

## EVALUATION OF ANTIOXIDANT CAPACITY OF ANTIOXIDANT-DECLARED BEVERAGES MARKETED IN ITALY

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### ABSTRACT

Clinical literature suggests a negative correlation between fruit juice consumption and the occurrence of various diseases. Consequently, many commercially available beverages are based on fruit juices or green tea extracts with specific additives that increase their antioxidant power. In order to fully estimate their potential antioxidant capacity, several products marketed in Italy were analyzed for total phenolics and flavonoids, DPPH· scavenging activity, TEAC, FRAP and ORAC-PYR. On average, fruit-based samples had more antioxidants than green teas, but specific additives significantly improved total antioxidant power. Differences between these samples and plain fruit juices were also evaluated. Total antioxidant supply remained almost constant during the entire shelf life of the products.

- Key words: antioxidant capacity, commercial juices, DPPH, ORAC-PYR, refrigerated storage, TEAC -

## INTRODUCTION

Reactive oxygen species (ROS) are unavoidable products of aerobic metabolism (HALLIWELL, 2006; 2007) which cause significant tissue damage and human diseases (SIES, 1985). A huge number of molecules called antioxidants that can break radical chain reactions and prevent cellular damage are widespread in nature (SANJUST *et al.*, 2008). Since the high antioxidant content of some foods and beverages (particularly fruit-based ones) has been clearly demonstrated (PELLEGRINI *et al.*, 2003; WU *et al.*, 2004), consuming such foods and beverages has been suggested to significantly reduce the risk of various diseases (GEY *et al.*, 1991; LA VECCHIA *et al.*, 2001). In particular, due to the high antioxidant content and "ready-to-drink" availability of fruit- and vegetable-based beverages, their antioxidant properties have been investigated with promising results (MULLEN *et al.*, 2007; ZULUETA *et al.*, 2007; SEERAM *et al.*, 2008; PISOSCHI *et al.*, 2009). Moreover, various researchers have also suggested their possible role in preventing oxidative damage (PANZA *et al.*, 2008) and diseases like cancer (HIRVONEN *et al.*, 2006), and in delaying the onset of Alzheimer's disease (DAI *et al.*, 2006).

Nowadays, in addition to traditional fruit juices and extracts, fruit-based beverages with declared antioxidant additives (such as vitamins, phenolics, flavonoids, etc.) and alleged anti-aging/antioxidant/free radical scavenger abilities are commercially available. The aim of the present study was to measure the total antioxidant power of these ready-to-drink beverages. Accordingly, several antioxidant fruit-based beverages commercially available in Italy were analyzed, both fresh and during storage, in order to quantify their antioxidant supply throughout shelf life. Some green-tea-based beverages were also included in this study because their composition differs only slightly from most antioxidant-declared beverages; they differ in the amount and variety of additives. To the best of our knowledge, there have not been any similar screenings of such products.

Many methods involving both electron transfer (ET) and hydrogen atom transfer (HAT) (HUANG *et al.*, 2005; PRIOR *et al.*, 2005) have been proposed to assess the antioxidant capacity of food samples. Differences between the results arising from these methods have been found (HUANG *et al.*, 2005; TABART *et al.*, 2009; ZULUETA *et al.*, 2009); therefore, in order to make this study as comprehensive as possible, many methods were used for each sample, both ET (FRAP, TEAC, DPPH· scavenging assays) and HAT (ORAC-PYR assay). In addition, the amounts of the main compounds usually related to antioxidant power (phenolics and flavonoids) were determined for each sample.

## METHODS AND MATERIALS

### Samples

All samples were purchased from local stores from September 2008 through February 2009. For each sample, information regarding the composition was taken from the packaging. Samples were stored in strict accordance with vendors' instructions (room temperature for all samples, except A stored at 4°C). After the packages were opened, samples were immediately analyzed or were kept at 4±2°C as suggested by vendors for the storage experiments.

Three natural and pasteurized citrus juices were analyzed for comparison; they were purchased from local stores in the same time period. They were exclusively red orange, orange and grapefruit juices; nothing else was added (including water or sugar). Samples were stored at 4°C.

### Chemicals

All of the reagents were of reagent grade, and were used without further purification. In particular, Trolox (cat. No. 56510), DPPH free radical (cat. No. D9132), Pyrogallol Red (cat. No. 223239), Rutin (cat. No. 84082), Folin-Ciocalteu's reagent (cat. No. 47641), APH (cat. No. 44,091-4), Gallic acid (cat. No. 48630) and ABTS (cat. No. 11557) were all purchased from Sigma-Aldrich-Fluka (Milan, Italy). Ascorbic acid Baker Teststrips (cat. No. 4409-01) were purchased from Baker (Phillipsburg, NJ, Usa).

### Total phenolic determination

The total soluble phenolic content was determined with Folin-Ciocalteu reagent according to a previously described method (SLINKARD and SINGLETON, 1977). Briefly, 1 mL of each sample was diluted with 2.5 mL Na<sub>2</sub>CO<sub>3</sub> 2% w/v. Following vortexing and one min incubation at 25°C, 0.25 mL 1 N Folin-Ciocalteu reagent was added. After vortexing again, the mixture was incubated at 25°C in the dark for 45 min. Absorbance at 760 nm was measured using an Ultro-Spec 2100 spectrophotometer (Amersham Bioscience, Milan, Italy). Gallic acid was used as the standard (linearity range 0.05-0.6 mM), and the results were calculated as gallic acid equivalent (GAE) using a standard curve.

### Total flavonoid determination

The total flavonoid content was measured by using a previously described method (ZHISHEN *et al.*, 1999). Briefly, 0.25 mL of sample were diluted with 1.25 mL H<sub>2</sub>O and 0.075 mL NaNO<sub>2</sub> (5% w/v). After 5 min incubation at 25°C, 0.15 mL AlCl<sub>3</sub> (10% w/v) was added. After 6 min 0.5 mL of 1 M NaOH and 0.275 mL H<sub>2</sub>O were added. Mixtures were vortexed and absorbance at

510 nm was measured. Rutin was used as the standard (linearity range 0.1-0.6 mM) and the results are expressed as Rutin Equivalent (RE).

DPPH· (1,1-Diphenyl-2-picrylhydrazyl radical) scavenging assay

DPPH assay was performed as already described (HUANG *et al.*, 2005). Briefly, 0.3 mL of sample were added to 0.7 mL of DPPH solution 25 mg/L in ethanol. Decrease in absorbance at 515 nm was followed for 30 min at 25°C. The remaining DPPH (%DPPH<sub>rem</sub>) was measured as follows: %DPPH<sub>rem</sub> = 100 × [DPPH]<sub>rem</sub> / [DPPH]<sub>T=0</sub>. Trolox was used for the calibration curve (linearity range 5- 50 µM), and results are expressed as Trolox Equivalents (TE).

Ferric reducing antioxidant power (FRAP)

FRAP was measured as described by HUANG *et al.*, (2005). Briefly, 2.5 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl were diluted in 25 mL of 0.1 M sodium acetate buffer (pH 3.6) and 2.5 mL of 20 mM FeCl<sub>3</sub>. After warming to 37°C, 0.2 mL of this solution were added to 0.77 mL H<sub>2</sub>O and 0.03 mL of sample. Mixtures were incubated for 6 min at 25°C and centrifuged at 8,000 *g* for 10 min. Absorbance at 593 nm was then measured. Trolox was used for the calibration curve (0.1-0.6 mM linearity range), and the results are expressed as Trolox Equivalents (TE).

Trolox Equivalent Antioxidant Capacity (TEAC) assay

The TEAC assay was performed according to RE *et al.*, (1999). Aqueous 2,2'-azinobis(3-ethylbenzothiazoline 6-sulphonate) (ABTS, 7 mmol) was diluted with 2.45 mmol of aqueous K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>. The mixture was incubated at 25°C for 16 h to give ABTS radical. This solution was then diluted with sodium phosphate buffer 75 mM (pH 7.4) until the absorbance reached 0.70±0.01 at 734 nm. 0.01 mL of sample was then diluted with 1 mL of this ABTS radical solution, and the differences in absorbance at 734 nm were measured after 6 min incubation at 25°C. Trolox was used for the calibration curve (linearity range 0.1-0.8 mM).

ORAC-PYR determination

ORAC-PYR was determined using the pyrogallol red method where APH was the radical releaser (ALARCON *et al.*, 2008). Briefly, 0.75 mL of 6.6 mM pyrogallol red solution in 75 mM potassium phosphate buffer (pH 7.4) and 0.125 mL of the sample were incubated at 25°C for 10 min. Then 0.125 mL of 0.153 mM APH solution in 75 mM potassium phosphate buffer (pH 7.4) were added and the decrease in absorbance at 540 nm was

followed for 35 min at 25°C. For each sample, the area under the kinetic curve was calculated (AUC<sub>net</sub>) by subtracting the area of the blank (AUC<sub>blank</sub>) from the area of the sample (AUC<sub>sample</sub>): AUC<sub>net</sub> = AUC<sub>sample</sub> - AUC<sub>blank</sub>. Trolox was used for the calibration curve (linearity range 0.1-0.8 mM), and the results are expressed as Trolox Equivalents (TE).

Statistical analysis

The results are the averages of at least six independent determinations, and the data are reported as the mean value±standard deviation (SD). The Pearson correlation coefficient (*r*) and the associated *p*-value were used to show correlation and significance. Probability values of *p* < 0.05 were considered statistically significant. All of the statistical analyses were performed using R 2.5.1 software (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS AND DISCUSSION

Total phenolic and flavonoid content

In order to fully evaluate the free radical scavenging power of commercial beverages that advertise their antioxidant content, the total phenolic and total flavonoid contents were determined, since these compounds have been positively correlated with antioxidant capacity (FANG *et al.*, 2009; FUKUSHIMA *et al.*, 2009). Vitamin C has also been correlated with the antioxidant power of commercial products. This information is usually reported on the packages and data are summarized in Table 1. The determinations of vitamin C content showed that the measured and declared vitamin C values were not significantly different, confirming the accuracy of the package-reported information (data not shown). This substance has already been extensively studied, and the present work focused on less investigated chemical compounds.

The results of total phenolic and flavonoid contents are reported in Table 2. This table shows that the concentration of total phenolics varied widely among samples. More than one order of magnitude difference was observed between the poorest (R, 0.63 mM GAE) and the richest (B, 17.97 mM GAE) beverage. In particular, green tea-based samples had significantly lower average total phenolic values. The richer beverages (B, C, and D) all came from the same producer, but had quite different compositions (complex mixtures of several fruit juices and tea extracts).

The results of the total flavonoid analyses were very similar; these data are quite well correlated with total phenolic data (*r* = 0.95). Flavonoids, on average, made up 15-20% of the total phenolic composition, except in Q in which the flavonoid content was less than 10%.

Table 1 - Composition of the beverages analyzed. All samples were stored at room temperature, and refrigerated storage was suggested after package opening. Only A was stored at 4°C.

Sample	Additives	Juice type
A	Vit. A (4 mg/L), C (0.3 g/L) and E (15 mg/L), coenzyme Q (0.015%)	Orange and mango juice
B	Vit. C (0.3 g/L) and E (25 mg/L)	Apple, orange, pomegranate, cherry juices. Apple, elderberry, and red grape extracts (0.15%)
C	Vit. C (25 mg/L), and E (25 mg/L), $\beta$ -carotene	Peach, mango, apple, grape, orange, Maracuja juices. Apple, green, and white tea extracts (0.11%)
D	Vit. C (0.3 g/L) and E (25 mg/L)	Red grape, apple, and blueberry puree. Elderberry juice. Green tea, red grape leaves, elderberry, and apple extracts (0.28%)
E	Vit. C (0.24 g/L)	Red orange (18%), pomegranate (16.2%), and elderberry (0.1%) juices
F	Vit. C (0.15 g/L)	Red grape (13.5%), apple (12.5%), plum (12%), elderberry (1%), cherry (0.5%), and blueberry (0.5%) juices. Grape peel extract
G	Vit. C (0.18 g/L), fibers, quercetin (0.013%)	Pear (15%), white grape (14%), apple (7%) juices. Food fiber (0.6%)
H	Vit. C (0.3 g/L), $\beta$ -carotene	White grape (11%), apple (10%) juices. Apricot (5%), mango (4%), and papaya (1%) purees
I	Vit. C (0.15 g/L), folic acid, lutein (0.005%)	Kiwi (15%), apple (12%), white grape (10%), and lime (3%) juices and purees
J	Vit. C (0.15 g/L) and E	Pink grapefruit (15%), white grape (2%) juices. Matè (0.05%) and ginseng (0.04%) extracts
K	Lipoic acid (58 mg/L), Vit. C (0.18 g/L)	<i>Paullinia sorbilis</i> and <i>Vitis vinifera</i> extracts
L	Vit. C (0.36 g/L) and Vit. E (48 mg/L), catechins (0.14 g/L)	Green tea extract (0.12%)
M	Vit. C (40 mg/L)	Green tea extract (0.2%)
N	Vit. C (0.4 g/L)	Green tea extract (0.168%)
O	Vit. C (0.14 g/L)	Green tea extract
P	Vit. C (1 g/L)	Green tea extract, polyphenols (200 mg/L). Peach and lemon juice
Q	Epigallocatechin gallate (0.18%), Vit. E (0.015%)	Green tea extract (0.2%)
R	Lemon juice	Green tea infusion (1%) and jasmine extract (0.1%)

Three samples (G, L, and Q) were declared to have flavonoid additives (quercetin, catechins and epigallocatechin-3-gallate, respectively). However, these additions were not always with a significantly higher total flavonoid or phenol-

ic content in the final product. In fact, while L was the green tea-based sample with the highest amount of total flavonoids, Q had the lowest percentage of flavonoids among the total phenolics of all the samples.

Table 2 - Total phenolic and flavonoid values in the beverages. Values are means $\pm$ SD ( $n = 6$ ). Samples refer to table 1.

Sample	Total phenolics GAE (mM)	Total flavonoids RE (mM)
A	2.90 $\pm$ 0.07	0.52 $\pm$ 0.06
B	17.97 $\pm$ 0.06	3.51 $\pm$ 0.03
C	17.64 $\pm$ 0.51	3.39 $\pm$ 0.07
D	14.09 $\pm$ 0.84	2.48 $\pm$ 0.15
E	5.05 $\pm$ 0.03	0.49 $\pm$ 0.01
F	13.56 $\pm$ 0.04	2.17 $\pm$ 0.06
G	4.63 $\pm$ 0.66	1.19 $\pm$ 0.02
H	4.26 $\pm$ 0.04	0.54 $\pm$ 0.01
I	4.14 $\pm$ 0.38	0.56 $\pm$ 0.01
J	2.57 $\pm$ 0.01	0.34 $\pm$ 0.01
K	2.76 $\pm$ 0.24	0.93 $\pm$ 0.04
L	6.39 $\pm$ 0.59	1.12 $\pm$ 0.09
M	0.87 $\pm$ 0.02	0.09 $\pm$ 0.01
N	3.11 $\pm$ 0.07	0.42 $\pm$ 0.01
O	3.18 $\pm$ 0.26	0.63 $\pm$ 0.13
P	5.83 $\pm$ 0.52	0.67 $\pm$ 0.07
Q	3.21 $\pm$ 0.35	0.19 $\pm$ 0.01
R	0.63 $\pm$ 0.03	0.08 $\pm$ 0.01

In this context, the importance of low amounts of additives should be taken into account. For instance, G declared only 0.04 mM of quercetin added, one order of magnitude less than Q (0.4 mM of epigallocatechin-3-gallate); the total flavonoid content of G, however, was one order of magnitude higher than that of Q. This finding suggests that additives had only a marginal effect on the flavonoid composition of the beverages studied. This is probably due to the low amounts added. Conversely, additive-containing samples showed the highest DPPH, TEAC, FRAP and ORAC (*vide ultra*) values, suggesting that the antioxidant activity is not the same in all flavonoids. These data however do suggest that additives are crucial for total antioxidant power.

#### Total antioxidant capacity

Since notable differences have been reported between ET-based and HAT-based methods for evaluating antioxidant capacity (in particular, ET methods could underestimate antioxidant content) (HUANG *et al.*, 2005; ZULUETA

et al., 2009), both types of assays were included in this screening. Accordingly, the antioxidant power for each beverage was determined with DPPH, FRAP, TEAC (ET-based), and ORAC-PYR (HAT-based) assays. The results are reported in Table 3.

There was more than one order of magnitude of difference between the highest (L = 12.2 mM TE) and lowest (R = 0.2 mM TE) FRAP values. Similar results were observed for the TEAC and DPPH assays.

The differences were even greater for the ORAC-PYR results. There were more than two orders of magnitude of difference between the highest (P = 104.9 mM TE) and lowest sample values (R = 0.6 mM TE).

Due to the quite complex compositions of the beverages studied, it was not possible to associate specific composition patterns with high or low antioxidant capacity. However, the beverages that were almost completely composed of green tea extracts had quite low antioxidant power. Excluding L, P, and Q (which contain specific additives), the green teas had a mean TEAC value of 3.3 mM TE, while the mean for all the beverages was 7.5 mM TE. Even larger differences were obtained with ORAC-PYR: 4.3 mM TE was the mean for green tea, while 45.9 mM TE was the overall ORAC-PYR mean. The mean for green tea was therefore one order of magnitude lower than that for all beverages.

It is worth noting that most of the green teas contained vitamin C as a flavouring agent (only Q and R did not contain it); samples L, P, and Q are green teas that also have specific phenolics added. The total antioxidant power of the latter beverages was higher than in the simple green teas, and also higher than the overall mean values. The ORAC-PYR values were 44.5, 104.9, and 76.7 mM TE, respectively. These results suggest that, unless the commercial green tea extracts contain specific additives, they are less effective antioxidant beverages than common fruit juices. This is probably due to their high water dilution.

Among the fruit juice-based beverages, there were marked differences in reducing power. Samples B, C, D, and F had FRAP, TEAC, and DPPH values that were about twice as high as those of A, E, G, H, I, J, and K. Excluding J and K (the poorest fruit juice samples), all of the beverages had comparable ORAC-PYR values. This is in good agreement with the fact that this method evaluates overall antioxidant capacity, while FRAP, TEAC, and DPPH evaluate the presence of reducing compounds.

The FRAP, TEAC, and DPPH assays are all reduction-based. In light of this fact, it is not surprising that they all gave quite similar results for the same sample, according to previously reported studies (THAIPONG *et al.*, 2006; SEERAM *et al.*, 2008; FANG *et al.*, 2009). In contrast, ORAC-PYR results were always clearly higher than the

Table 3 - Total antioxidant power (TE mM) of the samples. Values are means±SD (n = 6). Samples refer to table 1.

Sample	DPPH	TEAC	FRAP	ORAC-PYR
	TE (mM)			
A	0.45±0.03	2.30±0.03	1.24±0.08	29.0±2.50
B	5.3±0.20	16.7±0.40	8.8±0.20	58.3±1.9
C	11.2±0.10	14.3±0.50	9.9±0.50	77.3±14.70
D	8.2±0.20	12.3±0.30	9.7±0.2	68.9±1.90
E	3.7±0.10	5.7±0.10	4.0 ±0.10	65.8±2.80
F	13.3±0.30	16.6±0.90	7.9 ±0.20	61.3±1.50
G	2.6±0.10	4.7±0.20	3.8±0.10	91.8±22.20
H	3.3±0.30	3.2±0.10	3.0 ±0.10	79.6±20.30
I	2.3±0.40	2.6±0.20	2.2 ±0.20	40.6±1.30
J	1.03±0.02	2.99±0.15	1.58±0.02	5.3±0.10
K	0.85±0.09	2.0±0.10	0.51±0.01	5.4 ±0.20
L	8.2±0.30	16.4±0.30	12.2±0.30	44.5±2.50
M	0.52±0.07	1.2±0.10	0.42±0.01	1.7±0.20
N	3.8±0.20	6.9±0.30	4.6±0.10	8.7±1.10
O	1.9±0.10	4.7±0.10	2.7±0.20	6.1 ±0.20
P	8.3±0.20	13.0±0.50	10.0±0.10	104.9±8.90
Q	5.6±0.40	9.7±0.70	6.4±0.10	76.7±1.50
R	0.14±0.02	0.49±0.07	0.19±0.01	0.60 ±0.04

mean values obtained with the ET-methods. This confirms that ORAC-PYR reports the total antioxidant power of samples, and not just the part due to the reducing compounds such as phenolics. These findings are in quite good agreement with previously reported studies that reported the risk that ET assays may underestimate total antioxidant capacity (TABART *et al.*, 2009; ZULUETA *et al.*, 2009).

The features of the methods used were further corroborated by their correlation coefficients versus total phenolic and total flavonoids contents (Table 4). The statistical analysis of the *p*-values associated with the Pearson test revealed that the ET assays were correlated with both the phenolics and flavonoids (*p* < 0.005 for DPPH and FRAP versus total flavonoids and *p* < 0.001 all other cases), according to published works with similar samples (THAIPONG *et al.*, 2006; FANG *et al.*, 2009; FUKUSHIMA *et al.*, 2009). In contrast, the HAT methods (like ORAC-PYR) are

Table 4 - Pearson correlation coefficient of the total phenolic, total flavonoid, and declared vitamin C content in DPPH, TEAC, FRAP and ORAC-PYR assays.

\*\*\* *p* < 0.001 (two-tailed); \*\* *p* < 0.005 (two-tailed); \* *p* < 0.05 (two-tailed); † *p* < 0.1 (two-tailed); § *p* > 0.1 (two-tailed)

Method	r		
	Total phenolics	Total flavonoids	Declared vitamin C
DPPH	0.76***	0.66**	0.21§
TEAC	0.81***	0.73***	0.30§
FRAP	0.73***	0.65**	0.41†
ORAC-PYR	0.49***	0.40†	0.44†

not directly related to reducing substances such as phenolics. They take into account the whole antioxidant capacity, which depends on chemicals that cannot always be regarded as reductants. In this perspective, lower correlation coefficients reported in the literature between ORAC-PYR and the total phenolics are not surprising (THAIPONG *et al.*, 2006; ALARCON *et al.*, 2008). In the present study the *p*-values for ORAC-PYR are much higher than those for the ET methods ( $p < 0.05$  versus total phenolics and  $p < 0.1$  versus total flavonoids). The correlation is weaker, but it is still good (Pearson *r* coefficient always  $> 0.4$ ). These data suggest that phenolics make up a significant portion of the total antioxidant content of the beverages analyzed.

Conversely, both ET and HAT methods showed a weaker correlation with the vitamin C content ( $p < 0.1$  for FRAP and ORAC-PYR, but  $p > 0.1$  for the other assays), suggesting that the addition of vitamin C (usually done as flavouring or acidifying agent) has less effect on the antioxidant properties. This is confirmed by the widespread use of vitamin C as a flavouring additive in many foods and beverages that have no antioxidant declaration (like salads, dairy and egg-based products or alcoholic beverages).

These results emphasize the importance of using both ET and HAT-based methods to obtain a complete estimate of the total antioxidant capacity of beverages. While the ET methods offer an easier approach, to obtain quick and quite good estimates of antioxidant power, a full antioxidant capacity assessment is possible in combination with HAT methods.

#### Comparison with natural samples

To better understand the values reported, some commercial citrus juices were also analyzed. The values for red orange, orange and grapefruit juices were 54.8, 21.1, and 9.1 mM TE ORAC-PYR, respectively. The first showed antioxidant activity comparable to the mean of fruit-based antioxidant beverages (53.1 mM TE ORAC-PYR), but the others were considerably lower. They were about one order of magnitude higher than the values for most of the green teas analyzed; excluding L, P, and Q (samples with phenolic additives), the ORAC-PYR mean for green teas was 4.3 mM TE. This result could be partially attributed to the high dilution of commercial antioxidant samples. Simple fruit juices do not usually contain added water. Comparable results were obtained with ET-based methods.

These facts were corroborated by other studies. ZULUETA *et al.* (2007) analyzed fruit and milk-based beverages marketed in Spain. Their samples were quite similar in composition to samples in this study, but did not contain specific antioxidant additives (except vitamins A, C, and E). The samples had a mean TEAC value of 2.5 mM TE. This value is considerably low-

er than that of the antioxidant beverages in the present study (mean values 7.5 mM TE), indicating that specific antioxidant additives increased the antioxidant capacity. While, the order of magnitude remained the same, the differences were not substantial. PELLEGRINI *et al.* (2003) measured the antioxidant capacity of common beverages and reported their best samples (some red wines, coffee, lemon and orange juices). The TEAC values were similar to values for the antioxidant beverages (or even higher for very concentrated samples like espresso coffee). Only a few antioxidant samples (such as B, C, D, F, L, Q, and P) showed an antioxidant power that was clearly higher than that of plain natural juices.

All of this information suggests that antioxidant power can be improved through the use of additives. Samples containing additives, however, did not always have a significantly higher antioxidant capacity than plain natural juices.

#### Refrigerated storage effect

The beverages analyzed are usually marketed in 0.75 liter or 1 liter packages, so consumers do not usually drink the entire contents immediately after opening. Producers of these beverages typically indicate a shelf-life of 3-4 days after the package is opened. Accordingly, samples were stored at 4°C as suggested by producers, simulating domestic storage, and the total antioxidant capacity was evaluated for a few days.

The results of the experiments revealed that no significant loss of activity was detected during the shelf-life (data not shown); ET (FRAP, TEAC, DPPH- scavenging assays) and HAT (ORAC-PYR assay) methods gave similar TE results for at least 3-4 days. The decrease in antioxidant power was negligible for at least 10 days after opening the package. Such a time span is substantially longer than the expiry date suggested by the producer, as microbial degradation usually takes place before. This definitely allows consumers to obtain a full benefit of the antioxidant power of the beverages.

## CONCLUSIONS

The results of this study show that, on average, fruit-based beverages had higher antioxidant power than green teas. The importance of additives has been outlined, because they can noticeably improve total antioxidant power. Simple fruit juices had a lower antioxidant content (justifying the typical higher price of antioxidant-declared beverages), but this difference was not always significant. Accordingly, consumers should exert extreme care when choosing beverages, since only specific and targeted additives (like polyphenols and catechins) in non-negligible amounts can significantly improve the antioxidant content of beverages when

compared to traditional fruit juices or tea extracts. In all cases, total antioxidant power was retained almost completely throughout the shelf life of the beverage. Clear differences were also reported between ET and HAT methods, confirming the importance of both approaches in order to fully estimate the antioxidant capacity of food samples.

## ABBREVIATIONS

DPPH	1,1-Diphenyl-2-picrylhydrazyl
TEAC	Trolox Equivalent Antioxidant Capacity
FRAP	Ferric Reducing Antioxidant Power
APH	2,2'-Azo-bis(2-amidopropane)dihydrochloride
ORAC	Oxygen Radical Absorbance Capacity
GAE	Gallic Acid Equivalents
TE	Trolox Equivalents
ET	Electron Transfer
HAT	Hydrogen Atom Transfer

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