recommendations, these results demonstrate that *M. integrifolia* nuts analyzed do not contribute with significant amounts (>15% de la RDA) of Na, K or Ca (Table 2). However, macadamia's cultivars can be an excellent source of Cur and Mn, and a good source of Mg, P, Fe and Zn.

Antioxidant activity, α-tocopherol and total polyphenols

In the determination of TPC statistically significant differences between the varieties in both years of study were observed (Table 1). HAES 344 presented the greatest TPC content and showed a statistically significant difference from the Cannon and San Joaquín cultivars in 2013, and with Cannon in 2014. The different origins of this varieties could explain the observed variations in the content of these minor compounds. The TPC was found around 80-95 mg/100g macadamia nut. The values agree with those reported in the literature (87 mg/100g) for dry macadamia nuts (Abe *et al.*, 2010).

Regarding α-tocopherol, the nuts from San

Joaquin cultivars showed the highest content and significant difference compared to HAES 344 and Cannon in both years, and stood out from the others with five times higher levels of α -tocopherol. In general, higher levels of α -tocopherol were observed in 2013, which could be related to the conservation conditions of nuts after harvest and other factors like the environmental conditions. Although not described specifically for macadamia, it is known that for other products, such as the olive, the fruit maturity index and storage period can decrease the content of α -tocopherol as well as their composition (Špika *et al.*, 2015). The data reported in the literature for α -tocopherol, according to Silva (2003), Kornsteiner *et al.*, (2005) and Wall (2010) did not detect α -tocopherol in macadamia nuts oil, however, Thomas and Gebhardt (2008) indicate that macadamia nuts contains 0.6 mg/100 g of α -tocopherol, which is lower than the amount observed in this study.

The samples had higher TAC in the harvest 2013, being the variety HAES 344 the one that showed the highest values in both years of study, with a significant difference from the other varieties. Furthermore, significant differences in consecutive years in each studied cultivar were observed (Table 1). Reports on the TAC of *M. integrifolia* whole nuts in different cultivars are limited. Contreras *et al.* (2011) reported 18.6 μ M TEAC/g on *M. integrifolia* nuts harvested in Colombia and Abe *et al.* (2010) reported 4.5 μ M TEAC/g in commercial *M. integrifolia* from Brazil. The values are lower than those observed in the present study. Moreover, Rodrigues *et al.* (2013) mentions that the antioxidant capacity of macadamia nuts is high and are superior to other nuts such as cashews, Brazil nuts and peanuts. The results are interesting, considering that the measure of the total antioxidant activity includes a variety of components. Besides phenols, other compounds may be responsible for the antioxidant activity displayed by the different nut types, such as tocopherols and minerals (Kornsteiner *et al.*, 2005). Current intake recommendations of α -tocopherol or vitamin E is 15 mg per day for women and men in adulthood and only 2.7 mg/day for children under 11 months (Institute of Medicine of the National Academies/Food and Nutrition Board, 2002/2005). According to these data, macadamia nuts of San Joaquín cultivars, will cover over 120% of the RDA (15 mg/day) of vitamin E per 100g, but a portion of 25g contribute up to 30% of the RDA in adults, males and females. It follows that especially the San Joaquín cultivar can be considered an excellent source of dietary α -tocopherol. Currently there are no definite recommendations about the use of polyphenols in the diet, although it is

recommended to consume 5 servings per day of foods containing them such as fruits and vegetables, in order to get the benefits associated with consumption of TPC (FAO/WHO, 2003). In the group of nuts, walnuts contain the highest TPC values (2499 mg GAE/100g). Comparatively with other nuts and edible seeds, analyzed macadamia nuts presented mean values between pinions, with 50 mg/100g, and almonds, with 114mg/100g (Abe *et al.*, 2010).

Correlations between TPC and TAC and α -tocopherol

At harvest 2013, it was observed that both levels α -tocopherol, TPC and TAC were higher than in 2014. The results of TPC and TAC, show that in the same year of harvest a positive correlation was observed (Pearson, r 2013=0.7904, r 2014= 0.9430) this is, a higher TPC increase the TAC in the same variety. However, we do not observed correlation within the alpha tocopherol content and TAC (Pearson, r 2013=-0.1232, r 2014= -0.0056). It is reported that TPC of macadamia oil (70 g/100g of the nut) is considerably low compared to other nut oils (Wall, 2010), but even so, it could be influencing the TAC. Wall (2010) reported that *M. integrifolia* varieties harvested in Hawaii, contain significant amount of tocotrienols and squalene, which would be responsible for the whole antioxidant capacity. Thus, the total antioxidant activity in macadamia nuts, appears to arise from a more complex and more dynamic interaction between a variety of essential nutrients like fatty acid composition and phytochemicals, which has not been established. The high TPC content and their correlation with observed antioxidant capacity indicate that these effects are not restricted to the lipidic fraction. Further, Kaijser *et al.* (2000) report that there is not a clear relationship between the stability of the oil and the contents of polyunsaturated fatty acids. It is more likely that the antioxidant capacity of the nut is influenced by factors such as the presence of others tocols, carotenoids and sterols (Kaijser *et al.*, 2000). The higher TAC in 2013, may be explained by favorable combinations of minor antioxidant components, other than α -tocopherol, which have not been addressed in this work.

Conclusion

The analyses results show that macadamia nuts are a rich source of nutrients. Minerals as K, P, Mg, Zn, Cu and Mn show good concentration, facing nutritional standards. The composition of macadamia nuts, with high content of MUFA, Mg, Mn, dietary fiber and TPC, suggests that these nuts harvested in

Paraguay, have cardio-protective potential and they are recommended under responsible consumption, as a part of health-healthy diet. The values of TPC observed in macadamia nuts (77.9-96.3 mg GAE/100g), known in vivo antioxidants, and dietary fiber content (>5 mg/100g), may contribute to the claimed lipid-lowering effects. It is always advisable to promote its responsible intake and it seems prudent to recommend the inclusion of nuts, like macadamia, in heart healthy diets, in substitution other high energy source foods. There is a correlation between levels of TPC and TAC in the nuts analyzed, but no correlation was observed between levels of α -tocopherol and TAC.

The presented data will be useful for nutritionists and epidemiologists to estimate polyphenol intake from macadamia nuts produced in Paraguay, in human populations. Our study may also provide a database for food scientists, food technologists, and the food industry in order to develop functional foods containing macadamia nuts. The results could also be used to complement Latin-American nutritional databases.

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