

Antioxidant Profile of Different Types of Herbal Infusions and Teas Commercially Available in Mexico

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Abstract

Different types of teas and herbal infusions were studied. Antioxidant properties and phenolics compounds were estimated. *Camellia sinensis* based teas presented the highest values for inhibition of free radicals such as DPPH[•] and ABTS^{•+}. The results showed different values between teas (*Camellia sinensis*) and herbal infusions (as *Hibiscus sabdariffa*). Green tea presented the highest values for inhibition of lipid oxidation (95.03 ± 3.34 to 31.68 ± 14.50 %) and polyphenolics content (0.112 ± 0.018 to 1.343 ± 0.068 GAE mg mL⁻¹). In HPLC test, suggested the presence of antioxidant compounds such as episyngic acid, procyanidin among others phenolic compounds. This study concluded that mexican teas and herbal infusions have potential to provide several benefits for human health, due to phenolic compounds present in them.

Keywords: antioxidant activity; polyphenolic compounds; herbal infusions and teas.

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1. Introduction

The extracts obtained from natural plants represent a novel source for obtaining high-quality and high-activity antioxidant with multiple applications in food pharmacological industries. In the literature some authors have reviewed the healthy and food technological benefits provide by several families of plants from different parts around the world [12,2]. In this sense, those plants represent an attractive alternatives to develop functional beverages by adding the active compounds obtained from these plants or by the consumption themselves. According to [10], tea is the second most widely consumed beverage around the world, which is rich in polyphenolic compounds contributing to the health benefits of tea. In addition, it has been observed that tea infusions have relatively higher phenolic content than those prepared from fresh traditional plants [16].

Due to the wide functional properties of the tea extracts/infusions, they have been applied in several studies as health promoting and food technological ingredients. In a recent study carried out by [14], the authors reported that leafy herbal tea have the ability to inhibit some cariogenic bacteria, when water extraction method is used to obtain them and therefore improve dental health simply by consumption of these extracts. In addition, it has been established the relationship between quinolinic acid peroxidation on brain and the free radical scavenging capacity of both, sour and green teas. Some authors reported that freshly prepared aqueous extracts form these teas can prevent the quinolinic acid-induced peroxidation *in vitro* at different levels and this findings were attributed to the presence of phenolic compounds such as catechin/galate for green tea, while for sour tea esculetin, dephinidin and cyaniding were described [13]. In terms of food technology, the results obtained for [16] suggest that tea extracts are good source for natural antioxidant, which can be used to preserved quality and nutritional aspects of rich-lipid food as mayonnaise, just to mention some benefits.

According to [4], it is very important to carry out researches regarding to the chemical composition and bioactivity of commercial teas in order to give the consumers scientific information about these functional beverages. Taken into account all above exposed, in the present study herbal infusions and teas commercially available in Mexico were characterized in their antioxidant profile and total polyphenolic content.

2. Materials and methods

2.1. Chemicals and samples

Specialized reagents as DPPH·, ABTS·+, Folin-Ciocalteu, Ethanol, Linoleic acid, Potassium ferricyanide, Amberlite XAD-16, Gallic acid and other standards were purchased from SIGMA ALDRICH with high purity or HPLC grade, as appropriate. Other reagents were used as reagent grade. Teas were obtained from a randomly purchase from local supermarkets and herbalist shops in the metropolitan area of Monterrey, Nuevo León, México.

2.2. Sample preparation

Each sample was homogenized in weight to 3 g. The samples were prepared by adding 250 mL of distilled hot water (equivalent to a cup) and the mixture was kept in constant agitation (200 rpm) with a magnetic stirrer for 5 min. After this time, samples were filtrated through Whatman #1 filter paper. Finally, tea infusions were

transferred to Falcon dark tubes and closed in a modified atmosphere with nitrogen and immediately frozen at -20 °C until use.

2.3. Functional characterization

Functional characterization was based on 1) the ability of each sample to scavenge free radicals, 2) by inhibiting lipid peroxidation, 3) for their ferric reducing antioxidant power. Two different free radical scavenging assays were adopted in order to determine the capacity of the tea infusions to scavenge free radicals namely: [1, 1-diphenyl-2-picrylhydrazyl (DPPH[•]) and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}).

2.3.1. DPPH[•] antioxidant assay

The DPPH[•] radical scavenging was determined using the methodology previously described by [17] with slight modifications. Briefly, 50 µL of each sample were mixed with 2.95 mL of DPPH[•] solution 60µM (DPPH[•] methanol), to obtain a total volume of 3 mL. The samples were homogenized for 10 seconds and left 30 minutes in the dark. Finally, absorbance was recorded at 517 nm and the results were expressed as gallic acid equivalents per milliliter of infusion (GAE mL⁻¹) according to the calibration curve prepared with the same standard.

2.3.2. ABTS^{•+} antioxidant assay

The ABTS^{•+} scavenging capacity assay was carried out according to the methodology proposed by [18]. A stock solution containing 2:1 volumes of 7mM ABTS solution and 2.45 mM of potassium persulfate was rested from 12 to 16 hours at room temperature and adjusted with absolute ethanol until reaching an absorbance of 0.700 ± 0.002 nm according to the original methodology. After that, the measuring cell was placed in the spectrophotometer, 50 µL of each diluted infusion (1:2) and 950 µL of the above resulting solution were added. After 1 minute, the absorbance was measured at 734 nm. The results were expressed as Trolox equivalents per milliliter (TE mL⁻¹) according to the calibration curve prepared with the same standard.

2.3.3. Inhibition of lipid oxidation

Inhibition of lipid oxidation was conducted using linoleic acid as the lipid source. This was made according to the method described by [21] with minor modifications. Linoleic acid solution was prepared as follows: 0.6 g of linoleic acid and 1.5 g of tween 20 diluted in 8 mL of ethanol (96 %). Then, 50 µL of each extract was mixed with 100 µL of the solution of linoleic acid and 1.5 mL of acetate buffer solution (0.02 M, pH 4.0). The controls contained 50 µL of acetates solution. All samples were homogenized and emulsions were incubated at 37 °C in order to induce the oxidation of linoleic acid, then immediately added 750 µL of a solution of FeCl₂ 50 mM (0.01 g of FeCl₂ were mixed with 0.017 g EDTA and diluted in 1 L of distilled water). An aliquot of 250 µL of the above mixture was taken and added 1 mL of 0.1 M NaOH in ethanol (10 %) to stop the oxidation reaction. Subsequently, 2.5 mL of 10 % ethanol were added and absorbance was measured at 237 nm using acetate buffer as blank.

2.3.4. Ferric reducing antioxidant power (FRAP)

The method of [7] was adopted to determine the reduction power in the samples with slight modifications. Briefly, 50 μL of each infusion was mixed with 120 μL of phosphate buffer (0.1M, pH 6.6). Then, 120 μL of potassium ferricyanide were added to the mixture, which was homogenized and incubated at 50 °C for 20 minutes. Afterward, 120 μL of trichloroacetic acid at 10 % were incorporated to the incubated mixture, followed by 410 μL of distilled water and 100 μL of ferric chloride. Finally, the absorbance was measured at 700 nm and results were expressed as gallic acid equivalents per milliliter (GAE mL^{-1}) according to the calibration curve prepared with the same standard.

2.4. Determination of phenolic compounds

Determination of total phenolic content by the Folin-Ciocalteu was carried out according to the methodology proposed by [11] with minor modifications. In this case, 250 μL of extract were added to 250 μL of Folin-Ciocalteu reagent and 250 μL of sodium carbonate. The mixture was homogenized and incubated at 40 °C for 30 minutes. Next, 2 mL of distilled water were added to the mixture and the absorbance at 750 nm was measured.

2.5. Determination of flavonoids

The determination of flavonoids was carried out according to the method proposed by [9]. A volume of 150 μL of each sample and 150 μL of sodium nitrite (5 %) were mixed, the mixture was homogenized and added 150 μL of aluminum chloride at 10 % and 1 mL of sodium hydroxide 0.1 M, homogenized and absorbance was measured at 510 nm.

2.6. Identification of phenolic compounds

High Performance Liquid Chromatography Resolution carried out for detection of phenolic compounds in samples. After purification with Amberlita XAD-16, samples were run on a HPLC-Varian equipment, Auto sampler Model 410 with mixing pump Varian Model 230I and a detector PDA Varian Model 330 computer. Samples were processed at a temperature of 30 °C, using a Grace Denali C-18 column 5 μM (250 mm x 4.6 mm). The flow rate was 1.2 mL/min, the number of phases used in the process was: pumping samples (methanol), percentage solvent (acetonitrile), and mixture of polar and non-polar solvents (acetic acid 3 %).

2.7. Statistical analysis

The significant differences between the mean values of different characteristics were determined using the Tukey test for multiple comparisons to the probability of 5 % ($p \leq 0.05$).

3. Results

3.1. Functional characterization of tea and herbal infusions

Phenolic compounds exhibit antioxidant capacity through the effects of free radical scavenging. Usually, antioxidant and free radical scavenging properties of several plant extracts or infusions are linked to

polyphenolic content. According to [24], those properties are the most widely accepted by researchers to attribute several health-benefits of the polyphenolic compounds. To determine free radical scavenging activity in teas and herbal infusions, two types of radical used DPPH and ABTS⁺, both methods work for estimating the scavenging activity of free radicals in samples.

3.1.1. DPPH antioxidant assay

Inhibition capacity of DPPH radical from herbal infusions and teas commercially available in Mexico were analyzed in this study. The values ranged from 94.33 ± 0.84 to 57.52 ± 5.9 GAE mg mL⁻¹ as follows: pomegranate > green > jasmine > white > peach > raspberry > black > roselle > lemon. The results of the inhibition capacity of DPPH· of the tea infusions are presented in Table 1.

3.1.2. ABTS⁺ antioxidant assay

The values obtained to inhibit the radical ABTS⁺ of herbal infusions and teas analyzed in this study ranged from 266.23 ± 0.21 to 52.89 ± 8.18 TE mg mL⁻¹ as follows: pomegranate > peach > raspberry > black > white > roselle > jasmine > green > lemon. The results of the inhibition capacity of ABTS⁺ of the tea infusions are presented in Table 1.

3.1.3. Inhibition of lipid oxidation

Although the analysis of inhibition of DPPH radicals and ABTS⁺ are widely used to measure the antioxidant capacity in vitro of extracts from several samples of plants, these analyzes can only give limited information about the antioxidant properties of these extracts in real biological systems. For this reason, an additional analysis must be carried out in order to simulate conditions on food systems. According to [8], the use of linoleic acid as the lipid source in this analysis can simulate the lipids present in biological systems. The inhibition of lipid oxidation of herbal infusions and teas were analyzed in this study and the values ranged from 95.03 ± 3.34 to 31.68 ± 14.50 % as follows: green > jasmine > pomegranate > peach > white > lemon > raspberry > black. Values for inhibition of lipid oxidation are expressed in Table 1.

3.1.4. Ferric reducing antioxidant power

The results of the reducing power of the samples are presented in Table 1. It was observed that the evaluated infusions are able to reduce Fe⁺³ to Fe⁺² in an independent way. Just as the polyphenolic content, the *Camelia sinensis*-based teas showed the highest values for reducing power (Table 1).

3.2. Determination of total phenolic content

The determination of the total polyphenolic compounds of herbal infusions and teas were analyzed, and the values ranged from 0.112 ± 0.018 to 1.343 ± 0.068 GAE mg ml⁻¹ as follows: jasmine > green > white > pomegranate > peach > black > raspberry > roselle > lemon. Values for these infusions polyphenols are expressed in Figure 1.

Table 1: DPPH[•] and ABTS^{•+} radical scavenging, FRAP, inhibition of lipid oxidation and main phenolic compounds within herbal infusions and teas

Commercially available name	Sample composition	DPPH	ABTS	FRAP (mg ml ⁻¹)	LOI (%)
		(GAE mg ml ⁻¹)	(TE mg ml ⁻¹)		
Lemon tea	<i>Cymbopogon citratus</i> Stapf. bit leaves	57.52 ± 5.91 e	52.89 ± 8.18 c	0.057 ± 0.011 e	55.63 ± 11.20 cd
Pomegranate tea	<i>Camelia sinensis</i> and Hibiscus sabdariffa, <i>Aspalathus linearis</i> bit leaves, orange peel and dehydrated pomegranate	94.33 ± 0.84 a	266.23 ± 0.21 a	0.385 ± 0.024 b	89.49 ± 2.64 ab
Raspberry tea	<i>Hibiscus sabdariffa</i> , <i>Malusa domestica</i> , <i>Rosa canina</i> , raspberry natural-identical flavor, citric acid and <i>Rubus idaeus</i>	86.38 ± 3.33 bc	264.85 ± 1.42 a	0.185 ± 0.012 c	32.66 ± 11.68 e
Peach tea	<i>Ceylon</i> black tea with peach flavor	90.14 ± 0.64 ab	266.16 ± 1.41 a	0.371 ± 0.035 cd	81.09 ± 8.77 b
Roselle tea	<i>Hibiscus sabdariffa</i> bit leaves	72.51 ± 9.21 d	219.25 ± 25.95 b	0.169 ± 0.023 d	46.21 ± 12.65 d
Black tea	<i>Camelia sinensis</i> bit leaves	84.14 ± 0.48 c	260.64 ± 23.25 a	0.150 ± 0.011 d	31.68 ± 14.50 e
Green tea	<i>Camelia sinensis</i> bit leaves	93.21 ± 0.13 a	214.22 ± 27.72 b	0.409 ± 0.015 a	95.03 ± 3.34 a
Jasmine tea	<i>Camelia sinensis</i> bit leaves and <i>Jasminum officinale</i>	91.84 ± 0.48 a	215.11 ± 21.46 b	0.384 ± 0.016 b	91.75 ± 4.91 a
White tea	<i>Camelia sinensis</i> bit leaves	90.89 ± 1.11 a	230.98 ± 17.67 b	0.374 ± 0.025 b	62.01 ± 6.71 c

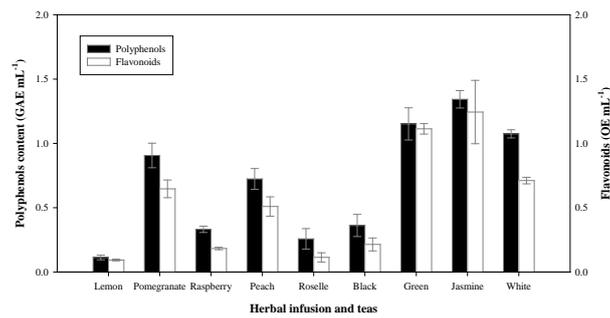


Figure 1: Total polyphenols and flavonoids content of different types of herbal infusions and teas

3.3. Determination of flavonoids

Determination of flavonoids of herbal infusions and teas was analyzed in this study and the values ranged from 1.24 ± 0.24 to 0.09 ± 0.007 QE mg ml⁻¹ as follows: jasmine > green > white > pomegranate > peach > black > raspberry > roselle > lemon. The content of flavonoids for these infusions is expressed together with the content of total polyphenols in Figure 1. The lowest values were recorded by the herbal infusion of lemon; however, the highest values were recorded by teas made from *Camellia sinensis*, as the results obtained in the determination of total polyphenols, mainly for jasmine, green and white teas.

3.4. Identification of phenolic compounds

Flavonoids, hydroxybenzoic acids and a epicatechin timer were identified as the main phenolic compounds in herbal infusions and teas. However, this analysis also revealed two or three more peaks on each sample that corresponded to other phenolic compounds not identified, which may contribute to samples biological activities together with the identified phenolic acids (Table 2).

Table 2: Main phenolic compounds identified in herbal infusion and teas

Phenolic compound	Terention time (minutes)	Lemontea	Pomegranate tea	Raspberry tea	Peach tea	Roselle tea	Black tea	Jasmine tea	White tea	Green tea
Epicatechin	15.707	✓								
Chlorogenic acid	12.43									✓
Syringic acid	15.679		✓	✓	✓	✓	✓	✓	✓	✓
Vanillinic acid	14.656	✓							✓	
Catechol	21.624									✓
Catechin	11.732		✓	✓						
Procyanidin B	13.312					✓				
Procyanidin C	16.462	✓	✓		✓			✓	✓	✓
Punicalagin	9.612			✓	✓	✓			✓	
Quercetin	22.049									
Resorcinol	7.843			✓						

4. Discussion

4.1. Functional characterization of herbal infusions and teas

The infusions of lemon tea had the lowest capacity with respect to inhibition of DPPH[•] radical, which were according to the findings reported by [14]. However, the *Camellia sinensis*-based teas had the highest values which were similar to those reported by [3], mainly in infusions based on wild fruits (especially stands out the pomegranate) and in the case of the green tea variety (such as jasmine). Inhibition capacity of DPPH[•] radical presents very similar results to the ones obtained by the ABTS^{•+} radical scavenging assay, which may indicate that the antioxidants present in herbal infusions and teas work in a very similar way to eliminate radicals formed in each assay.

As in the previous analysis, lemon infusions had the lowest values for the ability to inhibit the radical ABTS^{•+}. Meanwhile, the teas with higher values were: pomegranate tea (composed by *Camellia sinensis* and *Hibiscus sabdariffa*), peach tea (made by Ceylon tea, a variety derived from black tea, which is one of the best varieties due to their organoleptic properties and a special management on the process), and the raspberry tea (made mostly of *Hibiscus sabdariffa* and a mixture of different plants and nuts). Although significant differences between samples were minimal, it is important that chemical interactions (synergism, antagonism and additional effects) among the various polyphenolic compounds may occur in tea and other complex food matrix [4].

Regarding to inhibition of lipid oxidation, green tea had the highest values for this assay, which are similar to those reported by [13]. It has been reported that in the presence of antioxidant compounds, such as polyphenolic compounds, the accumulation of lipid oxidation products should be minimal until the antioxidants present in the samples have been "killed" [8]. In this sense, we can assume that polyphenolic compounds present in the samples analyzed are able to avoid peroxidation of linoleic acid under the test conditions.

According to the results for FRAP assay, similar effect was observed by [4] who reported the highest values of FRAP for *Camellia sinensis* when evaluated different brazilian teas, which matches with the total polyphenolic content in the analyzed samples. In counterpart, the lowest values were recorded for lemon tea, which were higher than those reported by [14]. FRAP values for roselle and green teas evaluated in this study were similar to those reported by [13].

These results above indicate suggest that Mexican herbal infusions and teas have antioxidant properties comparable with those exhibited by several plant infusions from Brazil, Portugal, Italy and Germany, among others countries, which indicates their potential benefits for human health.

For phenolic content, infusions from lemon tea presented the lowest values of these compounds, which were similar to the reporter by [4]. In general, *Camellia sinensis*-based extracts exhibited higher values with respect to other samples except for the black tea. These findings are consistent with [1], who observed the same trend for green, white, and black tea, obtained from the same crop. Furthermore, since the black tea is the result of the oxidation of the polyphenols in the leaf through a multi-stage enzymatic process carried out mainly by polyphenol oxidases and peroxidases, the results obtained in this study could be attributed to such bioprocesses,

which usually affect the content of catechins in tea [16]. Higher values were presented by jasmine tea, which is composed of *Camellia sinensis*-based tea supplemented with 5 % of jasmine flowers (*Jasminum officinale*), and this result could be related to the effect of addition, due to the presence of some phenolic compounds such as 3, 4-dihydroxy 3, 4 phenethyl, protocatechic acid, among others present in the latter one [19]. Although pomegranate tea is composed of *Camellia sinensis* and contains material from different plants, it was one of the teas with lower content of polyphenols. This discrepancy can be explained because the chemical composition of teas not only depends on the quality of the original plant, the geographical origin, and climatic conditions, but also the pathophysiological and environmental conditions, water stress, and soil composition, among others [4]. Similar results were recorded for infusion of raspberry, which has lower polyphenolic content compared to other infusions, with the exception of roselle and lemon. Finally, the values obtained by the infusion of roselle were similar to those reported by [6] for a drink based on *Hibiscus sabdariffa*, which causes a significant increase in the antioxidant potential of human plasma increase.

According [4], the different *Camellia sinensis*-based teas contain gallic acid, chlorogenic acid, catechin, epicatechin, procyanidin B2, quercetin, and caffeine. Moreover, [5] reported different compounds present in fractions obtained from an infusion from *Cymbopogon citratus* leaves (main component in lemon tea), such as tannins, phenolic acids (caffeic and *p*-coumaric acid derivatives). On the other hand, some authors suggest the presence of quercetin, luteolin, a luteolin glycoside and chlorogenic acid using TLC and HPLC fingerprint analysis of an extract of *H. sabdariffa* flowers [20]. Differences (Table 2) could be explained due to chemical components in samples depend on several environmental and technological factors such as geographic origin and manufacturing processes. According to [15], the market related to functional beverages still remaining small and fragmented. Therefore, infusions in the form of tea commercially available in Mexico can be considered as a good resource for bioactive compounds, which can be used as the formulation of a functional beverage or for various health benefits.

5. Conclusion

The variety of tea polyphenols extracted has become a topic of great interest for its role associated with health benefits. Our work demonstrates the power and the phenolic antioxidant present in beverages profile commercially available, providing the knowledge to evaluate their potential to the consumer anticipating their health benefits and food technology. However, more investigation about identification of phenolic compounds in samples must be done.

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