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Total Polyphenol Content and Antioxidant Capacity of Commercially Available Tea (*Camellia sinensis*) in Argentina

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Tea, *Camellia sinensis* (L.) O. Kuntze (Theaceae) is cultivated in Argentina in the northeastern region (provinces of Misiones and Corrientes), between 26° and 28° south latitude, the southernmost area of the world where tea is cultivated. The objective of this work was to determine the total polyphenol content and the *in vitro* antioxidant capacity of green and black tea cultivated and industrialized in Argentina. Twelve samples of eight brands were analyzed. The total polyphenol content was determined according to the International Organization for Standardization method (ISO) 14502-1 for the determination of substances characteristic of green and black tea. The antioxidant capacity was determined by the ferric thiocyanate method (FTC) and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay. Green tea showed a higher polyphenol content than black tea. The total polyphenol concentration in green tea was found to vary from 21.02 ± 1.54 to 14.32 ± 0.45% of gallic acid equivalents (GAE), whereas in black tea, the polyphenol content ranged from 17.62 ± 0.42 to 8.42 ± 0,55% of GAE ($P < 0.05$). A similar profile was observed for the antioxidant capacity determined by both methods. The antioxidant activities were well correlated with the total polyphenol content ($r^2 = 0.9935$ for the ferric thiocyanate method and $r^2 = 0.9141$ for the 1,1-diphenyl-2-picrylhydrazyl free-radical scavenging assay). This is the first systematic screening for the quantification of polyphenols and antioxidant activity in tea commercialized in Argentine markets. The results obtained herein allow one to conclude that Argentine tea is of very good quality when compared to teas from other sources.

KEYWORDS: ***Camellia sinensis*; tea; polyphenols; antioxidant; FTC; DPPH; Argentina**

INTRODUCTION

Apart from water, tea, *Camellia sinensis* (L.) O. Kuntze (Theaceae) is probably the most widely consumed beverage in the world (*1*). Even though the tea plant is cultivated all over the world, it grows best in tropical and subtropical areas with adequate rainfalls, good drainage, and a slightly acidic soil (*2*). The ecophysiology of the commercially grown tea plant is closely linked with the climate to which it adapts (*3*).

Commercially grown teas are hybrids of two distinct ecotypes: the Assam-type (var. *assamica*) and the China-type (var. *sinensis*) (*4*). Because of the distinctive difference in the ecology of their origins, the two ecotypes and their hybrids exhibit considerable variation in their ecophysiology. For example, var. *sinensis* is known to be a stronger ecotype than var. *assamica*, being resistant to both cold and hot drought conditions. However, var. *sinensis* is considered to be inferior in both quantity and quality of yield (*5*).

In Argentina, tea is cultivated in the northeast part of the country, between the 26° and 28° south latitude in the provinces of Misiones and Corrientes, the southernmost region of the world. About 10% of the production is commercialized in local markets, whereas the remaining 90% is exported. China and India make up a great proportion of the world's production (nearly 48.5% during 2005), and Argentina represents 2% of the international market. Over the last few years, the countries that have headed the demand of Argentine tea were the United States (55.2%) and Chile (14.3%) (*6*).

The catechins present in green tea are commonly called polyphenols. Green and black teas are processed differently during manufacturing. Fresh green tea leaves, which are very rich in catechins, are not fermented; they are withered, and catechin oxidation by polyphenol oxidase is prevented by steaming (Japan) or by panning (China) (*2*), processes that essentially maintain the polyphenols in their monomeric forms. The major catechins found in green tea are (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin-3-gallate, and (−)-epigallocatechin-3-gallate. Black tea leaves are subjected to crushing and a full fermenting process where catechin derivatives are oxidized, resulting in the formation of the polymeric compounds, thearubigins and theaflavins (*7*). The chemical structures of these catechins or polyphenols are shown in **Figure 1**. Catechins and other polyphenols have antioxidant activities (*8*). They act as antioxidants *in vitro* by sequestering metal ions and by scaveng-

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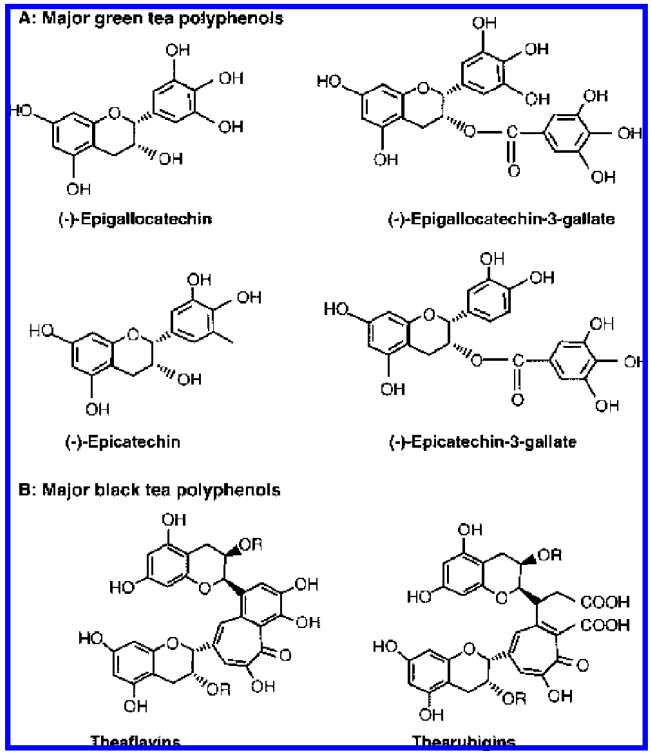

Figure 1. Chemical structures of major polyphenols present in tea. (**A**) Major polyphenolic constituents present in green tea (epicatechin derivatives). (**B**) Major polyphenolic constituents present in black tea. R = galloyl group.

ing reactive oxygen and nitrogen species (*9, 10*). The role of free radicals in injury to small-for-size grafts was investigated in rat liver explants. A polyphenol extract from *Camellia sinensis* decreased liver graft injury and increased the survival of small-for-size liver grafts, most likely by scavenging free radicals (*11*). The antioxidant capacity of polyphenols present in teas has been reported to be both chemoprotective and therapeutic (*12*). The ability to inhibit the effects of various mutagens in cultured cell lines has also been observed (*13*). They may also work indirectly as antioxidants through their effects on the activity of transcription factors and enzymes (*14*). The antioxidant properties of green tea and its constituent catechins were evaluated in a number of diseases associated with reactive oxygen species, such as cancer and cardiovascular and neurodegenerative diseases. Several epidemiological studies as well as studies in animal models have shown that green tea can confer protection against various cancers such as those of the skin, breast, prostate, and lung. Green tea has also been shown to be hypocholesterolemiant (*15*), to prevent the development of atherosclerotic plaques (*16*), to have antidiabetic effects in animal models of insulin resistance, and to have antibacterial, anti-inflammatory, and anti-HIV activities (*1, 17*−*19*). Among age-associated pathologies and neurodegenerative diseases, green tea has been shown to confer significant protection against Parkinson's disease, Alzheimer's disease, and ischemic damage (*20*). The increasing interest in its health benefits has led to the inclusion of green tea in the group of beverages with functional properties. The amount of tea polyphenols has been regarded as a quality indicator of tea (*21*). Information based on parameters, chemical standardization, and biological assays are complementary indicators of the quality of tea, regarding its biological activities. To date, no studies have been reported on the phenolic compounds and antioxidant capacity of tea from Argentina.

MATERIALS AND METHODS

Samples. Twelve commercial samples, consisting of tea leaf bags of eight different brands (A−H) were purchased in the supermarket in Buenos Aires city. Three of the samples were green tea (g.t.), and nine were black tea. Brands A, B, and C had both green tea and black tea. Brands D, E, F, G, and H corresponded to black tea only. For each commercial tea sample studied, three bags were sampled.

Chemicals and Reagents. For the determination of the total phenolic content (TPC), Folin−Ciocalteu's phenol reagent (Merck Chemicals Argentina, Buenos Aires), gallic acid (99% purity, Sigma Argentina), and anhydrous sodium carbonate (99% purity, Anedra Argentina) were used. For the determination of the antioxidant activity, 1,1-diphenyl-2-picrylhydrazyl, linoleic acid, ascorbic acid, and trizma base (Sigma Argentina), and $FeCl_2$ and ammonium thiocyanate (Merck Chemicals Argentina) were employed.

Extraction of Polyphenols. The method described by the International Organization for Standardization (ISO) 14502-1 was used (*22*). Briefly, 0.200 ± 0.001 g of each sample was weighed in an extraction tube, and 5 mL of 70% methanol at 70 °C was added. The extract was mixed and heated at 70 °C on a vortex for 10 min. After cooling at room temperature, the extract was centrifuged at 200*g* for 10 min. The supernatant was decanted in a graduated tube. The extraction step was repeated twice. Both extracts were pooled and the volume adjusted to 10 mL with cold 70% methanol. One milliliter of the extract was diluted with water to 100 mL.

Determination of Total Polyphenol Content. The total polyphenol content (TPC) was determined by spectrophotometry, using gallic acid as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1 (*22*). Briefly, 1.0 mL of the diluted sample extract was transferred in duplicate to separate tubes containing 5.0 mL of a 1/10 dilution of Folin−Ciocalteu's reagent in water. Then, 4.0 mL of a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm was measured against water. The TPC was expressed as gallic acid equivalents (GAE) in g/100 g material. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 10 to 50 μg/mL (Pearson's correlation coefficient: $r^2 = 0.9996$).

Determination of the Antioxidant Activity by the Ferric Thiocyanate Method. The antioxidant capacity was determined by the ferric thiocyanate method (FTC) (*23*). A volume of 0.8 mL of extracts with different TPC was mixed with 0.05 M phosphate buffer at pH 7 and 2.5% linoleic acid in ethanol to obtain 4 mL of solution. The resulting solutions were incubated at 38.5 °C in a glass flask. Aliquots were removed at regular intervals, and $FeCl_2$/ammonium thiocyanate solution was added in order to allow any peroxides resulting from the oxidation of linoleic acid to react, forming a complex that can be detected spectrophotometrically at 500 nm (Shimadzu UV 2101). This step was repeated every 24 h until the control (phosphate buffer plus linoleic acid) reached its maximum absorbance value. Therefore, high absorbance values indicated high levels of lino1eic acid oxidation. Phosphate buffer was used as the reaction blank. The total antioxidant activity was expressed as the average of three independent determinations carried out in duplicate. The percentage inhibition of lipid peroxidation of linoleic acid was calculated by applying the following equation: inhibition of lipid peroxidation (%) = $100 - [(A_s/A_0) \times 100]$, where A_0 is the absorbance of the control reaction (linoleic acid alone, 100% peroxidation), and A_s is the absorbance obtained in the presence of the sample extract or positive control of antioxidant activity (1 mg/mL ascorbic acid). The inhibitory concentration 50 (IC_{50}) values were calculated from data obtained graphically, using a mathematical method based on the principle of the right-angled triangle: $IE_{50} = D - [(A - 50\%\ \text{max response})X]/Y$, in which A is the immediately higher response of 50% max response; B is the immediately lower response of 50% max response; D = log concentration corresponding to A response; C = log concentration corresponding to B response; $X = D - C$; and $Y = A - B$ (*24*).

Determination of the Free Radical Scavenging Activity by the 1,1-Diphenyl-2-picrylhydrazyl Free-Radical Scavenging Assay. Scavenging activities of the extracts on the stable free radical DPPH were assayed using the modified Blois' method (*25*), in which the bleaching rate of DPPH is monitored at a characteristic wavelength in presence of the sample. A volume of 0.1 mL of an aqueous dilution of the extracts

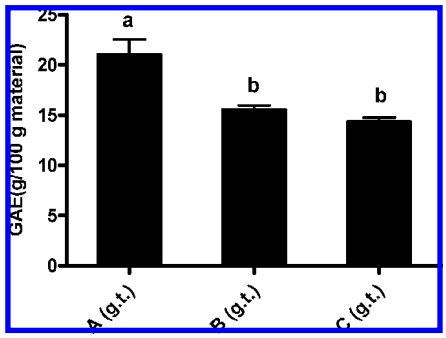

Figure 2. Total polyphenol content expressed as gallic acid equivalents (GAE; g/100 g plant material) in green tea (g.t.) bags from different brands. Bars represent the mean ± SEM of three independent experiments carried out in duplicate. Letters a and b indicate significative differences between samples according to one way ANOVA and Student−Newman−Keuls' tests; $p < 0.05$. Capital letters indicate different brands.

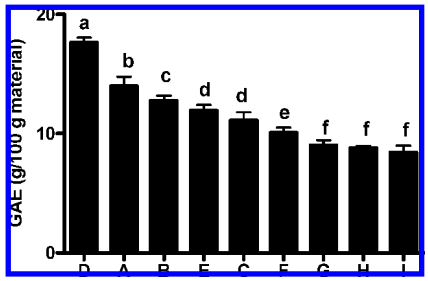

Figure 3. Total polyphenol content expressed as gallic acid equivalents (GAE; g/100 g plant material) in black tea bags from different brands. Bars represent the mean ± SEM of three independent experiments carried out in duplicate. Different letters indicate significative differences between samples according to one way ANOVA and Student−Newman−Keuls' tests; $p < 0.05$. Capital letters indicate different brands.

was mixed with 0.5 mL of a 500 μM DPPH solution in absolute ethanol and 0.4 mL of a 0.1 M Tris-ClH buffer at pH 7.4. The mixture was kept for 20 min in darkness, and then the absorbance was read at 517 nm. The percentage of decrease of DPPH bleading was calculated by measuring the absorbance of the sample and applying the following equation: % of inhibition = $[1 - (A_s/A_0)] \times 100$, where A_s is the absorbance of sample (i.e., extracts), and A_0 is the absorbance of the DPPH solution. Ascorbic acid solutions of different concentrations were used as positive controls for antioxidant activity.

Statistical Analysis. Data were expressed as the means ± standard error of the mean of three independent experiments carried out in duplicate. A one-way ANOVA with the *a posteriori* Student−Newman−Keuls' test was used to evaluate the significancy of results. A probability (p) value <0.05 was considered significant.

RESULTS AND DISCUSSION

Polyphenols Content. The Folin−Ciocalteu assay is one of the oldest methods developed to determine the content of total phenols (*26*). In this work, the total polyphenol content of 12 samples of green and black tea bags cultivated and industrialized in Argentina, belonging to eight different brands, was analyzed. Only three brands (A−C) had green and black tea in the market, whereas the remaining nine brands (D−I) contained black tea only. Results are presented in **Figures 2** and **3**. As shown in **Figure 2**, the total polyphenol content in green tea was found to vary from 21.02 ± 1.54 in brand A to 14.32 ± 0.45% GAE in brand C. No significant differences were found between brands B and C (15.53 ± 0.43% GAE vs 14.32 ± 0.45% GAE).

As shown in **Figure 3**, the total polyphenol content in black tea bags ranged from 17.62 ± 0.42% GAE to 8.42 ± 0.55% GAE.

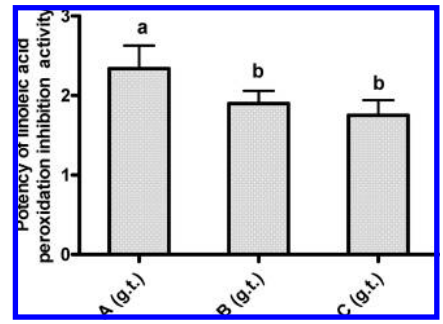

Figure 4. Potency of linoleic acid peroxidation inhibition of green tea (g.t.) bags. Bars represent the mean ± SEM of three independent experiments carried out in duplicate. Letters a and b indicate significative differences between samples according to one way ANOVA and Student−Newman−Keuls' tests; $p < 0.05$. Capital letters indicate different brands. Potency was calculated as follows: $P = -\log IC_{50}$.

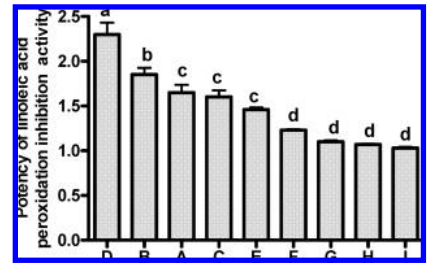

Figure 5. Potency of linoleic acid peroxidation inhibition of black tea bags. Bars represent the mean ± SEM of three independent experiments carried out in duplicate. Different letters indicate significative differences between samples according to one way ANOVA and Student−Newman−Keuls' tests; $p < 0.05$. Capital letters indicate different brands. Potency was calculated as follows: $P = -\log IC_{50}$.

The highest polyphenol content was found in the black tea sample from brand D, 17.62 ± 0.42% GAE, an amount even superior than those obtained for green tea bags from brand B (g.t.) and C (g.t.) (15.53 ± 0.43% GAE and 14.32 ± 0.45% GAE respectively, $p <$ 0.05 one way ANOVA and Student−Newman−Keuls' tests). Black tea bags from brands A and B had 12.74 ± 0.46% GAE and 13.97 ± 0.79% GAE, respectively. No significant differences were found between brands E (11.92 ± 0.48% GAE) and C (11.10 ± 0.69% GAE). The polyphenol content of brand F (10.05 ± 0.44% GAE) was higher than that obtained for brands G, H, and I (9.01 ± 0.43% GAE, 8.77 ± 0.19% GAE, and 8.42 ± 0.55% GAE, respectively), without any significant differences being found among them (**Figure 3**). The differences found between brands could be due to a postmaturation process where black tea continues to ferment (*27*). The oxidation of phenolic compounds in all types of teas during the storage period has been previously reported (*28*). The values obtained in this work for Argentine tea were comparatively higher than those reported for different brands commercialized in Malaysia, which showed % GAE values of 19.13 ± 0.37 and 11.37 ± 1.48 for green tea and 8.49 ± 0.80 and 6.06 ± 0.54 for black tea (*29*). Other authors have reported the total polyphenol content in Australian black tea bags, which had an average of 16%, a value comparatively higher than the average obtained in this work (11.51%), but in that case, different extraction and quantification methods were employed (*30*).

In this work, the total polyphenol content found in green tea bags was higher than that obtained in black tea bags, with the exception of brand D, which showed higher values than green tea bag samples: B (g.t.) and C (g.t.). This finding may indicate that polyphenols could have oxidized during the fermentation

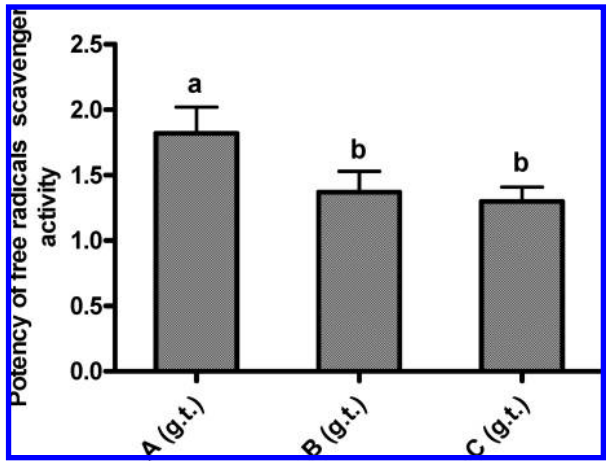

Figure 6. Potency of scavenging activity on free radical DPPH of green tea (g.t.) bags. Bars represent the mean ± SEM of three independent experiments carried out in duplicate. Letters a and b indicate significative differences between samples according to one way ANOVA and Student−Newman−Keuls' tests; $p < 0.05$. Capital letters indicate different brands. Potency was calculated as follows: $P = -\log IC_{50}$.

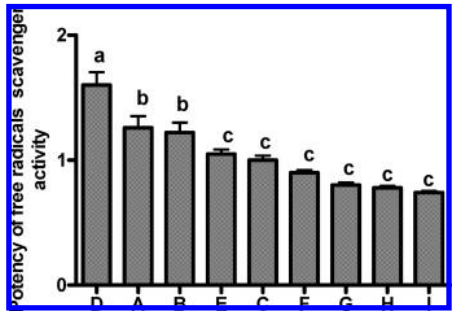

Figure 7. Potency of scavenging activity on free radical DPPH of black tea bags. Bars represent the mean ± SEM of three independent experiments carried out in duplicate. Different letters indicate significative differences between samples according to one way ANOVA and Student−Newman−Keuls' tests; $p < 0.05$. Capital letters indicate different brands. Potency was calculated as follows: $P = -\log IC_{50}$.

stage of green tea processing, thus obtaining a product of lower quality regarding the polyphenol content.

Usually, teas originating from Indian or Sri Lankan varieties (*Camellia sinensis* var. assamica) have higher polyphenol contents (ca. 30%) than those from the Chinese variety (*Camellia sinensis* var. sinensis, ca. 20%) (*31, 32*).

The applied research and the technological development linked with the production of tea began in 1957 in Argentina. This research has allowed the optimization of the production, obtaining specimens that are more resistant to plagues and diseases and that tolerate low temperatures. The handling of the soil with a superficial tilling each 5−6 years assures a perfect balance between the soil and the plant in a semiforest environment, with abundant organic matter and low luminosity. The different types of pruning, the cycles between them, the mechanized harvest, and the handling of the postharvest material have been well determined. This controlled process allows the arrival of the raw material to the drying process to occur with a minor degree of deterioration, thus obtaining a material of good quality (*33*).

The total polyphenol content of green tea bags from brand A (g.t.) obtained herein 21.02 ± 1.54% GAE was simmilar to that reported for green tea bag samples from China (21%−23%), one of the main tea producers of the world (*30*).

Antioxidant Activity. Both direct and indirect methods were applied to determine the antioxidant activity (AOA) in tea samples. As a rule, the data obtained with indirect methods must be positively correlated with data obtained by a direct method (*34*).

FTC Test. The AOA was expressed as IC_{50} (AOA expressed as inhibition percentage corresponding to the reduction of the peroxidation of linoleic acid of 50%). Results were then transformed into potency ($-\log IC_{50}$) values.

Brand A (g.t.) showed the higest antioxidant activity followed by brands B (g.t.) and C (g.t.). No significant differences were observed between the last two brands (**Figure 4**).

According to the AOA, black tea samples could be divided into four groups. The first, brand D, had the highest AOA, followed by brand B, and then followed by a group of three brands (A, C, and E). The last group (F, G, H, and I) had the lowest antioxidant activity without any significant differences between them ($p < 0.05$) (**Figure 5**).

The ascorbic acid solution (1 mg/mL) employed as positive control rendered AOA values of 50 ± 5%.

DPPH Test. In this test, the AOA was also expressed as IC_{50} (AOA expressed as inhibition percentage corresponding to a reduction of the absorbance of DPPH of 50%). Results were then transformed into potency ($-\log IC_{50}$) values.

Brand A (g.t.) showed the higest antioxidant activity followed by brands B (g.t.) and C (g.t.). No significant differences were found between the last two brands (**Figure 6**).

According to the AOA, black tea samples could be divided into three groups. The first, brand D, had the highest AOA, followed by brand A and brand B, both with simmilar AOA. The third group corresponded to brands C, E, F, G, H, and I, which showed the lowest antioxidant activity without any significative differences among them ($p < 0.05$) (**Figure 7**).

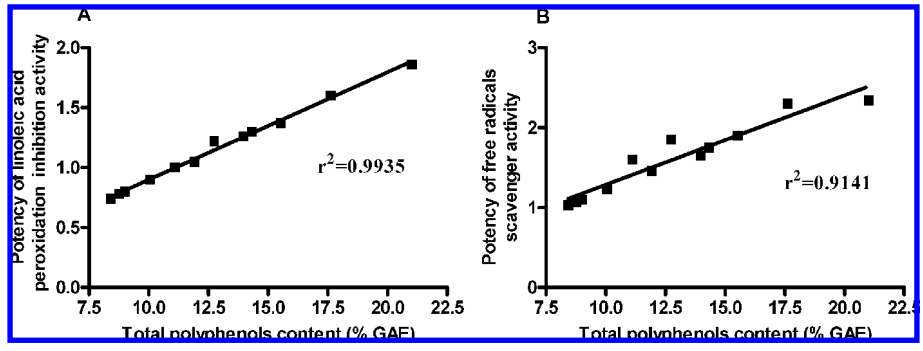

Figure 8. Correlation between the potency of linoleic acid peroxidation inhibition (**A**) or scavenging activity on the free radical DPPH (**B**) and total polyphenol content. Results represent the mean ± SEM of three independent experiments performed in duplicate. % GAE = gallic acid equivalents g/100 g plant material.

The ascorbic acid solutions employed as positive control at the following concentrations 0.1 μg/mL; 1.0 μg/mL; 10 μg/mL; 100 μg/mL; and 1.000 μg/mL rendered AOA values of 0; 6.3 ± 0.5%; 63.6 ± 5.0%; 74.0 ± 5.0%; and 83.3 ± 7.0%, respectively.

Correlation between the Antioxidant Capacity and the Total Polyphenol Content. Numerous examples of the application of the Folin−Ciocalteu assay to characterize natural products may be found in the literature. In most cases, total phenols determined by this method are correlated with the antioxidant capacities confirming the value of the Folin−Ciocalteu test (*34*). A new enzymatic method involoving the use of horseradish peroxidase and 4-aminoantipyrine has recently been used in a comparative study of total polyphenol content of tea. Significant differences were found in the results obtained by the Folin−Ciocalteu and enzimatic methods indicating that the enzymatic method needs further standardization (*35*).

In this work, a high correlation was demonstrated between the total polyphenol content and antioxidant capacities by both methods. Pearson's correlation coefficients (r^2) were 0.9935 and 0.9141 for the FTC and DPPH assays, respectively (**Figure 8A**, **B**).

The total polyphenol content and the antioxidant activity are both parameters of quality for tea regarding its biological properties, and both assays should be applied for the quality control of manufactured and imported teas. According to the results obtained, tea from Argentina is of very good quality in comparison to tea from other sources. This is the first systematic survey of teas from the Argentine market.

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