Advances in Food Science and Engineering, Vol. 1, No. 1, March 2017
https://dx.doi.org/10.22606/afse.2017.11002

Antioxidant Capacity of Commercial Flavored Waters

María Luisa Pérez-Rodríguez[1], Rebeca López-Froilán, Ángela Casado[2]

[1]Departamento de Nutrición y Bromatología II. Bromatología. Facultad de Farmacia. Universidad Complutense de Madrid, 28040 Madrid, Spain.

[2]Departamento de Medicina Celular y Molecular. Centro de Investigaciones Biológicas (CSIC). Avda. Ramiro de Maeztu, 9. 28040 Madrid, Spain.

Email: peromalu@ucm.es

Abstract: Thirty two samples of flavored water commercialized in Spain were evaluated for their antioxidant capacities by three methods: AOP based in cupric ion reducing antioxidant capacity (CUPRAC), free radical scavenging capacity by N,N-dimethyl-p-phenylenediamine (DMPD), and total polyphenols content performed by Folin-Ciocalteu reagent. Flavored waters are a kind of soft drinks formulated with mineral or bottled water, flavorings (extracts, juices or aromas) and additives which mainly are acidifying agents and sweeteners. The samples were distinguished in three groups in function of the ingredient that provide the flavor. The antioxidant capacity was higher in waters with plant extracts followed by those with aromas and finally by waters with a small amount of juices. In spite of the low antioxidant capacity, regular use of flavored water could contribute substantially to the total of dietary antioxidants. This kind of drinks does not contain alcohol or stimulants and neither typically contain sugar. This represents a certain advantage over traditional soft drinks.

Keywords: Antioxidant capacity, flavored waters, beverages, DMPD assay.

1 Introduction

Bottling and commercializing water represents an important sector of the world economy [1].There is now a greater awareness of the need and benefits of drinking water. Good hydration is essential for the proper functioning of the human body. Growing concern about the calories in soft drinks has led the industry to innovate in the formulation of water and beverages. The result is refreshing products with water and flavors, preservatives, sweeteners and juices and/or plant extracts that consumers associate with healthy alleged actions and that provide singular tastes and smells appreciated by them.

Today, a significant part of marketed water is flavored. Its mineral composition has been recently studied by Barroso et al. [2]. They concluded that flavored waters can be an adequate alternative to consumers that do not like natural water. The different ingredients added to natural waters hardly influence its mineral composition. Some preservatives, acidifying agents and sweeteners are not hazardous if consumed with moderation [2].

Moreover, the presence of flavors, juices, extracts and even bioactive compounds in this kind of drinks could also provide some antioxidant capacity to the beverages, an important feature at the prevention of multiple diseases.

Diet constitutes the main external contribution to body defenses against oxidative damage. It provides cells specific antioxidants that are able to scavenge multiple types of free radicals contributing to maintain cellular health. Some flavored waters include health claims on their labels in this sense.

In comparison with other beverages which are rich in polyphenols like coffee, wine, beer, tea or cacao, they do not suffer from certain drawbacks such as the alcoholic content in wine and beer, or the presence of stimulant compounds such as caffeine in coffee and tea and theobromine in chocolate drinks [3].

By the other hand the methodology for evaluating natural antioxidants must be carefully interpreted according to the system and to the analytical method used to determine the extent and end-point of oxidation [4]. Several methods have recently been developed for measuring the total antioxidant capacity of food and beverages; these assays differ in their chemistry (generation of different radicals and/or target molecules) and in the way end points are measured [5].

DMPD assay is a free radical scavenging method developed by Fogliano et al. [6]. The principle of this assay is that, at an acidic pH and in the presence of suitable oxidant solution, DPMD can form a stable and colored radical cation ($DMPD^{\cdot+}$) [6]. As the odd electron of the colored radical cation becomes paired off in the presence of a hydrogen donor, its absorbance decreases. Therefore, the extent of decolorization is directly related to the antioxidant capacity of the solution being investigated. This reaction has been widely used to assess the ability of compounds to act as hydrogen donors [7].

The aim of this study was to determine the antioxidant capacities of the flavored waters on the Spanish market. This information would be of consumer's interest in order to complete their knowledge about the advantages/disadvantages on the consumption of these beverages.

For the evaluation of antioxidant capacity free radical scavenging capacity by DMPD assay and measurement of the total polyphenols performed by Folin-Ciocalteu reagent were initially used. In addition the AOP kit based on CUPRAC assay was also performed. We have refused to determine the flavonoid content of flavored waters because recent studies have stated that they had no flavonoids, in detectable amounts, in their composition [8].

2 Materials and Methods

2.1 Chemicals and Reagents

Glacial acetic acid (chemically pure), Folin-Ciocalteu reagent and sodium carbonate were purchased from Panreac (Barcelona, Spain). Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and potassium acetate were obtained from Merck (Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DMPD (N,N-dimethyl-p-phenylenediamine dihydrochloride), citric acid, L-ascorbic acid, and gallic acid, were purchased from Sigma-Aldrich (St. Louis, MO. USA). Methanol HPLC quality was obtained from Lab Scan(Gliwic, Poland).

Samples were purchased in various local supermarkets in Madrid, Spain. These were classified by the major component that gives their taste. This gives us three groups called juice (J), extract (E) and aroma (A) (Table 1).

Flavored waters containing added carbonic acid or whose source is sparkling mineral water were previously degassed with an ultra sound bath at 25ºC.

Table 1: Classification of samples

	Description	Identification
Juice	Concentrated lemon 5%, added carbon dioxide, natural flavor, calcium lactate, chloride, acesulfame K, sucralose, magnesium sulfate, vegetable oil, glycerol esters of wood rosins, sucrose acetate isobutyrate, artifical colouring; quinoline yellow.	1 J[G]
	Lemon and lime juice from concentrate, α tocopherol, dimethyl dicarbonate, acesulfame K, potassium sorbate	2J
	Apple juice concentrate and pear, α tocopherol, acesulfame K, dimethyl dicarbonate, potassium sorbate	3J
	Peach and passion fruit juice concentrate, pear juice, α tocopherol, acesulfame K, dimethyl dicarbonate, potassium sorbate	4J
	Juice from concentrate lemon, citric acid, sucralose, acesulfame K, vitamins B3, B5, B6, biotin, B9, B12	5J
	Juice from concentrate peach, citric acid, sucralose, acesulfame K, vitamin: B3, B5, B6, biotin, B9, B12	6J
	Aloe vera juice 0.01%,wild berries flavor, citric acid, acesulfame K, sucralose, ascorbic acid	7J
	Lime juice 0.5% and 0.5% kumquat juice, citric acid, glucose-fructose syrup, sodium citrates, sodium benzoate	8J
	0.5% strawberry juice, 0.1%, cranberry juice, 0.1% blackberry juice, citric acid, glucose-fructose syrup, sodium citrates, sodium benzoate	9J
Extract	Extract 0.04% of melissa, chamomile, lime and mint, citric acid, ascorbic acid, vitamin B3, potassium sorbate, dimethyl dicarbonate, fructose	10 E
	Aloe vera extract 0.1%, ascorbic acid, citric acid, vitamin B 3 potassium sorbate, dimethyl dicarbonate, fructose., dietary fiber 0.7%	11 E

	Green tea extract, 2.3% concentrated fruit of lemon and apple, citric acid, natural lemon aroma, sparkling mineral water.	12 E[G]
	Rooibos extract and hibiscus extract, citric acid, sucralose, acesulfame K, sulphite ammonia caramel	13 E
	Green tea extract, citric acid, sucralose, acesulfame K, sulphite ammonia caramel, chlorophylls	14 E
	Ginseg extract, lemon flavor, citric acid, ascorbic acid, acesulfame K, sucralose	15 E
	Ginseg extract, raspberry juice, apple and pear with concentrate 2.3%, sparkling mineral water, citric acid.	16 E
	Extracts of natural fruits (strawberry & kiwi), natural mineral water, malic acid, flavorings, potassium sorbate, sodium benzoate, and aspartame.	17 E[G]
	Green tea extract, peach juice concentrate, natural mineral water, citric acid, fructose, carbonic acid, sodium citrates, aspartame, acesulfame K, ascorbic acid.	18 E[G]
	White tea extract (lemon, lavender, mint), lemon juice concentrate, natural mineral water, citric acid, sodium citrates carbonic acid, fructose, acesulfame K, ascorbic acid.	19 E[G]
	Polyphenols, citric acid, acesulfame K, sucralose, ascorbic acid,β Carotene.	20 E[*]
Aroma	Flavors (pineapple and cactus), citric acid, acesulfame k,sucralose.	21 A
	Flavor (lemon), citric acid, acesulfame K, sucralose.	22 A
	Flavors (lemon), citric acid, dimethyl dicarbonate, sodium benzoate, acesulfame K, sucralose.	23 A
	Flavors (clementine), citric acid, dimethyl dicarbonate, sodium benzoate, acesulfame K, sucralose.	24 A
	Flavors (apple), citric acid, dimethyl dicarbonate, sodium benzoate , acesulfame K, sucralose	25 A
	Flavors (orange-peach), citric acid, dimethyl dicarbonate, sodium benzoate, acesulfame K, sucralose	26 A
	Flavors(lemon), citric acid, acesulfame K, sucralose, ascorbic acid	27 A
	Orange and peach flavor, ascorbic acid, citric acid, acesulfame K, sucralose.	28 A
	Apple flavor, citric acid, acesulfame K, sucralose, ascorbic acid, 0.5% soluble fiber	29 A
	Apple and lychee flavor, citric acid, ascorbic acid, sugar. Acesulfame K, sucralose.	30 A
	Lemon and lime flavor, citric acid, sugar, ascorbic acid.	31 A
	Flavor of lemon, tea, and mango, citric acid, acesulfame K, sucralose, ascorbic acid, β carotene.	32 A[*]

* Flavored waters have claims on antioxidants on labeling

[G] These samples contain carbonic acid or they are sparkling mineral waters

2.2 Total Polyphenols

Total polyphenols were determined by a colorimetric assay based on procedures described by Singleton and Rossi [9] with some modification. Briefly, the amount of sample used depended on the composition of the sample: 2 ml were used when they contained extract (≥0,1%) or showed antioxidant properties on the label, 5 ml if they contained extract and 10 ml for the other samples. They were mixed with 50 ml of water and 5.0 ml of the Folin–Ciocalteu reagent, and 20 ml of 200 g/l (w/v) sodium carbonate solution was added; the flask was then filled to the mark with distilled water (100 ml). The mixture was incubated in the dark at a temperature of 25ºC for 30 min before reading the absorbance at 750 nm, in 10 mm pathlength plastic cuvettes using a Lambda EZ 210 UV-visible spectrophotometer (Perkin Elmer, Massachusetts, USA), using a blank of reagents as the reference. Results were expressed as mg of gallic acid equivalents (GAE) /l, a standard curve being prepared using pure gallic acid.

2.3 DMPD.+ Assay

The method described by Fogliano et al. [6] was modified to adapt this method to the laboratory equipment. Briefly, DMPD 100 mmol/l was prepared by dissolving 209 mg of DMPD in 10 ml of deionized water; 2.5 ml of this solution was added to 250 ml of acetate buffer 0.1 M, pH 5.25. Colored radical cation ($DMPD^{\cdot+}$) was obtained by adding 0.5 ml of 0.05M ferric chloride (final concentration of DMPD 0.1 mmol/l).

To achieve the same conditions as in the method of Fogliano et al. [6], once oxidized radical cation was obtained, it was transferred into test tubes, 4 ml in each. The tubes were stirred in a water bath with agitation at a temperature of 25ºC for 10 minutes to reach the stability of radical cation, 200 µ l sample or standard were added to each tube and kept in the same bath for 20 minutes, in order to produce the reaction. Then, the absorbance was measured at 505 nm. The inhibition of absorbance was calculated using the following equation:

$$\%\ inhibited\ absorbance = \left(1 - \frac{A}{A_0}\right) * 100 \quad (1)$$

A_0 is the absorbance of uninhibited radical cation (calculated from the average of 5 different measurements of the absorbance´s radical cation). A is the absorbance measured 20 minutes after the addition of antioxidant samples.

The results were expressed as mmol trolox equivalents/l (TEAC) and mmol ascorbic acid equivalents/l (VCEAC), by using trolox and ascorbic acid, respectively, as standards for calibration. To calculate the results of the samples, it is necessary to take into account their dilution factor.

2.4 AOP Kit

It was used Kit BIOXYTECH® AOP- 490TM (AOP kit), from DELTACLON. The assay was based on the commercial AOP method of Da Cruz [10]. For the photometric assay, F16 MaxiSorp microplates (Nunc, Roskilde, Denmark) of 350 µl/well and a microplate reader with a 490-nm filter were used (Bio-Tek ELx808, Bio-Tek Instruments, Winooski, VT, USA). 200 µl of each sample diluted 1:40 with the R1 reagent (containing bathocuprine) were put into each well and a first reading at 490 nm was taken. After the addition of 50 µl of the R2 reagent (containing dissolution of Cu (II)), the reaction mixture was incubated 3 min at 25ºC. The reaction was stopped by the addition of 50 µ l of stop solution, and a second reading at 490 nm was taken. The difference between the two readings was used in the calculations. Distilled water was used instead of sample or standard for blanks. Results were compared with a standard curve obtained with trolox and then expressed in mmol trolox equivalents/l [11]. They were also compared with a standard curve obtained with ascorbic acid and then expressed in mmol ascorbic acid equivalents/l too. In the AOP method the absorbance at 490 nm was linear from 0.0625 up to 1 mmol trolox equivalents/l (Trolox: y =0.379 x – $2.8*10^{-3}$, $R^2 = 0.996$, $p < 0.01$; and ascorbic acid: y = 0.314x – $2.9*10^{-2}$, $R^2 = 0.982$, $p < 0.01$). Therefore, the kit AOP assay showed a high linearity and results demonstrated the assay was linear.

2.5 Statistical Analysis

Data were analyzed using SPSS (SPSS version 15.0 for windows) software. Analysis of variance (ANOVA) and Duncan's multiple range method were used to compare any significant differences between samples. Values were expressed as mean ± standard deviation. Differences were considered significant at $p < 0.05$. All the analyses were carried out in triplicate.

3 Results and Discussion

Table 1 represents the labeled ingredients information in flavored waters. Their classification was carried out according to the component that provided the flavor, so the three groups, juice, extract and aroma. As shown in Table 1, flavored waters classified into the juice group had a content of less than 5% in concentrated fruit juice.

By looking carefully at each one of the groups, it can be see that most of the samples from the first group are composed of different fruit juices (lemon, apple, peach…) and a very low proportion of them for the juice of a medicinal plant such as *Aloe vera*. Referring to the presence of extracts in the second group, they are mainly extracts of tea, melissa, rooibos, hibiscus, Aloe *vera*, ginseng and a final sample included in this group because it contains polyphenols from unspecified origin in their composition. The latter group is composed of samples of different fruit flavors, most of them lemon flavorings.

These waters also need other ingredients, without positive relation with well-being and health, but necessary to assure the desired quality for the producer and consumers, and the safety of the product, such as, acidifying agents, sweeteners and preservatives [2].

The main sweeteners found in the 32 flavored waters are acesulfame potassium and sucralose, exceptionally samples 30A and 31A contain sugar and 9J glucose syrup. As preservatives it can be found potassium sorbate or potassium benzoate. Some also contain antioxidants such as α tocopherol (3 J and 4J), β -carotene (20 E and 32 A) and ascorbic acid that was present in the 28% of the samples. Only three samples have colorants in their composition: quinoline yellow (1 J), sulphite ammonia caramel (13

E and 14E) and chlorophyll (14E). Finally, it is noteworthy that citric acid is present in 81% of the samples as acidifying.

The concentration of polyphenols in the flavored waters was determined by the Folin-Ciocalteu method and the antioxidant capacity was performed by the DMPD assay. Figure 1 shows the results obtained by both methods.

The Folin-Ciocalteu assay (total polyphenols) was not supposed to characterize antioxidant capacity, in reality this method seems to be one of the best for rough estimating antioxidant capacity of food samples [12].

Total polyphenols content in flavored waters ranged from 0 to 218.7 mg GAE/ l, been lower than the respective pure extracts, juices and aromas present in the samples, as expected by the dilution factor. If we compare the values obtained for samples with lemon flavor (between 0-33.9 mg GAE / l) with those found by Wu et al. [13] in lemon juice or concentrate (1.80 mg GAE / ml), one can correlate them with the low concentrations of both ingredients present in the samples. If samples contain tea extracts or medicinal plants, the total polyphenol values increase significantly (as shown in Figure 1) between 21 and 218.7 mg GAE / l in the sample 11E.

If we compare the total polyphenols content in flavored waters with the values of total polyphenols obtained in their respective sources of added extracts, such as for example Aloe vera 0.23 mg GAE/g dry matter [14], ginseng roots 5-8.2 mg GAE/100g [15] and green tea 659.2 mg GAE/100g fresh matter [16], we can conclude that the concentration of extracts in the waters studied is low.

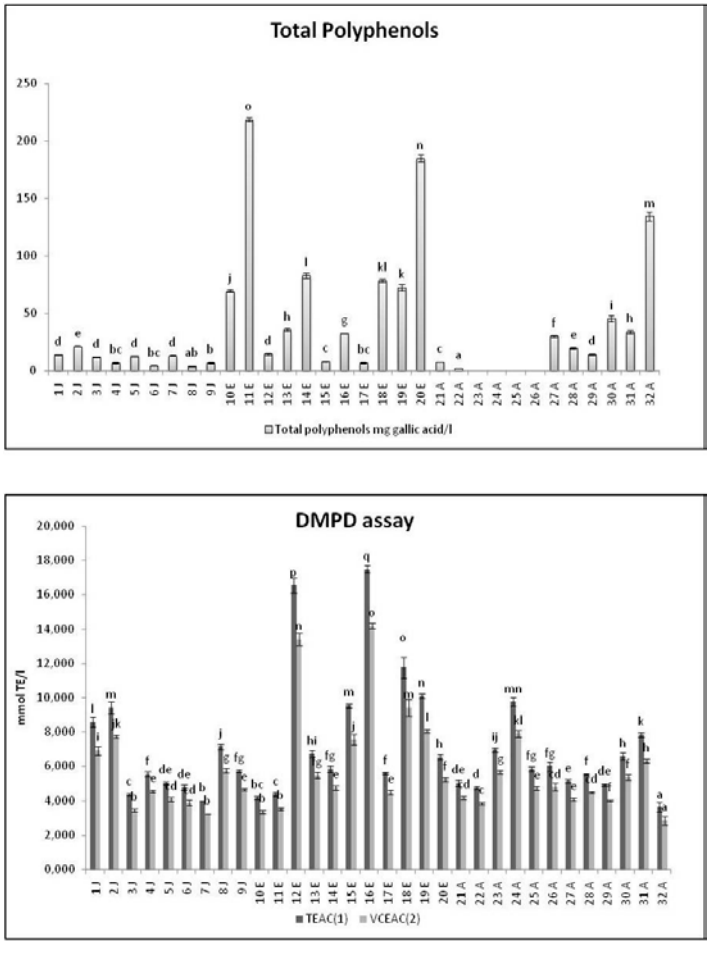

Figure 1. Total polyphenols content ▪ mg gallic acid/l. The standard deviation was <3%, the results expressed as mmol trolox equivalent/l (■ TEAC) and mmol ascorbic acid equivalents/l (□ VCEAC) of flavored waters determined by the DMPD assay. The standard deviation was <5%.

The water 11E includes *Aloe vera* extract. According to Zheng and Wang [14], *Aloe vera* presents a value of total polyphenols content of 0.23 mg GAE / g dry matter.

It is also necessary to emphasize the low value obtained in samples containing extracts of ginseng, 15E and 16E (21.24 and 32.7 mg GAE / l, respectively). They are very low compared with the value obtained in extracts of ginseng roots determined by Kim et al. [15] that ranged from 5 to 8.2 mg GAE / g depending on the method of extraction used, or the value of the extract from the leaves of ginseng by Jung et al. [16] the values varying between 932-2333.2 mg GAE/100g, depending on the extracting solvent. This fact shows that the concentration of these extracts is very low in both flavored waters.

The value found in sample 13 E, containing rooibos extract, was 35 mg GAE / l. Rooibos leaves contain 659.2 mg GAE / 100g fresh matter [17]. Green tea infusions can contain 1216 GAE / per cup (240 ml) [18], higher than the content in sample 14E (82.9 mg GAE/l). Soft drinks containing the same extract of green tea also showed a higher concentration, 0.8 mg GAE / ml [19].

It is also noteworthy that both mentioned samples (13 E and 14 E) come from the same brand and contain a colorant, sulphite ammonia caramel (E 150d), which has a total polyphenol content of 78.75 mg GAE / kg, as determined by Brenna et al. [3].

Referring to the total polyphenol content, samples containing either tea extract, herbs and even fruits have a higher value than other flavored waters, except for samples 20E and 32A that claim functional properties on their labels, with total polyphenol values among the highest (184,735 and 134,284 mg GAE /l respectively).

It is also important to highlight that the total polyphenols contained in flavored water are due to several compounds, because the Folin-Ciocalteu reagent is non-specific to phenolic compounds, as it can be reduced by many non-phenolic compounds (e.g. aromatic amines, sulfur dioxide, ascorbic acid, Cu (I), Fe (II), etc.), and for that reason it is not suitable for determination of "total phenolic content" unless interfering species are considered [20].

The DMPD results were higher than expected due to their content in total polyphenols. Figure 1 shows that the content of polyphenols obtained in the studied samples did not correlate well with the method DMPD (R^2=0.01). Moreover if we compare the values obtained in the determination of antioxidant capacities by the method DMPD with those obtained by Fogliano et al. [6] using the DPPH method (0.2-268.89 mg trolox / l) it can be seen that those obtained by the DMPD are much higher.

This fact could be due to some ingredient in flavored water, which reacts with the radical because DMPD assay reflects the ability of radical hydrogen-donors to scavenge the single electron from $DMPD^{\bullet+}$ [21]. The most repeated component in the flavored water samples is citric acid. To confirm this fact, a calibration curve for this organic acid was made, from an aqueous solution of 23.79 mmol/l. Standards were performed between 1 and 2.1 mmol/l. A linear relationship among concentration of citric acid and DMPD cation radical was found (Figure 2).

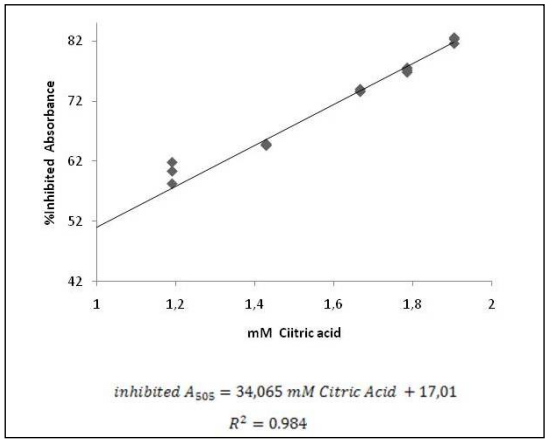

Figure 2. Calibration curve between citric acid and inhibited DMPD

These high values of antioxidant capacity in DMPD method were also observed previously ([22] and [23]) in fruit juices, compared to the other free radical scavenging methods ABTS and DPPH [23]. The

authors performed the fractionation of the juice components to determinate that the organic acids showed an antioxidant activity with DMPD assay, while organic acids did not have it with the other methods used, ABTS and DPPH. Citric acid has an important activity neutralizing the DMPD radical [23].

The citric acid is a metal chelator [24], it could react with the ferric ion used to obtain the $DMPD^{\bullet+}$ and it can be produced a displacement of the equilibrium towards DMPD, the reduced form (colorless).

In order to verify the citric acid interference the AOP kit was used. This assay was chosen because the citric acid also interferes with other methods for measuring antioxidant capacity, as in the case of FRAP, according to Prior et al. [25]. Nevertheless, this interference is not produced with the AOP method because copper, free and in phenanthroline complexes, has a lower redox potential than iron, so its reactions are more selective. Sugars and citric acid are not oxidized in the AOP method [25].This is the reason why in the samples containing juice and in most of those classified as aroma antioxidant capacity was not detected by the AOP method, while the values obtained by DMPD were high.

Results can be observed in Figure 3. Flavored waters showed antioxidant capacity by the AOP assay when their total reducing substances content was higher than 30 mg GAE/l. Samples in the extract group showed the highest results in AOP assay and total polyphenols content. The correlation between AOP assay and total polyphenols had an R^2 =0.7464.

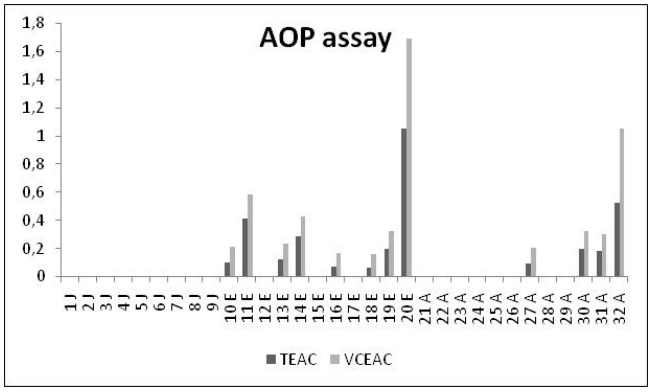

Figure 3. The results expressed as mmol trolox equivalent/l (■TEAC) and mmol ascorbic acid equivalents/l (■ VCEAC) of flavored waters determined by AOP assay

If we compare these results with those obtained by Kamel et al. [1], it can be observed that they are similar in spite of the different methodology used. They applied an electrochemical method in which they used a biosensor of guanine and adenine [1]. In their work, the results ranged from 1.6 and 8.0 mg ascorbic acid/l (0.01 and 0.05 mM ascorbic acid or VCEAC). These results are the same order of magnitude as those obtained in this study in the analysis with the AOP kit.

These results show that the DMPD method should be used with caution when evaluating the total antioxidant capacity in food products which are rich in organic acids, especially citric acid.

AOP is a fast method and it allows the measurement of standards and samples in one assay. The redox reaction producing colored species is carried out at pH 7 buffer as opposed to the basic conditions (pH 10) of the Folin-Ciocalteu assay [26] or to the acidic conditions (pH 5.25) such as the DMPD method.

However, this method has a disadvantage: it is expensive in comparison with the other methods used in this study. Nevertheless, it is possible to avoid the cost of these reagents if they are prepared at the concentration described by Da Cruz [10] and the method employed is the CUPRAC-BCS instead of the AOP method or if it is the CUPRAC method, as developed by Apak et al. [27]

4 Conclusion

Water is at the top of the ranking of the most consumed drinks all over the world. Today, a significant part of marketed water is flavored. In spite of the low antioxidant capacity found in this study, regular use of flavored water could contribute substantially to the total of dietary antioxidants. In addition, this

kind of drinks does not contain either alcohol or stimulants and also typically they do not contain sugar. This represents a certain advantage over traditional soft drinks, presenting as a market for healthier drinks.

Acknowledgments. The authors are thankful to Dr. Carlos Campos for his technical assistance in determination of antioxidant capacity by the AOP method. We also are thankful to the *Instituto Universitario de Lenguas Modernas y Traductores* (IULMyT) of the Complutense University for the language revision of the manuscript.

References

1. A.H. Kamel, C. Delerue-Matos, F.T.C. Moreira, and M.G.F .Sales, "Electrochemical determination of antioxidant capacities in flavored waters by guanine and adenine biosensors" Electrochimica Acta, , vol. 56, no. 24, pp. 8954-8961, 2011.
2. M.F. Barroso, Silva A., S. Ramos, M.T. Oliva-Teles, C. Delerue-Matos, M.G.F. Sales and M.B.P.P. Oliveira "Flavoured versus natural waters: Macromineral (Ca, Mg, K, Na) and micromineral (Fe, Cu, Zn) contents" Food Chemistry, vol. 116, no. 2, pp. 580-589, 2009.
3. O.V.Brenna, E.L.M. Cepp.i and G.Giovanelli (2009). "Antioxidant capacity of some caramel-containing soft drinks" Food Chemistry, vol. 115, no. 1, pp. 119-123, 2009.
4. C. Sanchez-Moreno "Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems" Food Science and Technology International, vol. 8, no. 3, pp. 121-137, 2002.
5. N. Pellegrini, M. Serafini, B. Columbi, D. Del Rio, S. Salvatore, M. Bianchi and F. Brigheti (2003). "Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays". The Journal of nutrition, vol. 133, no. 9, pp. 2812-2819, 2003.
6. V. Fogliano, V. Verde, G. Randazzo and A. Ritienei "Method for Measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines Journal of Agricultural and Food Chemistry, vol. 47, no. 3, pp. 1035-1040, 1999.
7. E. Venditti, T. Bacchetti, T. Tiano, P. Carloni, L. Greci and E. Damiani "Hot vs. cold water steeping of different teas: Do they affect antioxidant activity?" Food Chemistry, vol. 119, no. 4, pp. 1597-1604, 2010.
8. M. F Barroso., J.P Noronha., C Delerue-Matos. and M.B.P.P Oliveira, Flavored Waters: Influence of Ingredients on Antioxidant Capacity and Terpenoid Profile by HS-SPME/GC-MS. Journal of Agricultural and Food Chemistry, vol. 59, no. 9, pp. 5062-5072, 2011.
9. V.L. Singleton and J.A. Rossi "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents" American journal of Enology and Viticulture, vol. 16, no. 3, pp. 144-158,1965.
10. G. Da Cruz "Use of bathocuproine for the evaluation of the antioxidant power in liquids and solutions".U.S. Patent No 6,613,577, 2 Sept. 2003.
11. C. Campos, R. Guzmán, E. López-Fernández and A. Casado . "Evaluation of the cooper(II) reduction assay using bathocuproinedisulfonic acid disodium salt for the total antioxidant capacity: The CUPRAC-BCS assay " Analytical Biochemistry, vol. 392, no. 1, pp. 37-44, 2009.
12. V. Roginsky and E.Lissi " Review of methods to determine chain-breaking antioxidant activity in food" Food chemistry, vol. 92, no. 2, p. 235-254, 2005.
13. X.Wu, G.R. Beecher, J.M. Holden,D.B. Haytowitz, S.E. Gebhard and R. L. Prior "Lipophilic and hydrophilic antioxidant capacities of common foods in the United States" Journal of Agricultural and Food Chemistry, vol. 52, no. 12,pp. 4026-4037, 2004.
14. W. Zheng and S. Y. Wang "Antioxidant activity and phenolic compounds in selected herbs" Journal of Agricultural and Food chemistry, vol. 49, no. 11, pp. 5165-5170, 2001.
15. S.J. Kim, H.N. Murthy, E.J. Hahn, H.L. Lee and K.Y. Paek "Parameters affecting the extraction of ginsenosides from the adventitious roots of ginseng (Panax ginseng C.A. Meyer)" Separation and Purification Technology, vol. 56, no. 3, pp. 401-406, 2007.
16. C.H.Jung, H.Seog M., I. W. Choi, M. W. Park and H.Y. Cho "Antioxidant properties of various solvent extracts from wild ginseng leaves" LWT - LWT-Food Science and Technology, vol. 39, no. 3, pp. 266-274, 2006.

17.K.M. Yoo, C.H. Lee, H. Lee, B. Moon and C.Y. Lee “Relative antioxidant and cytoprotective activities of common herbs” Food Chemistry, vol. 106, no. 3, pp. 929-936, 2008.
18.A.K.Atoui, A.Mansouri, G. Boskou and P. Kefalas “Tea and herbal infusions: Their antioxidant activity and phenolic profile” . Food Chemistry, vol. 89, no. 1, pp. 27-36, 2005.
19.N.P. Seeram, M.Aviram, Y. Zhang, S.M. Henning, L. Feng, M. Dreher and D.Heber “Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States” Journal of agricultural and Food Chemistry, vol. 56, no. 4, pp. 1415-1422, 2008.
20.L.M. Magalhaes, M.A. Segundo, S. Reis and J.L.F.C.Lima “Methodological aspects about in vitro evaluation of antioxidant properties" Analytica Chimica Acta, vol. 613, no. 1, pp. 1-19, 2008.
21.I. Gülçin “Antioxidant activity of l-adrenaline: A structure-activity insight” Chemico-Biological Interactions, vol. 179, no. 2-3, pp. 71-80, 2009.
22.R. Pernice, G.Borriello, R.Ferracane, R.C.Borrelli, F. Cennamo and A. Ritieni “Bergamot: A source of natural antioxidants for functionalized fruit juices” Food Chemistry, vol. 112, no. 3, pp. 545-550, 2009.
23.M. I. Gil, F. A. Tomás-Barberán, B. Hess-Pierce, D. M. Holcroft, and A. A. Kader, “Antioxidant Activity of Pomegranate Juice and Its Relationship with Phenolic Composition and Processing,” Journal of Agricultural and Food Chemistry., vol. 48, no. 10, pp. 4581-4589, 2000.
24.E. A. Decker, C. C.Akoh and D. B. Min “Antioxidant mechanisms" in Food Lipids: Chemistry,Nutrition and Biotechnology. CRC press 2002, pp. 517-542.
25.R.L. Prior, X. Wu and K. Schaich “Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements” Journal of Agricultural and Food Chemistry, vol. 53, no. 10, pp. 4290-4302, 2005.
26.R. Apak, K. Guclu, M. Ozyurek, and S.E. Karademir “Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay” Microchimica Acta, vol. 160, no. 4, pp. 413-419, 2008.
27.R. Apak, K. Guclu, M. Ozyurek, and S.E. Karademir ”Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric iron reducing capability in the presence of neocuproine: CUPRAC method“ Journal of Agricultural and Food Chemistry, vol. 52, no. 26, pp. 7970-7981, 2004.

