Radical Scavenging, Antioxidant and Ferric Reducing Activities of Commercial Mineral Water Enriched with Fruit and Ready to Drink Flavoured Teas

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Abstract: The antioxidant properties of the commercial mineral water enriched with fruit and flavoured teas ready to drink were evaluated and compared with total phenolics, flavonoids, antocyanins and ascorbic acid content. Spectrophotometrical methods were used to evaluate total phenols, flavonoids and antocyanins content. Vitamin C content was also determined by titration with DIF. To screen the antioxidant properties DPPH and ABTS radical method, β-carotene bleaching test and FRAP assay were used. Mineral water enriched with fruit contains significantly higher level of total phenolics comparing with analyzed ready to drink flavoured teas. Generally, mineral water enriched with fruit exhibited a stronger radical scavenging activity compared to flavoured teas in both DPPH and ABTS test. Using the FRAP assay that measured the reducing ability of antioxidants flavoured ready to drink tea ranged from 1.42 to 3.80 mM Fe(II) while mineral water enriched with fruit ranged from 7.01 to 38.46 mM. Mineral water enriched with fruit also exhibited a higher inhibition of linoleic acid oxidation particularly sample FWE4 that showed IC_{50} values of 0.70 and 1.61 v/v at 30 and 60 min of incubation. Collectively, our results improve the knowledge concerning the antioxidant properties of different commercially ready to drink teas and fruit enriched mineral waters available on the Italian consumer market that could be effectively considerd as functional drinks.

Keywords: Mineral water enriched with fruits, Flavoured teas ready to drink, Antioxidant properties, Polyphenol content, Technological parameters.

INTRODUCTION

Reactive oxygen species (ROS) such as super oxide anion, hydroxyl radical and hydrogen peroxide result from the cellular redox process. At low or moderate concentrations, ROS exert beneficial effects on cellular responses and immune function but higher levels are implicated in the development of chronic and degenerative diseases [1]. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced *in situ*, or externally supplied through foods and/or supplements. These antioxidants act as free radical scavengers by preventing damages caused by ROS [1].

In recent years, there is an increasing interest in finding dietary antioxidant that can (sacrificially) scavenge reactive oxygen/nitrogen species (ROS/RNS) to stop radical chain reactions, or that can inhibit the reactive oxidants from being formed in the first place (preventive) [2]. Dietary water-soluble antioxidants, including polyphenolic compounds, vitamin C, are believed to be the effective nutrients in the prevention of these oxidative stress related diseases [3]. The most effective components seem to be phenolic compounds of many raw plant materials, particularly in herbs, seeds and fruits. Being a polyphenol-rich food with health benefits, it has become a more common element in food marketing. The public is highly aware of the term "antioxidant", which has been defined by the Institute of Medicine of the National Academy of Sciences as follows: "a substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiologic function in humans." Their metal-chelating capabilities and radical- scavenging properties have enabled phenolic compounds to be thought of as effective free radical scavengers and inhibitors of lipid peroxidation [4]. Especially flavonoids have attracted considerable interest because of their associated health properties, and these have been summarized in several papers [1, 5, 6].

Therefore, the marketing of many so-called "super foods" is commonly based on their antioxidant potential. In fact, a number of antioxidant foods claim to have superior antioxidant activity with health benefits based on *in vitro* antioxidant assays, and a limited number also have clinical evidence demonstrating effects on physiological function that can be related to oxidant protection.

Following the understanding of the benefits of antioxidant compounds, customers ask the food industry to develop beverages rich in antioxidants. Among them new products that are taking place recently are functional waters. The commercial fruit enriched mineral water and the ready to drink herbal teas has contributed towards the development of

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a new area of functional and healthy drinking. These are a drinks enriched with substances known to be beneficial in terms of protection against oxidative stress. New dietary habits aspire towards products which combine health and wellbeing with good taste and flavour. Fruit enriched water bases its strengths on the lightness, a unique and original balance of herbs and fruits with well-known health properties. Tea is one of the most widely consumed beverages in the world, next only to water. It has been consumed for centuries in the forms of unfermented (green tea), semi-fermented (oolong) and fermented (black tea) by ancient cultures for its medicinal properties [7]. Black tea is commonly consumed in the West whereas the consumption of green tea is especially popular in Asia, mainly for its health benefits [8]. Many studies have shown that polyphenolic compounds extracted from green tea leaves are good antioxidants against lipid peroxidation in phospholipid bilayers and in biological system [4, 9]. Recently, various kinds of fruit and flavoured teas are very popular in many European countries and in USA. These teas are obtained by the addition of natural aromas and dry fruits or herbs to the tea leaves in the last stage of processing.

Considering the increasing interest in the health properties of ready to drink tea and fruit enriched mineral water, the main goal of this dissertation consisted in improving the knowledge concerning the antioxidant properties of different commercially ready to drink teas and fruit enriched mineral waters available in the worldwide consumer market. Particularly, the aims of the present paper were to verify the relationships between the antioxidant properties and the poliphenols, flavonoids and antocyanins content. The poliphenols and flavonoids content was determined by Folin-Ciocalteau assay and the flavonoids content was determined spectrophotometrically using a method based on the formation of a flavonoid-aluminium complex, while antocyanins were determined using optical spectroscopy. The antioxidant activity was evaluated by two radical scavenging assay, DPPH and ABTS, using the β-carotene bleaching test and by FRAP assay to monitor the ferric reducing power. Furthermore, several technological parameters such as color density, polymeric color, percent polymeric color and pH were also determined.

MATERIALS AND METHODS

Reagents and Apparatus

Ethanol, sodium nitrite, NaOH, Na_2CO_3, $AlCl_3$, $FeSO_4$, $FeCl_2$, $FeCl_3$, chloroform, sodium carbonate, potassium persulphate, perchloric acid, HCl, $NaIO_3$, KCl and $H_2S_2O_5$ were obtained from VWR International s.r.l. (Milano, Italy). L-Ascorbic acid, β-Carotene, quercetin, chlorogenic acid, tripyridyltriazine (TPTZ), ABTS solution, Trolox, butylated hydroxytoluene (BHT), propyl gallate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), linoleic acid, Tween 20, Folin-Ciocalteu reagent, acetate buffer, ferrozine, EDTA, ascorbic acid, were purchased from Sigma-Aldrich S.p.a. (Milan, Italy). All the reagents used in the study were of analytical grade. Spectrophotometric determinations were carried out on UV-Vis Jenway 6003 spectrophotometer (Carlo Erba, Milan, Italy) with cuvettes of 1 cm in length.

Fruits Enriched Mineral Water and Ready to Drink Flavoured Teas

Samples of fruits enriched mineral water (FEW) and ready to drink flavoured teas (RDT) samples brands available on the Italian market were randomly purchased during supermarkets. All samples contained citric acid, sugar and natural flavors. All samples collected had more than 20 days and 6 month respectively, of residual shelf-life. The ingredients of each analyzed samples were reported in Table **1**. All

Table 1. Ready to Drink Flavored Tea and Fruit Enriched Mineral Water Ingredients and Calories

Sample	Natural extract	Juice	Sweetener	Stabilizing	Kcal/100mL
RDT1	Lime (0.50%), lemon (0.50%), verbena (0.50%), chamomile (0.50%), licorice (0.50%), orange (0.50%), lemon (0.50%), sage (0.50%), rosemaryn (0.50%), mentha (0.50%)	-	-	-	35.1
RDT2	Hibiscus (0.1%), Aspalathus linearis (rooibos) (0.1%), black-currant (0.1%)	Peach juice (0.1%)	-	-	33.75
RDT3	Jasmine (0.1%), green tea (1%)	Lemon	-	-	35.7
FEW1	Hawthorn (Crataegus oxycanta flowering tops 0.20%), pansy (0.30%)	Cranberry (12%), Lemon (3%)	Sucralose	-	28.3
FEW2	Juniper (0.02%)	Pineapple (12.6%), grapefruit (4.2%), orange (3.2%)	Sucralose	Pectin	29.3
FEW3	-	Grapefruit (12.24%), strawberry (4.08%), pink grapefruit (2.04%) Lemon (1.63%)	Sucralose	-	27.0
FEW4	Hawthorn (Crataegus oxycanta flowering tops 0.45%), black-currant (0.1%)	Orange (17%), blood orange (3%)	Sucralose	Pectin	28.8

RDT: Ready to drink flavored tea; FEW: Fruit enriched mineral water.

the samples were tested immediately after the product was opened, using pure products or various dilutions as needed to conduct the experiment.

Determination of Total Phenols and Flavonoids Content

The total phenolics content of FEW and RDT was determined by the Folin-Ciocalteau method [10]. Briefly, 100 μL of diluted (1:2) samples were mixed with 0.5 mL Folin-Ciocalteau reagent, 1 mL of distilled water and 1.5 mL of 20% Na_2CO_3. The absorbance was measured at 765 nm after 2h incubation at room temperature. Chlorogenic acid was used as a standard and the total phenolics content was expressed as mg/mL. The flavonoids content was determined using a method based on the formation of a flavonoid-aluminium complex [11]. One mL of diluted (1:2) samples were added to a 10 mL volumetric flask. One mL of 2% (w/v) $AlCl_3$ was added and the solution was then mixed. After 15 minutes it was read the absorbance at 510 nm. The total content levels of flavonoids were determined in triplicate and expressed as quercetin equivalents as mg/mL.

Determination of Total Anthocyanins

Presence of anthocyanins was determined as described by Giusti *et al* [12]. Samples were mixed directly with equal volumes of 25 mM potassium chloride solution (pH 1.0) and 0.4M sodium acetate buffer (pH 4.5). Absorbance was measured at 510 and 700 nm. Data were expressed in milligrams of anthocyanins per 100 mL of sample using a molar extinction coefficient of 26900, molecular weight of 449.2, and an absorbance of A = [(A_{510}-A_{700}) pH 1.0 - (A_{510}- A_{700}) pH 4.5].

Determination of Vitamin C Content

Analysis of total ascorbic acid levels in mineral water enriched with fruits and ready to drink flavoured teas was determined by titration with DIF (2,6-dichlorophenolindophenol sodium salt hydrate) using the official method described by the AOAC [13].

Determination of Color Density, Polymeric Color and Percentage Polymeric Color

Indices for density, polymeric color and a small/large/ equal percentage of polymeric color in the samples were determined using the method of Giusti *et al* [14]. Samples were diluted (1:2) with a 25 mM solution of potassium chloride at pH 1 until the absorbance at 520 nm was 1. Diluted samples were transferred into two cuvettes; 0.2 mL of sodium metabisulfite solution (0.90 M) was added into one cuvette, and 0.2 mL of distilled water was added into the other. After 15 min, the absorbance of both samples was measured at 420, 520, and 700 nm and compared with a blank containing distilled water. The following parameters were calculated:

Color density = [(A420 nm – A700 nm) + (A520 nm – A700 nm)] x 2;

Polymeric color = [(A420 nm – A700 nm) + (A520 nm – A700 nm)] x 2;

% polymeric color = (polymeric color/color density) x 100.

ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Activity Assay

Radical scavenging activity was determined according to the technique reported by Blois *et al*. [15]. An aliquot of 1.2 mL of $1x10^{-4}$ M DPPH solution in ethanol and 300 μL of sample at different dilutions in water (not diluted, 1/2, 1/3, 1/4, 1/5) were mixed. The bleaching of DPPH was determined by measuring the absorbance at λ= 517 nm. The DPPH radicals scavenging activity was calculated according to the following equation:

% inhibition=[1-(A_0-A_1/A_2) x 100]

(A_0= sample reading; A_1= solvent+sample reading; A_2= negative control reading).

Antioxidant Capacity Determined by Radical Cation ($ABTS^{+}$)

ABTS assay was based on the method developed by Re *et al*. [16]. ABTS radical cation ($ABTS^{+}$) was produced by the reaction of a 7 mM ABTS solution with 2.45 mM potassium persulphate. The $ABTS^{+}$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. After the addition of 25 μL of samples at different dilutions in water (not diluted, 1/2, 1/3, 1/4, 1/5) or Trolox standard to 2 mL of diluted $ABTS^{+}$ solution, absorbance at λ= 734 nm was measured at exactly 6 min. The scavenging ability of the sample was calculated according to the following equation :

ABTS scavenging activity (%) = [(A_0 – A) / A_0] x 100

where A_0 is the absorbance of the control reaction and A is the absorbance in the presence of samples.

Antioxidant Activity by β-carotene Bleaching Test

Antioxidant activity was determined also using the β-carotene bleaching test [17]. Briefly, 1 mL of β-carotene solution was added to 0.02 mL of linoleic acid and 0.2 mL of 100% Tween 20. Then 5mL of the emulsion were transferred into test tubes containing 200 μL of samples at different dilutions in water (not diluted, 1/2, 1/3, 1/4, 1/5) and transferred at 45 °C in a water bath for 60 min. The absorbance was measured at λ= 470 nm. Propyl gallate was used as positive control. The antioxidant activity (AA) was measured in terms of successful bleaching β-carotene by using the following equation: AA= [1- (A_0-A_t)/($A°_0$- $A°_t$)] x 100 where A_0 and $A°_0$ are the absorbance values at the initial incubation time for samples/standard and control, respectively, while A_t and $A°_t$ are the absorbance values measure in the samples/standard and control respectively at t = 30 min and t = 60 min.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP method measures the change in absorbance that occurs when the TPTZ (2,4,6-tripyridyl-*s*-triazine)-Fe^{3+} complex is reduced to the TPTZ-Fe^{2+} form in the presence of antioxidant compounds [18]. Briefly, the FRAP reagent contained 2.5 mL of 10 mM tripyridyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.3 M acetate buffer (pH 3.6) was freshly prepared. Samples were diluted 1/4 in water. An aliquot of 0.2 mL of solution was mixed with 1.8 mL of FRAP reagent and the absorption of the reaction mixture was measured at λ= 595 nm.

Ethanolic solutions of known Fe (II) concentration, in the range of 50–500 μM ($FeSO_4$), were used in obtaining the calibration curve. The FRAP value represents the *ratio* between the slope of the linear plot for reducing Fe^{3+}–TPTZ reagent by samples compared to the slope of the plot for $FeSO_4$.

Statistical Analysis

All experiments were carried out in triplicate. Data were expressed as means ± standard deviation (S.D.). The concentration giving 50% inhibition (IC_{50}) was calculated by non-linear regression with the use of Prism GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA). The dose-response curve was obtained by plotting the percentage inhibition versus concentration. Differences concerning parameters were analyzed by the one-way ANOVA test to compare group means ($\alpha = 0.05$). The R^2 values thus obtained were used to predict relationships. To complete the statistical analysis a multicomparison Bonferroni's test was performed.

RESULTS

pH, Color Density, Polymeric Color and Percent Polymeric Color

Results of pH, color density, polymeric color and percent polymeric color are reported in Table **2**. No differences were detected for pH values in teas and waters which ranging from 2.91 to 3.42 for FEW1 and FWE2, respectively. Similar values of color density index were observed for all samples except FEW1 which showed a color density index of 2.7. A high % value of ~~%~~ polymeric color around 87% were observed for all samples except for FEW1 and RDT2. Interestingly, sample FEW1 which was characterized by the highest color density index conversely presented a lower % polymeric color.

Total phenolics, Flavonoids, Antiocyanins and Vitamin C Content

The total phenolics content widely varied in the studied samples and ranging from 0.12 to 0.69 mg/mL for teas and from 1.05 to 3.54 mg/mL in fruits enriched mineral water (Table **3**). The highest results were obtained with sample FEW1 which contain lime, hawthorn, pansy as natural extract and some fruit juice such as cranberry and lemon. This mineral water was also characterized by the highest total flavonoids content (1.33 mg/mL). No differences were found in other analyzed samples with values ranging from 0.06 to 0.80 for RDT1 and FEW4, respectively. Comparison between total phenolics and flavonoids content is reported in Fig. (**1**). Statistical analysis revealed a positive correlation between total phenolics content and total flavonoids content for all analyzed samples. The antocyanins are included in the list of natural products known to work as powerful antioxidants. In our analysis the highest total antocyanins content was found in sample FEW1 (23.54 μg/mL) which contain

Table 2. pH, Color Density Index and % Polymeric Color of Analyzed Samples

Sample	pH	Color Density Index	% Polymeric Color
RDT1	3.11	0.19	83.33
RDT2	3.02	0.76	43.36
RDT3	3.03	0.34	88.39
FEW1	2.97	2.70	26.85
FEW2	3.42	0.51	88.04
FEW3	3.03	0.60	86.53
FEW4	3.20	1.10	86.59

RDT: Ready to drink flavored tea; FEW: Fruit enriched mineral water.

Table 3. Total Vitamin c, Phenolics, Flavonoids and Anctocyanins Content

Sample	Vitamin C (mg/100 mL)	Phenolics (mg/mL)	Flavonoids (mg/mL)	Antocyanins (μg/mL)
RDT1	0.001	0.12	0.06	ND
RDT2	0.004	0.69	0.49	2.01
RDT3	0.002	0.49	0.21	ND
FEW1	0.008	3.54	1.33	23.54
FEW2	0.023	2.89	0.25	ND
FEW3	0.005	2.88	0.46	0.80
FEW4	0.083	1.05	0.80	1.80

RDT: Ready to drink flavored tea; FEW: Fruit enriched mineral water. ND: not detected

hawthorn and pansy as a natural extract and also cranberry and lemon juice while in ready to drink teas the highest value was measured in for sample RDT2 (2.01 μg/mL) which contain *Hibiscus*, red tea and blackcurrant as natural extracts and also peach juice. Vitamin C content ranged from 0.001 to 0.004 mg *per* 100 mL of sample in flavoured teas and from 0.005 to 0.083 mg *per* 100 mL of fruit enriched mineral water. Interestingly, sample FEW4 which contains orange (17%) and blood orange (3%) showed the highest vitamin C content.

Radical Scavenging Activity Assays

DPPH is a stable free radical widely used to test the ability of beverages to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of food matrix. A dose-response relationship was observed for all tested samples Fig. (**2**). The IC_{50} value for radical scavenging activity by flavoured teas and fruits enriched mineral water are summarized in Table **4**. Generally, mineral water enriched with fruits exhibited a stronger radical scavenging activity than flavoured teas in DPPH test. Among analyzed teas RDT2 exhibited the highest radical scavenging activity with IC_{50} value of 78.50 v/v while among the fruits enriched mineral water FEW2 exhibited the most promising value (IC_{50} value of 13.09 v/v). FEW2 showed an interesting radical scavenging ability using, in conjunction, a different method. In fact in ABTS test this sample inhibited ABTS radical with a percentage of 65.37% when it was tested as a not diluted sample. With the same concentration FEW1 scavenged the ABTS radical with a percentage of 42.32%. All flavoured teas exhibited a lower ability in scavenging ABTS radical.

β-Carotene Bleaching Test

All analyzed samples were also able to inhibit the discoloration of β-carotene in dose-dependent manner Fig. (**3**). Generally all fruit enriched mineral water exhibited the highest inhibition of linoleic acid oxidation particularly sample FWE4 which showed IC_{50} values of 0.70 and 1.61 v/v at 30 and 60 min of incubation. Among the teas, sample RDT3 showed the highest inhibition of linoleic acid oxidation with IC_{50} values of 8.91 and 11.74 v/v at 30 and 60 min of incubation.

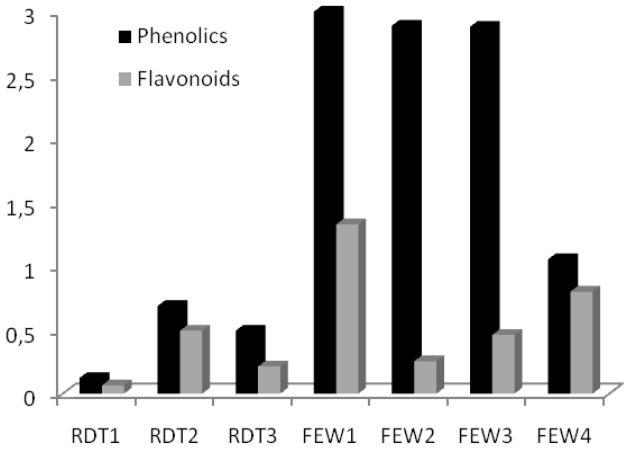
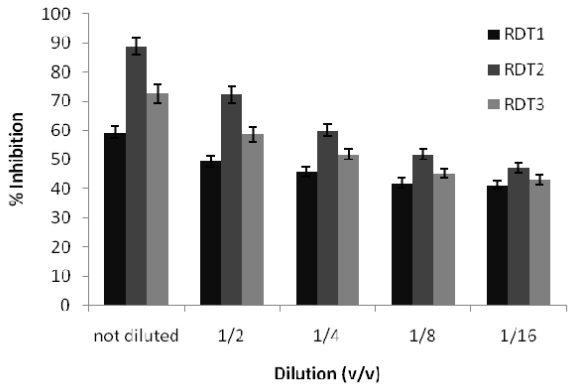
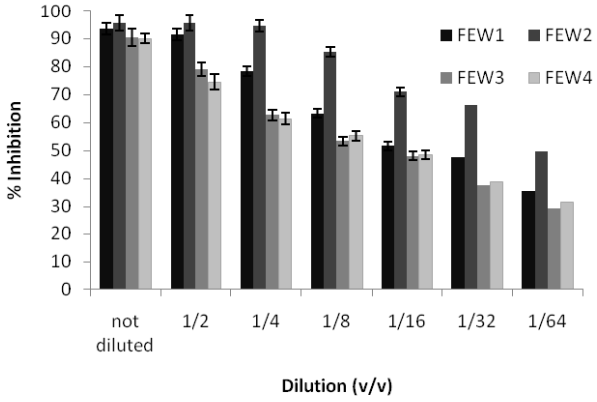


Fig. (1). Comparative total phenolics and total flavonoids content in ready to drink flavored tea (RDT); and fruit enriched mineral water (FEW). Each value is express as mean ± SD (standard deviation) (*n* = 3).



(**A**) Ready to drink flavored tea (RDT)



(**B**) Fruit enriched mineral water (FEW)

Fig. (2). DPPH radical scavenging activity of (**A**) ready to drink flavored tea (RDT) and (**B**) fruit enriched mineral water (FEW). Data are expressed as mean ± SD (standard deviation) (*n*= 3)

Total Antioxidant Activity (FRAP)

The FRAP assay measures the reducing ability of antioxidants that react with ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex and produce a colored ferrous tripyridyltriazine (Fe^{2+}–TPTZ). A dose–response relationship was observed for all tested samples. Using this assay, the FRAP value of samples ranged from 1.42 to 3.80 μM Fe(II)/L for ready to drink flavoured teas and from 7.01 to 38.46 μM Fe(II)/L for f mineral water enriched with fruit.

DISCUSSION

Food choice behavior is driven by the optimization of both nutrition and enjoyment derived from food choices. However, food choice is not just one dimensional, but a complex human behaviour influenced by many interrelating factors. Those influences are described in the Universal food choice model [19].Usually there are three dimensions influencing the consumer's food choice decision making process: 1) properties of the food; 2) person-related factors (e.g. cultural); 3) purchasing environmental factors. The future of

Table 4. Antioxidant Activity of Ready to Drink Flavored Tea and Fruit Enriched Mineral Water

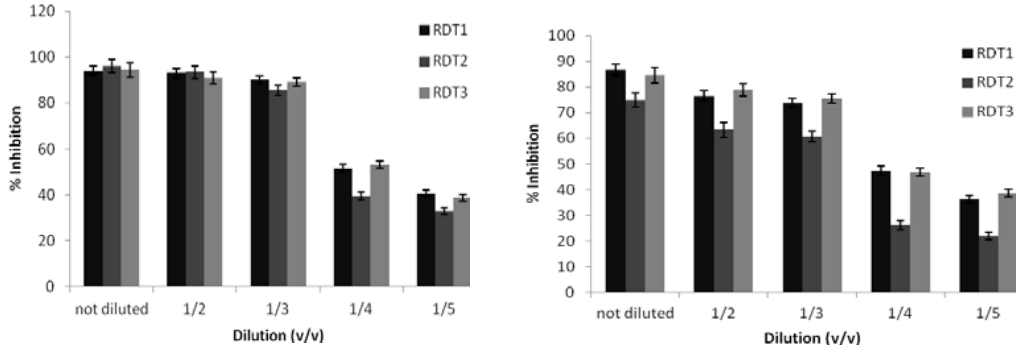
Sample	DPPH Assay (IC_{50} v/v)	ABTS Assay % Inhibition Using Not-diluted Sample	β-carotene Bleaching Test (IC_{50} v/v)		FRAP Assay (mM Fe(II))
			t30	t60	
RDT1	427.51±2.7	17.46±0.9	9.37±0.3	11.19±0.9	1.42±0.02
RDT2	78.51±1.4	12.18±0.7	14.09±0.5	20.36±1.2	3.80±0.04
RDT3	207.12±2.2	4.34±0.08	8.91±0.2	11.74±0.8	3.62±0.02
FEW1	50.93±1.3	42.33±0.7	2.51±0.09	7.02±0.5	20.93±1.1
FEW2	13.09±0.7	65.37±1.1	56.36±1.4	60.45±1.7	38.46±1.8
FEW3	67.62± 2.3	23.80±0.9	0.81±0.08	49.76±1.4	9.23±0.8
FEW4	59.39± 2.2	14.34±0.2	0.70±0.04	1.61±0.06	7.01±0.5

RDT: Ready to drink flavored tea; FEW: Fruit enriched mineral water. Data are expressed as mean ± S.D. (n = 3); DPPH Radical Scavenging Activity Assay; Antioxidant Capacity Determined by Radical Cation ($ABTS^{+}$); β-Carotene bleaching test; Ferric Reducing Antioxidant Power (FRAP). Ascorbic acid was used as positive control in DPPH (IC_{50}=5.0 μg/mL) and ABTS (IC_{50}=1.7 μg/mL); Propyl gallate was used as positive control in β-carotene bleaching test (IC_{50}=1.0 μg/mL at 30 and 60 min incubation) while BHT was used as positive control in FRAP (63.2 μM Fe(II)/g).Differences within and between groups were evaluated by one-way analysis of variance test (ANOVA) followed by a multicomparison Bonferroni's test. DPPH: One-way ANOVA *** p< 0.0001 (F= 9156, R^2= 0.999) Bonferroni Multiple Comparison Test **p< 0.001 except *p< 0.01 (RDT2 *vs* FEW3), ° p< 0.05 (FEW1, FWE3 *vs* FEW4); ABTS: One-way ANOVA *** p< 0.0001 (F= 410.3, R^2= 0.994) Bonferroni Multiple Comparison Test ** p< 0.001 except * p< 0.01 (RDT2 *vs* RDT3), ° p< 0.05 (RDT1 *vs* FEW3), • p>0.05 (RDT1 *vs* RDT2, FEW4, RDT2 *vs* FEW4); β-carotene bleaching test 30 min: One-way ANOVA ***p< 0.0001 (F= 2002, R^2= 0.998) Bonferroni Multiple Comparison Test ** p< 0.001 except ° p< 0.05 (FEW1 *vs* FEW4), • p>0.05 (RDT1 *vs* RDT3, FEW1 *vs* FEW3, FEW3 *vs* FEW4); β-carotene bleaching test 60 min: One-way ANOVA *** p< 0.0001 (F= 2660, R^2= 0.999) Bonferroni Multiple Comparison Test ** p< 0.001 except • p>0.05 (RDT1 *vs* RDT3); FRAP: One-way ANOVA *** p< 0.0001 (F= 540.5, R^2= 0.996) Bonferroni Multiple Comparison Test ** p< 0.001 except ° p< 0.05 (FEW4 *vs* RDT2, RDT3), • p>0.05 (RDT1 *vs* RDT2, RDT3, RDT2 *vs* RDT3, FEW3*vs* FEW4).

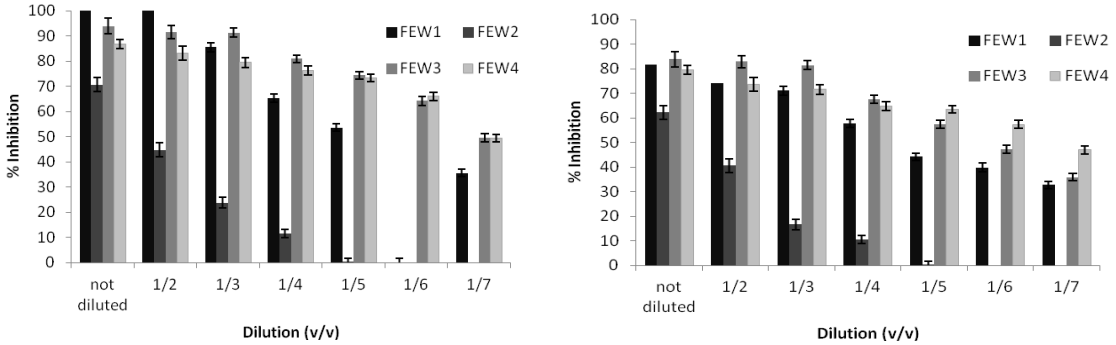
functional foods and beverages will depend on the extent to which they are accepted by consumers as part of the daily diet [20, 21]. A functional beverage can be defined as a drink product that is non-alcoholic, ready to drink and includes in its formulation non-traditional ingredients. This includes herbs, vitamins, minerals, amino acids or additional raw fruit or vegetable ingredients, so as to provide specific health benefits that go beyond general nutrition. Ready to drink teas, sports and performance drinks, energy drinks, enhanced fruit drinks, soy beverages and the quite recent developed fruit enhanced water, among others, are some of the product segments rolled out as functional beverages in the market space. Functional beverages have become popular due to its appeal to consumers who are seeking specific health benefits in their foods and beverages with their "healthiness-on-the-go" idea. Both convenience and health have been identified as important factors when consumers make decisions about purchasing foods and beverages. Functional drinks are promoted with benefits such as heart health, improved immunity and digestion, joint health, satiety, and energy-boosting [21, 22]. Currently, there are a number of commercial ready-to-drink polyphenol-rich bevera-ges, which base their marketing strategies on antioxidant potency.

Polyphenols are included in the list of natural compounds known as healthy components due to the results of a larger number of *in vitro* and *in vivo* studies that confirmed their biological activity [5, 20, 21]. Tea remains the most consumed rich polyphenolics functional drink in the world. A number of *in vitro* studies analyzed the antioxidant activity of different type of tea or different blend tea founding considerable variations. In the present study four commercial fruit enriched waters and three flavoured teas ready to drink were studied and compared with total phenolics, flavonoids, antocyanins and ascorbic acid content.

Our results are in agreement with previous studies. In fact, Pękal *et al*. [23] analyzed four fruit teas found a total phenolic content ranging from 123 to 141 mg gallic acid equivalent/ dry weight. A high variety in total phenolics content was also observed in 51 kinds of herbal and teas infusions made in China [24]. The phenolics content of three ready to drink teas, namely iced green tea, iced black tea and iced white tea from different brands, was measured by Seeram *et al.* [24] founding 0.8, 0.4 and 0.9 gallic acid equivalent (mg/mL). The same study reported the radical scavenging ability on DPPH of three ready to drink teas, specifically, ice green tea, iced black tea and iced white tea from different brands found an average of 22.3, 13, and 15.4%. The percentages of DPPH radical scavenging effect of fruit and flavoured tea infusions was investigated also by Pękal *et al*. [23] Blue fruit tea containing bilberry, blackcurrant, blackberry, raspberry, fruits of the forest which include, raspberries, cherries, blackberries, redcurrants and tropical fruit teas, in which pineapple and grapefruit peels are added, exhibited a percentage of radical scavenging effect comparable to typical black teas of *C. sinensis.* An analysis of the results revealed that in DPPH evaluations, the antioxidant properties of the tea infusions studied increased in the order: fruit tea<flavoured black tea<premium black tea. An analysis of FRAP such as, ice green tea, iced black tea and iced white tea all ready to drink from different brands revealed a mean of 1.7, 0.5 and 0.9 (μmol of FE/mL). [24]. Fu *et al*. [25] reported the ferric reducing antioxidant power of 23 Chinese tea infusions following a mean FRAP value of 11.979 mol Fe(II)/L. Furthermore, Robinson *et al*. [26] reported that there is no relationship between the market price of tea and its antioxidant activity. In fact the cheaper types of tea (e.g. Inter Spar Green Tea, Lemon Flavoured Green Tea Ceylon



(**A**) Ready to drink flavored tea (RDT) 30 min (dex) and 60 min (sin)



(**B**) Fruit enriched mineral water (FEW) 30 min (dex) and 60 min (sin)

Fig. (3). Lipid peroxidation inhibition using the β-carotene-linoleic acid system (**A**) ready to drink flavored tea (RDT) and (**B**) fruit enriched mineral water (FEW) after 30 min (DEX) and 60 min (SIN) incubation. Data are expressed as mean ± SD (standard deviation) (*n*= 3).

Black Tea) had lower antioxidant capacity than the expensive varieties (e.g. Java Green Tea, Green Blend, Darjeeling Black Tea) [27]. Community and culture studies suggests that consumption of green and black tea beverages may bring positive health effects. One hypothesis explaining such effects is that the high levels of flavonoids in tea can protect cells and tissues from oxidative damage by scavenging oxygen-free radicals. Chemically, the flavonoids found in green and black tea are very effective radical scavengers. The tea flavonoids may therefore be active as antioxidants in the digestive tract or in other tissues after uptake. The antioxidant activity of tea polyphenols is mainly due to their ability to scavenge free radicals and to increase the activity of some detoxifying enzymes within the small intestine, liver and lungs [28]. Thus, tea consumed throughout the world is believed to be not only a popular beverage but also an antioxidant agent available in everyday life. Recently flavoured and fruit teas are introduced in the market. These beverages, which are popular because of their fragrance and lower amounts of caffeine, could be a good source of compounds with antioxidant properties. Further-more, they are characterized by the lesser astringent and bitter taste perceived upon black tea consumption. Our samples contain a variety of natural extracts and juice that enhance the antioxidant potential of analyzed teas since they possess antioxidant activity [28-30]. As previously reported there is a great interest from food industries in creating functional beverages. Recently, flavoured mineral waters were introduced in the market. Lemon and orange juice are the most common ingredients introduced in analyzed samples along with other natural extracts (e.g. hawthorn, pansy, juniper and blackcurrant) and other friut juices (e.g. cranberry, grapefruit and strawberry) are used as functional ingredients. Our samples are characterized not only by high levels of polyphenols and flavonoids but also by an high anthocyanins content. Actually, there is an intense interest in the anthocyanin content of foods because of the possible health benefits, such as reduction of coronary heart disease, improved visual acuity, antioxidant and anticancer activities [31, 32]. With regards to total phenolic content in mineral water, previously Fàtima Barroso *et al*. [33] reported that the values of flavoured waters distributed in the Portuguese market, ranged from 0.29 to 284 mg gallic acid equivalent/L in flavoured lemon and apple water, respectively. In disagreement with our results the same flavoured waters had no flavonoids in detectable amounts in their composition. Investigation on their antioxidant activities pointed out a higher reducing power (0.14-11.8 mg of gallic acid/L) and radical scavenging activity (0.29-211.5 mg Trolox/L) of flavoured waters compared with the corresponding natural ones. The reducing power determination of these flavoured waters revealed that mineral water flavoured with apple exhibited the highest reducing power with value of 154.04 mg gallic acid/L. Polyphenols were considered responsible for the found bioactivity.

CONCLUSIONS

In conclusion, this study applied to *in vitro* antioxidant capacity testing to reflect the multiple antioxidant capacity tests using different reagents to provide a more complete profile of antioxidant activity. The present research demon-

strates for the first time the antioxidant power of mineral water enriched with fruit and ready to drink flavoured teas. Beverages with nice tasting fruits are frequently consumed and curiously, are often drunk without considering their potential impact on our health. Although the content of bioactive components found in the studied waters and teas is of a certain interest, the consumer cannot expect outstanding improvements to their health, by only intaking these beverages. In fact bio-availability differs greatly from one polyphenol to another, and for some compounds it depends on dietary source [34]. The stability of pure phenolic compounds on the *in vitro* gastro-intestinal digestion was extensively studied [35-37] showing that polyphenols contained in the liquid matrices are promptly bio-accessible comparing to polyphenols contained in solid matrices such as fruit and vegetables. For the above mentioned reason the present work could contribute to a better understanding of the effects derived from the consumption of fruit beverages and teas on human health. This assumption could be preliminary to human trials.

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CONFLICT OF INTEREST

None declared.

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