

Assessing and Comparing the Total Antioxidant Capacity of Commercial Beverages: Application to Beers, Wines, Waters and Soft Drinks Using TRAP, TEAC and FRAP Methods

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Abstract: This work measures and tries to compare the Antioxidant Capacity (AC) of 50 commercial beverages of different kinds: 6 wines, 12 beers, 18 soft drinks and 14 flavoured waters. Because there is no reference procedure established for this purpose, three different optical methods were used to analyse these samples: *Total Radical trapping Antioxidant Parameter* (TRAP), *Trolox Equivalent Antioxidant Capacity* (TEAC) and *Ferric ion Reducing Antioxidant Parameter* (FRAP). These methods differ on the chemical background and nature of redox system. The TRAP method involves the transfer of hydrogen atoms while TEAC and FRAP involves electron transfer reactions. The AC was also assessed against three antioxidants of reference, *Ascorbic acid* (AA), *Gallic acid* (GA) and 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (*Trolox*).

The results obtained were analyzed statistically. *Anova* one-way tests were applied to all results and suggested that methods and standards exhibited significant statistical differences. The possible effect of sample features in the AC, such as gas, flavours, food colouring, sweeteners, acidity regulators, preservatives, stabilizers, vitamins, juice percentage, alcohol percentage, antioxidants and the colour was also investigated. The AC levels seemed to change with brand, kind of antioxidants added, and kind of flavour, depending on the sample. In general, higher ACs were obtained for FRAP as method, and beer for kind of sample, and the standard expressing the smaller AC values was GA.

Keywords: Antioxidant capacity, beer, beverages, FRAP, soft drinks, TEAC, TRAP, water, wine.

1. INTRODUCTION

Humans cannot exist without oxygen but oxygen is intrinsically dangerous to their existence. The major production of ATP in the cell is an oxygen dependent process, carried out in the mitochondria *via* the electron transport chain. It involves the oxidation of energy-rich molecules and the transfer to oxygen (O₂) of the redox power in NADH and FADH₂, produced in several steps. This univalent reduction of oxygen generates reactive intermediates, including the superoxide anion radical, hydrogen peroxide and the extremely reactive hydroxyl radical. These seem to be the main responsible for oxygen toxicity [1-3].

The defense network *in vivo* against these radicals [4] includes various antioxidants (AOs). Some are proteins and enzymes, while others are small molecules [5]. They may act as (i) preventing antioxidants by suppressing the formation of reactive oxygen and nitrogen species, (ii) scavenging antioxidants by removing active species rapidly before the active species attack biologically essential molecules, and (iii) by repairing damages (*de novo* antioxidants), clearing the wastes, and reconstituting the lost function [6]. A body

deficiency in these AOs can lead to oxidative stress and, subsequently to the oxidation of biomolecules [3, 7], accelerating the aging process and contributing to the development of chronic degenerative diseases, including coronary heart disease and cancer [2, 3, 7-10]. Exogenous AOs intake can play a crucial role in body protection against such diseases [11], as suggested by epidemiological data [12].

Foods obtained from plant processing methods constitute an important source of dietary AOs [13-15], with great emphasis for polyphenol compounds [16]. These include daily beverages such as wines, beers, soft-drinks, flavored waters, and juices. Some advantages coming from drinking these have also been highlighted in the literature. Drinking wine moderately may help preventing cardiovascular diseases and some types of cancers [3, 17-21]. This has been associated to the high levels of polyphenols in wines, because they act as free radical-scavenging compounds, participating in the second line defense *in vivo* [6]. A similar condition was attributed to beer, although with lower levels of polyphenols [22-24]. Drinks derived from fruits and vegetables are also rich in polyphenolic compounds [25-27], although less biologically available than in wines because they are strongly bonded. In addition to their AO activity, dietary AO are thought to have several beneficial activities by behaving as antibacterial and antiviral agents [28].

The overall AO contribution in each beverage is very difficult to identify. The extent to which sample dilution

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factor affects AC values is weakly understood [29]. Several protocols used to test AOs in foods consist of oxidizing a substrate under standard conditions, with or without food sample, and evaluating the activity by various appropriate methods to determine how much oxidation is inhibited at a relevant endpoint [28]. Several antiradical methods have been described, being TRAP, TEAC and FRAP methods the one most widely in the literature for this purpose [30-39].

TRAP method was developed by [39], and employs peroxy, RO_2 , radicals produced by the thermal decomposition of an azo compound [7, 23, 31, 34] such as, AAPH (2,2'-azobis (2- methylpropionamide)) in the presence of the radical cation $\text{ABTS}^{+\bullet}$ (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate)). The colour is measured, changing from dark blue to colourless in the presence of an antioxidant. This is detected by UV-Vis spectrophotometry at 734 nm [31, 40] and measures the contribution of all antioxidants in the sample, indicating its total antioxidant potential.

TEAC assay was first reported by [39] and based on the activation of metmyoglobin with hydrogen peroxide in the presence of ABTS, [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic)acid], to produce $\text{ABTS}^{+\bullet}$, in the presence of antioxidants. *Re et al.* [41] simplified this assay and described it as the production of the greenish blue $\text{ABTS}^{+\bullet}$ by persulfate oxidation of ABTS. Maximum absorptions are observed at 645 nm, 734 nm and 815 nm; as well as 415 nm [41, 42]. The addition of an antioxidant to the medium allows the evaluation of the AC through the decolorization degree as percentage inhibition of the $\text{ABTS}^{+\bullet}$ radical.

FRAP assays were developed by [30]; and measure the antioxidant power of a substrate by monitoring the reduction of the Fe^{3+} -TPTZ (ferric-tripyridyltriazine) complex to Fe^{2+} -TPTZ (ferrous-tripyridyltriazine). This reduction is conducted by the antioxidants in the sample. The colour of the complex changes from yellow to an intense violet blue, with absorption maximum at 593 nm [7, 35, 43]. FRAP is analyzed over time until all iron is reduced.

Overall, all these methods measure the ability of the sample matrix to inhibit radical species, i.e., quantify the AC. This is the global contribution from all AOs, including their probable synergic and antagonic effects. Furthermore, two requisites are needed to compare beverages in terms of AC: a representative sampling of each kind of beverage and a suitable method to carry out these measures. Sampling depends on the marketed products of the same kind and on their variable composition. This composition may be roughly estimated in the labeled data, because plant extracts or intentionally added AOs are indicated there. A more advantageous method is difficult to select since the obtained AC is a global measure and a direct comparison between the results obtained by different methods may not be possible. Each method relies on a different chemical background for which the contribution of each AO (showing a specific activity/sensitivity to each method), inside the sample matrix will lead to different results. The interfering effect from co-existing compounds in each assay is also variable from sample to sample and the standard used to express the AC may also lead to different results. Hence, for typifying the commercial beverages in terms of their AO power, all samples should be analysed by different methods and against

different reference standards. Regular AOs used as standards include AA, GA or Trolox.

Thus, this work aims to assess the AC of marketed beverages (soft drinks, beers, wines and waters) and compare the obtained values. To validate the obtained results, several optical assays against different antioxidants as standards are used to analyse the same samples. The most common antioxidants were selected for this purpose: AA, GA and Trolox. The effect of several parameters from each group of beverages was evaluated to assess the possibility of managing the AC content of each group of samples.

2. MATERIALS AND METHODS

2.1. Reagents

All reagents were *pro analysis (p.a.)* grade and deionised water of $0,054 \mu\text{S cm}^{-1}$ was employed. AA (Riedel-de-Haën), GA (Fluka) and Trolox (Fluka) were used as standards. Sodium acetate tri-hydrated (Merck), AAPH (Aldrich), ABTS (Fluka), acetic acid (Merck), sodium persulfate (Fluka), dipotassium hydrogen phosphate (Merck), phosphoric acid (Panreac), iron chloride hexahydrate (Merck), TPTZ (Fluka) and hydrochloric acid (Carlo Erba) were used.

2.2. Samples

A total of 50 samples purchased in local supermarkets were included in this study: 18 soft-drinks, 14 natural and flavoured waters, 6 white wines, and 12 beers. Soft drinks (samples no. 1 to 18) were from 6 different brands and had orange, lemon, apple or pineapple flavours; three of these were without gas, and four had gas. The wine samples (no. 19 to 24) were all white wines from different brands; three were green and three were red wines. The water samples (no. 25 to 38) were from three different brands and were natural or with flavours (lemon, mango, strawberry, pear/green tea, cereals, kiwi, lemon and magnesium, apple/white tea, pineapple, and strawberry/guava). Beers (samples no. 39 to 50) were from 8 different brands and were single, stout, black, blonde, brune, and optime brune.

2.3. Equipment

Absorbance values were measured by a Thermo Scientific Evolution 300 UV-Vis spectrophotometer ($\pm 0,001$ units of absorbance) using plastic or quartz cells of 1 cm optical path and 3 mL capacity. The pH was monitored by a combined glass electrode connected to a Crison[®] GLP pH meter. A thermostatic shaker bath was used to keep samples at 37 °C during the analysis of FRAP. Solids were weight with a Sartorius BP 211 D ($\pm 10^{-6}$ g). Volumes were rigorously measured by Gilson[®] automatic micropipettes of adjustable volume.

2.4. Methods

2.4.1. TRAP Assay

The TRAP assay was performed according to [31] for being suitable for aqueous based drinks, with some modifications, A mixture of ABTS and AAPH was prepared

to generate the radical $ABTS^{\bullet+}$. For this purpose, a solution of 75 μM ABTS and 2 μM AAPH was prepared in 50 mM acetate buffer pH of 4.3. This solution was incubated at 45 °C during 1 h and then cooled to room temperature. 2400 μL of this solution was added to 800 μL in the optical-cell. Absorbance readings were made at 734 nm after 15 minutes. TACs were calculated after the linear regression between the absorbance and the concentration of the standard solutions: AA (5 to 140 μM), GA (10 to 200 μM) and Trolox (5 to 140 μM). The AC was expressed in the same units as standards [31].

2.4.2. TEAC Assay

The method was used as described by [20]. $ABTS^{\bullet+}$ was produced by mixing 7mM ABTS with 2.45 mM potassium persulfate, in the dark, at room temperature, for 12-16 h. This solution had an intense blue-green colour and was diluted up to 500.0 mL with phosphate buffer before use, until the absorbance of 1.300 ± 0.05 at 734 nm was reached (approximately 15.0 mL). 1000 μL of sample was added to 2000 μL of this $ABTS^{\bullet+}$ solution, and the corresponding absorbance measured after 15 minutes at 734 nm. Concentration ranges of 15-140, 10-250 and 5-140 μM were used for AA, GA and Trolox, respectively. The TACs of the samples were determined as described for TRAP assay.

2.4.3. FRAP Assay

The FRAP assay was carried out according to the procedure described by [4, 28]; with some modifications. Briefly, the reagent contained 20.0 mL of 20 mM TPTZ solution, 20.0 mL of 20 mM iron (III) chloride solution and 12.0 mL of deionised water. TPTZ solution was prepared in 40 mM HCl and dissolved at 50 °C. 200 μL of sample was diluted in 1500 μL of the previous solution and 1300 μL of phosphate buffer. Readings were taken after 210 minutes at 593 nm, when the signal was stable. Samples were maintained in a thermostatic shaker bath at 37°C during the experiment. The standard solutions ranged 0.83-33.33 μM for AA, 0.83-16.67 μM for GA and 0.83-33.33 μM for Trolox. Once again TACs were determined as in the previous assays.

2.5. Statistical Analysis

All the results of the AC were analyzed by the Statistical Package for Social Science (SPSS, version 15.0). The descriptive analysis of each variable covered several statistical parameters, such as average, variance, standard deviation, minimum and maximum value, graphic representation in box whiskers [44], and identification of outliers. The confidence interval was 99 %. All observations were made independently.

TACs were different for method and standard used and for kind of sample and its composition. The analysis of the variance (ANOVA) using the *One-Way Anova* test was employed to identify homogenous subgroups between groups of samples. This test analyzed the effect of the dependent variable, testing if one dependent variable average of a parameter was equal to another. The application of this test was conditioned to ACs showing a normal distribution (normality) with variances between each group equal among

themselves (homoscedasticity). Normality was verified by the Shapiro-Wilk test, when $p > 0.01$. AC was converted in logarithmic values when experimental data did not show a normal distribution. The homogeneity of variances was measured by the *Levene* test. Homoscedasticity was verified when $p > 0.01$.

3. RESULTS AND DISCUSSION

3.1. Samples Characterization

From all the samples used in this study, 36 % were soft drinks, 28 % waters, 24 % beers and 12 % wines. All these were organized in terms of their % distribution by several factors that could affect the observed ACs. The presence of gas, flavours, food colouring, sweeteners, acidity regulators, preservatives, stabilizers, vitamins, juice percentage (%), alcohol percentage (%), antioxidants and the colour were considered for this purpose (Fig. 1). The ACs were determined by TRAP, TEAC, and FRAP assays and evaluated against the standards AA, GA and TR (Tables 1-4). In general, the results pointed out that all drinks displayed AC properties, although the values have a plenty variation with the sample, method and standard. In general, the ACs were higher for beers, analysed with the FRAP method and measured against either AA or Trolox. Lower ACs were obtained for waters, using the TRAP method against trolox.

3.2. ACs in Soft Drinks

The AC values of soft drinks for the 3 different methods and for the 3 different standards are indicated in Table 1. AC values ranged from 101 μM to 7359 μM . Generally, the highest values were obtained with TEAC method. This method also showed the narrower distribution of ACs, with 3006 μM of amplitude. Regarding standards, AA and Trolox values were higher than GA. Considering the 50 beverage samples, numbers 1, 5 and 9 displayed the highest values of AC in TRAP and FRAP methods. These samples had in common the presence of an apple flavour. Samples 13 to 18 displayed the lowest ACs, observed with FRAP method. These samples had in common the added gas or were carbonated.

Many parameters may have influenced the wide range of ACs observed. These include commercial brand, flavour, gas, food colouring, sweeteners, acidity regulators, preservatives, stabilizers, vitamins, juice percentage, colour, method and standard. Statistical analysis was conducted over these and results pointed out three parameters played a significant effect: flavour, juice percentage and colour, with significant differences between methods and standards.

ACs regarding flavours in soft drinks followed a normal distribution with p between 0.01 and 0.984 depending of the method or the standard used. Apple flavour presented the higher average AC value (7359 μM), while orange presented the lowest one (101 μM). The higher amplitude range was observed for apple flavour, ranging from 476 μM to 7359 μM . Sample 15 was a severe outlier for FRAP method when compared with GA and AA and a moderate outlier when compare with Trolox. *Levene* test confirmed homoscedasticity and *F* test presented a significance of

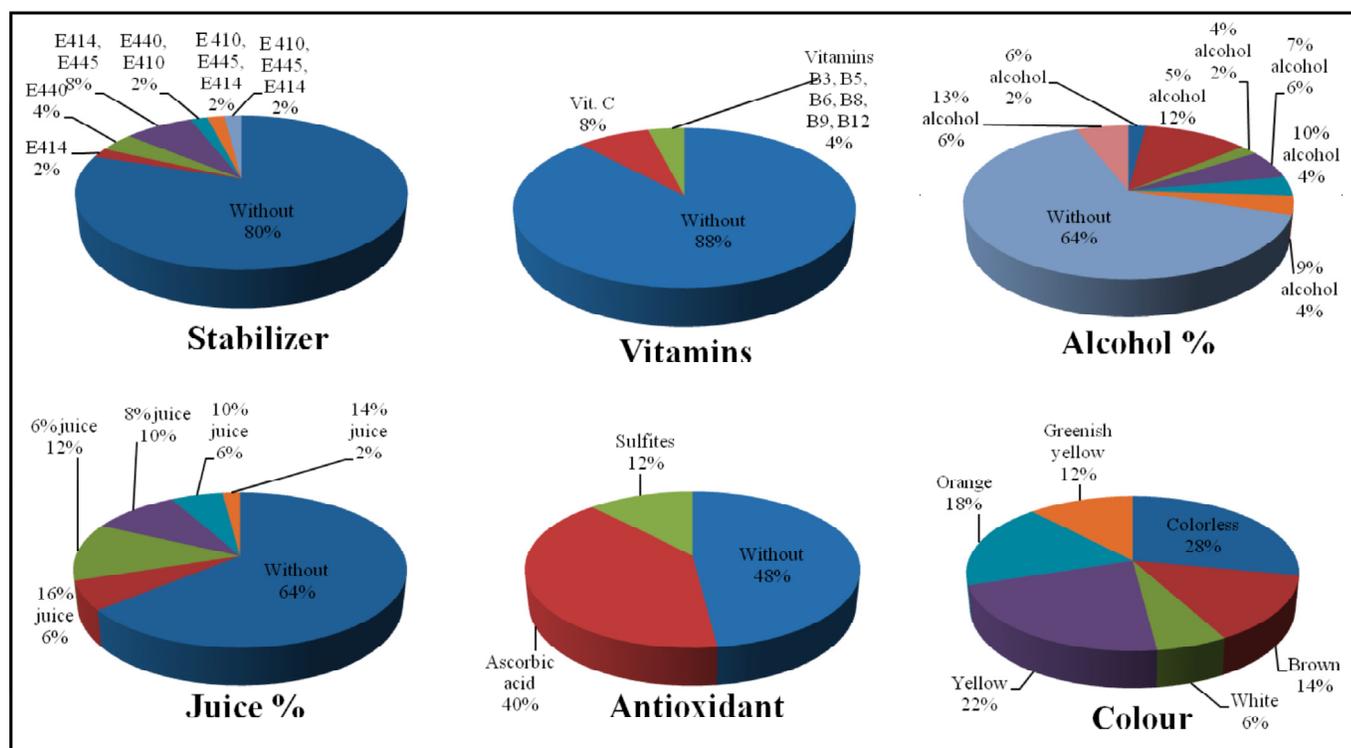


Fig. (1). Percentage (%) distribution by constitution parameter of the analyzed samples.

Table 1. AC Values of Marketed Soft Drinks

| N.º | Methods | | | | | | | | |
|-----|--------------------|-------------|-------------|--------------------|------------|-------------|--------------------|----------|-------------|
| | TRAP | | | TEAC | | | FRAP (t=210 min) | | |
| | Concentration (µM) | | | Concentration (µM) | | | Concentration (µM) | | |
| AA | GA | Trolox | AA | GA | Trolox | AA | GA | Trolox | |
| 1 | 1,709 ± 146 | 1,260 ± 168 | 1,286 ± 126 | 2,942 ± 11 | 2,244 ± 12 | 3,620 ± 15 | 1,455 ± 42 | 603 ± 18 | 1,462 ± 42 |
| 2 | 670 ± 2 | 177 ± 1 | 557 ± 2 | 3,367 ± 8 | 2,710 ± 9 | 4,228 ± 11 | 948 ± 59 | 397 ± 26 | 952 ± 60 |
| 3 | 555 ± 4 | 143 ± 1 | 454 ± 3 | 3,382 ± 45 | 2,727 ± 49 | 4,250 ± 64 | 1,072 ± 36 | 451 ± 16 | 1,076 ± 36 |
| 4 | 658 ± 11 | 173 ± 3 | 546 ± 10 | 3,363 ± 66 | 2,706 ± 73 | 4,223 ± 95 | 1,024 ± 34 | 430 ± 15 | 1,028 ± 34 |
| 5 | 7,359 ± 224 | 7,067 ± 259 | 5,982 ± 194 | 3,562 ± 13 | 2,924 ± 15 | 4,507 ± 19 | 1,421 ± 134 | 588 ± 58 | 1,427 ± 135 |
| 6 | 628 ± 10 | 164 ± 3 | 520 ± 8 | 3,780 ± 24 | 3,162 ± 26 | 4,818 ± 34 | 998 ± 78 | 418 ± 34 | 1,001 ± 8 |
| 7 | 412 ± 11 | 101 ± 3 | 328 ± 10 | 3,845 ± 5 | 3,234 ± 6 | 4,911 ± 8 | 1,041 ± 41 | 437 ± 18 | 1,045 ± 4 |
| 8 | 646 ± 26 | 170 ± 8 | 536 ± 23 | 3,812 ± 27 | 3,197 ± 29 | 4,863 ± 38 | 978 ± 120 | 410 ± 52 | 982 ± 12 |
| 9 | 5,725 ± 170 | 5,181 ± 197 | 4,568 ± 148 | 3,678 ± 77 | 3,051 ± 84 | 4,673 ± 110 | 1,162 ± 229 | 476 ± 99 | 1,168 ± 230 |
| 10 | 648 ± 33 | 170 ± 10 | 537 ± 29 | 2,632 ± 56 | 1,905 ± 61 | 3,178 ± 80 | 972 ± 123 | 407 ± 53 | 976 ± 123 |
| 11 | 548 ± 22 | 141 ± 7 | 448 ± 20 | 3,315 ± 61 | 2,653 ± 67 | 4,153 ± 87 | 1,045 ± 22 | 439 ± 9 | 1,049 ± 22 |
| 12 | 625 ± 6 | 163 ± 2 | 517 ± 5 | 2,898 ± 61 | 2,197 ± 67 | 3,559 ± 87 | 1,044 ± 17 | 438 ± 7 | 1,048 ± 17 |
| 13 | 442 ± 15 | 110 ± 4 | 354 ± 13 | 3,718 ± 74 | 3,095 ± 81 | 4,729 ± 106 | 507 ± 59 | 235 ± 28 | 820 ± 95 |
| 14 | 643 ± 28 | 169 ± 8 | 533 ± 25 | 2,857 ± 29 | 2,151 ± 32 | 3,500 ± 42 | 350 ± 50 | 160 ± 24 | 565 ± 81 |
| 15 | 646 ± 4 | 170 ± 1 | 535 ± 4 | 3,731 ± 66 | 3,109 ± 73 | 4,748 ± 95 | 533 ± 103 | 247 ± 49 | 862 ± 167 |
| 16 | 649 ± 4 | 170 ± 1 | 538 ± 3 | 2,915 ± 80 | 2,215 ± 87 | 3,583 ± 114 | 403 ± 76 | 185 ± 36 | 651 ± 123 |
| 17 | 412 ± 5 | 101 ± 1 | 328 ± 4 | 2,921 ± 50 | 2,221 ± 55 | 3,591 ± 72 | 404 ± 125 | 186 ± 60 | 652 ± 203 |
| 18 | 656 ± 6 | 173 ± 2 | 545 ± 6 | 2,696 ± 72 | 1,975 ± 78 | 3,270 ± 102 | 401 ± 81 | 185 ± 39 | 648 ± 132 |

0.007 which suggested that at least one group of samples was different from the others. *Post-Hoc* tests pointed out two homogeneous average sub-sets for a 0.01 *subset of alpha*. The different group that showed higher ACs was that of apple flavoured samples.

Regarding juice content, the average ACs values lied between 1500 and 3004 μM . The higher AC values were observed for samples with 16% of juice percentage. This group also presented the widest amplitude of AC variation. Only one sample had 14% of juice content, and therefore the corresponding ACs were not representative of this group. ANOVA tests showed two different sets of samples being samples with 16% of juice percentage significantly different from the others.

The intrinsic colour of the samples was correlated to their juice content. Brown samples had 16% of juice, yellow samples had 10%, white samples had 6% and orange samples had 8%, also corresponding to apple, pineapple, lemon and orange flavours. Thus, the statistical analysis pointed out the same results, with the brown samples displaying significant statistical differences.

3.3. ACs in Waters

Natural waters (samples 25, 32 and 36) have a simple composition for which they did not show AC properties, displaying the lowest values of AC (Table 2). The opposite behaviour was displayed by samples claiming ascorbic acid addition (samples 37 and 38), presenting the highest values of AC in all methods and standards. Samples 33 and 34 also showed high ACs, but the observed behaviour was not consistent in all methods and standards. These samples were flavoured waters, meaning that the high ACs could be

attributed to their flavours, vitamins and/or minerals (fruit flavours are expected to attribute water some antioxidant properties).

The amplitude of variation of ACs was 1684, 752 and 873 μM for FRAP, TRAP and TEAC, respectively. To correlate the sample properties with the statistical differences, the factors evaluated were: brand, flavors, sweeteners, acidity regulators, preservatives, vitamins, antioxidant, method and standard. The only significant statistical difference was obtained for brand, antioxidants and standard, this last one depending on the method in use.

In terms of brand, one specific brand showed the highest and the more disperse AC values, mostly for FRAP with AA and Trolox standards. Generally, ACs obtained with FRAP method were higher in all brands. The average ACs varied between 220 and 629 μM . Normality and homoscedasticity was verified with significances of 0.035 and 0.930 for normality test and 0.01 for variance test. ANOVA test was used to check if the observed differences were significant. The F test indicated a significance of 0.000, suggesting that at least one of the groups was significantly different from the others. ANOVA one-way identified two sub-sets of homogeneous averages, with C brand displaying statistical significant differences from the other two.

The only samples with claimed AA were numbers 37 and 38, both flavored waters from C brand. Samples 9 and 10 were outliers. These were flavored waters of B brand with lemon and apple flavors, respectively. Considering samples without AA, FRAP assays gave the highest ACs and most dispersed values. As normality and homoscedasticity was not verified, the AC was transformed in log AC. The *Shapiro-Wilk* test for the transformed variable displayed a

Table 2. AC Values of Marketed Drinking Waters

| N.º | Methods | | | | | | | | |
|-----|---------------------------------|--------------|-------------|---------------------------------|--------------|--------------|---------------------------------|---------------|-----------------|
| | TRAP | | | TEAC | | | FRAP (t=210 min) | | |
| | Concentration (μM) | | | Concentration (μM) | | | Concentration (μM) | | |
| | AA | GA | Trolox | AA | GA | Trolox | AA | GA | Trolox |
| 25 | 43 \pm 1 | 44 \pm 1 | 20 \pm 1 | 117 \pm 4 | 30 \pm 4 | 108 \pm 6 | ND | ND | ND |
| 26 | 75 \pm 10 | 74 \pm 10 | 43 \pm 7 | 154 \pm 2 | 71 \pm 2 | 162 \pm 3 | 704 \pm 55 | 347 \pm 27 | 726 \pm 58 |
| 27 | 67 \pm 31 | 66 \pm 30 | 37 \pm 22 | 265 \pm 1 | 193 \pm 1 | 321 \pm 1 | 732 \pm 83 | 361 \pm 41 | 754 \pm 86 |
| 28 | 85 \pm 8 | 84 \pm 8 | 50 \pm 6 | 169 \pm 2 | 87 \pm 2 | 183 \pm 3 | 184 \pm 34 | 91 \pm 17 | 184 \pm 35 |
| 29 | 94 \pm 9 | 93 \pm 8 | 57 \pm 6 | 275 \pm 3 | 203 \pm 3 | 334 \pm 4 | 885 \pm 137 | 436 \pm 67 | 913 \pm 142 |
| 30 | 103 \pm 12 | 101 \pm 11 | 63 \pm 9 | 223 \pm 3 | 146 \pm 3 | 260 \pm 4 | 839 \pm 148 | 413 \pm 73 | 865 \pm 154 |
| 31 | 66 \pm 1 | 65 \pm 1 | 36 \pm 1 | 169 \pm 1 | 88 \pm 1 | 184 \pm 2 | 138 \pm 98 | 68 \pm 48 | 135 \pm 102 |
| 32 | 47 \pm 1 | 48 \pm 1 | 23 \pm 1 | 112 \pm 4 | 24 \pm 4 | 101 \pm 5 | ND | ND | ND |
| 33 | 89 \pm 15 | 87 \pm 14 | 53 \pm 10 | 601 \pm 6 | 462 \pm 6 | 742 \pm 8 | 898 \pm 96 | 443 \pm 47 | 927 \pm 100 |
| 34 | 222 \pm 9 | 214 \pm 8 | 147 \pm 6 | 676 \pm 45 | 545 \pm 49 | 849 \pm 64 | 107 \pm 45 | 53 \pm 22 | 103 \pm 46 |
| 35 | 40 \pm 3 | 41 \pm 3 | 18 \pm 2 | 256 \pm 5 | 182 \pm 5 | 307 \pm 7 | 634 \pm 68 | 313 \pm 34 | 652 \pm 71 |
| 36 | 49 \pm 1 | 49 \pm 1 | 24 \pm 1 | 116 \pm 2 | 30 \pm 2 | 108 \pm 3 | ND | ND | ND |
| 37 | 722 \pm 9 | 694 \pm 8 | 493 \pm 6 | 709 \pm 1 | 581 \pm 1 | 897 \pm 2 | 1.684 \pm 228 | 830 \pm 112 | 1.737 \pm 238 |
| 38 | 770 \pm 10 | 740 \pm 9 | 527 \pm 7 | 677 \pm 1 | 546 \pm 1 | 851 \pm 2 | 1.649 \pm 246 | 813 \pm 121 | 1.701 \pm 257 |

significance of 0.01 and *Levene* test a significance of 0.02. The F test has a significance of 0.000, indicating that the two groups were statistically different.

The effect of the standard was considered for each method since it was not possible to find a normal distribution, even when all ACs were transformed by means of several mathematical approaches (Fig. 2). For TRAP method, the means were higher when Trolox was used as standard and lower for AA. The assumptions of normality are verified, with values of p between 0.01 and 0.051. The test of homogeneity of variance resulted in a significance of 0.628. The ANOVA test indicated 2 sub-sets in which the average AC for AA is considered to be statistically different and lower than the average of the standard Trolox. The behavior for FRAP method was similar to that of TRAP, with 2 sub-sets of homogeneous averages and AA displaying the lower ACs with statistical differences from those presented by other standards. In TEAC method there were no statistical differences between the standards.

3.4. ACs in Wines

The ACs of wine samples ranged from 495 to 22160 μM (Table 3). TEAC method provided the higher values for all

standards used. There were no significant differences between samples 19, 20 and 21 that were green wines and samples 22, 23 and 25, the mature wines. Only gas and alcohol content could be analysed as possible reasons for reaching different ACs. However, none of these originated significant differences between the observed groups. Only the use of different standards was responsible for producing statistically different ACs (Fig. 3).

The mean AC against each standard was 16818 μM for GA, 444 μM for Trolox and 6267 μM for the AA. The GA was by far the standard that gave the highest values, varying within the range 9976 - 22160 μM . On the contrary, Trolox was the one with the lowest ACs, with a variation of 702 μM . In *Shapiro-Wilk*, this assumption was true for all patterns and their significance varied from 0.152 to 0.228 for AA to the GA. The observed values were not distributed normally but when an equal number of observations is made in each group homoscedasticity and normality may be ignored (Everitt e Brian assumptions). The F test presented a significance value of 0.000, meaning that at least one group of samples was different from the others. The Anova test suggested 3 subsets of alpha 0.01, indicating that each standard provided statically different results.

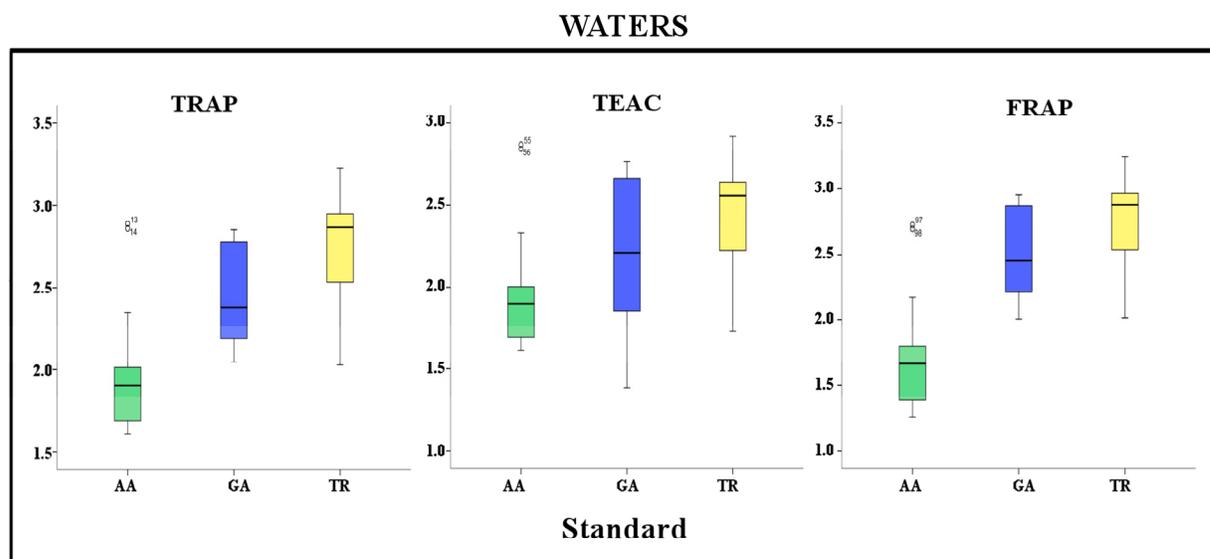


Fig. (2). Effect of standard on water samples.

Table 3. AC Values of Marketed Wines

| N.º | Methods | | | | | | | | |
|-----|---------------------------------|-----------------|-----------------|---------------------------------|------------------|-------------------|---------------------------------|-----------------|-----------------|
| | TRAP | | | TEAC | | | FRAP (t=210 min) | | |
| | Concentration (μM) | | | Concentration (μM) | | | Concentration (μM) | | |
| AA | GA | Trolox | AA | GA | Trolox | AA | GA | Trolox | |
| 19 | 6,849 \pm 94 | 6,479 \pm 108 | 5,541 \pm 81 | 16,920 \pm 1 | 13,644 \pm 1 | 21,263 \pm 1 | 1,498 \pm 322 | 495 \pm 123 | 1,658 \pm 333 |
| 20 | 7,506 \pm 75 | 7,237 \pm 87 | 6,110 \pm 65 | 17,483 \pm 106 | 14,260 \pm 116 | 22,066 \pm 152 | 2,246 \pm 518 | 782 \pm 199 | 2,432 \pm 536 |
| 21 | 7,763 \pm 18 | 7,533 \pm 21 | 6,332 \pm 16 | 16,404 \pm 623 | 13,079 \pm 683 | 20,526 \pm 890 | 3,040 \pm 562 | 1,086 \pm 215 | 3,254 \pm 581 |
| 22 | 4,438 \pm 4 | 4,408 \pm 4 | 3,647 \pm 3 | 17,548 \pm 13 | 14,332 \pm 15 | 22,160 \pm 19 | 1,762 \pm 106 | 596 \pm 41 | 1,932 \pm 110 |
| 23 | 7,744 \pm 117 | 7,511 \pm 135 | 6,315 \pm 101 | 17,258 \pm 849 | 14,014 \pm 930 | 21,745 \pm 1212 | 1,126 \pm 139 | 353 \pm 53 | 1,273 \pm 144 |
| 24 | 6,360 \pm 46 | 5,914 \pm 53 | 5,117 \pm 40 | 13,573 \pm 331 | 9,976 \pm 363 | 16,481 \pm 474 | 1,611 \pm 139 | 539 \pm 53 | 1,776 \pm 144 |

3.5. ACs in Beers

Beers are made with water, non malt cereals, hops and malt. These materials point out the possibility of having high ACs in beers. Table 4 shows the AC values obtained by all methods and against all standards. The ACs obtained by the FRAP method were 2000 times higher than those obtained by TRAP and TEAC assays. Sample 40 presented the higher values whatever the method or the standard. This sample was a dark beer. In general, dark beers presented higher values of AC compared to light (regular) beers from the same brand.

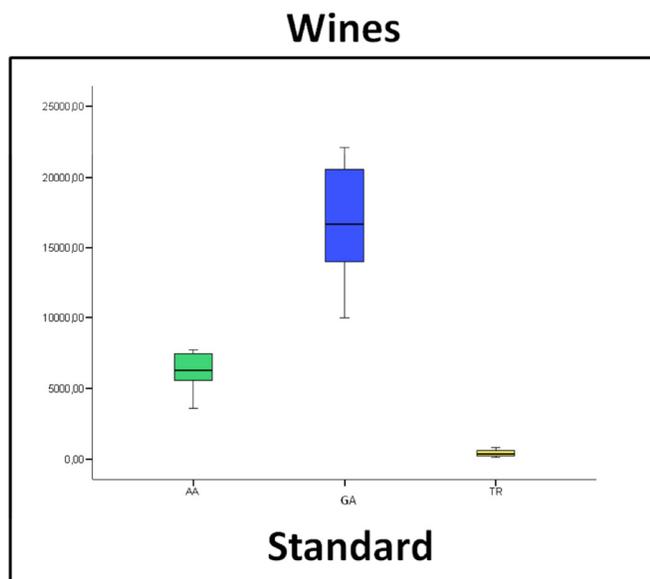


Fig. (3). Effect of standard on wine samples.

Different factors were observed to justify the different ACs. These were brand, kind (dark or light), colorants, alcohol, colour, method and standard. The only significant statistical difference was obtained for method and standard (Fig. 4). Regarding the standards, Trolox showed much higher values of AC than the other standards. Regarding the

method, TEAC was the one providing much lower ACs than the other ones. All these differences were confirmed statistically.

3.6. Main Factors Affecting AC

Fig. (5) displays the distribution of the ACs considering the factors that played a more relevant role on the observed values. Thus, we examined the impact of dilution factor, type of beverage, and the method and standard used for this purpose. Comparing the different kind of beverage, it was obvious that beers presented the highest values of AC comparing with the other analyzed drinks; their AC values reached almost 35000 μM . The lowest ACs were obtained with flavoured waters. Here, the levels of antioxidant species are probably very low because the antioxidants come only from the added flavours. Sulphites are the typical antioxidant compounds in wines and they can justify some of the AC recorded for this kind of drink. In addition, it is well known that wines are rich in polyphenolic species, a very important group of naturally occurring antioxidants. The higher values of ACs in wines were about 8000 μM . Soft-drinks displayed higher ACs than waters but these were much lower than in wines.

Comparing the different methods used, FRAP assays gave 10 times higher ACs than the other methods. It reached more than 61000 μM . The ACs of the other methods were always below 1000 μM .

In general terms, AA and Trolox were responsible for the higher ACs, with GA originating lower values. The highest AC when AA was used as standard was about 27000 μM ; for Trolox 25000 μM was the top AC. AC values for GA were the lowest ones, a little over the value of 10000 μM .

4. CONCLUSIONS

The results confirmed that the method and the standard used in the analysis of beverages played a significant role on

Table 4. AC Values of Marketed Beers

| N.º | Methods | | | | | | | | |
|-----|---------------------------------|---------------|-----------------|---------------------------------|--------------|---------------|---------------------------------|-------------------|--------------------|
| | TRAP | | | TEAC | | | FRAP (t=210 min) | | |
| | Concentration (μM) | | | Concentration (μM) | | | Concentration (μM) | | |
| AA | GA | Trolox | AA | GA | Trolox | AA | GA | Trolox | |
| 39 | 1,502 \pm 11 | 452 \pm 5 | 1,771 \pm 15 | 712 \pm 82 | 189 \pm 26 | 709 \pm 102 | 912,652 \pm 5 | 276,876 \pm 48 | 819,004 \pm 174 |
| 40 | 1,999 \pm 7 | 670 \pm 3 | 2,437 \pm 9 | 1099 \pm 31 | 313 \pm 10 | 1190 \pm 38 | 2214,550 \pm 13 | 607,287 \pm 8 | 1805,985 \pm 38 |
| 41 | 1,603 \pm 23 | 496 \pm 10 | 1,906 \pm 30 | 630 \pm 112 | 163 \pm 36 | 606 \pm 139 | 1103,622 \pm 88 | 307,612 \pm 32 | 910,816 \pm 119 |
| 42 | 1,767 \pm 11 | 568 \pm 5 | 2,125 \pm 15 | 799 \pm 15 | 217 \pm 5 | 817 \pm 19 | 305,608 \pm 13 | 373,694 \pm 10 | 1108,212 \pm 28 |
| 43 | 1,555 \pm 9 | 475 \pm 4 | 1,842 \pm 12 | 659 \pm 14 | 172 \pm 4 | 643 \pm 17 | 1196,702 \pm 77 | 568,898 \pm 37 | 1170,485 \pm 177 |
| 44 | 1,971 \pm 16 | 657 \pm 7 | 2,398 \pm 21 | 883 \pm 38 | 244 \pm 12 | 921 \pm 47 | 1619,138 \pm 8 | 769,127 \pm 4 | 1591,780 \pm 45 |
| 45 | 1,835 \pm 54 | 598 \pm 24 | 2,217 \pm 72 | 894 \pm 18 | 248 \pm 6 | 935 \pm 22 | 1451,257 \pm 179 | 689,553 \pm 85 | 1424,352 \pm 25 |
| 46 | 1,727 \pm 248 | 550 \pm 108 | 2,072 \pm 332 | 982 \pm 47 | 276 \pm 15 | 1045 \pm 59 | 1707,373 \pm 44 | 810,950 \pm 21 | 1679,777 \pm 9 |
| 47 | 1,510 \pm 95 | 456 \pm 41 | 1,782 \pm 127 | 651 \pm 64 | 170 \pm 21 | 632 \pm 80 | 1328,664 \pm 606 | 631,446 \pm 287 | 1302,091 \pm 124 |
| 48 | 1,281 \pm 5 | 355 \pm 2 | 1,475 \pm 6 | 709 \pm 52 | 188 \pm 17 | 705 \pm 65 | 890,612 \pm 194 | 423,814 \pm 92 | 865,222 \pm 64 |
| 49 | 1,542 \pm 18 | 470 \pm 8 | 1,825 \pm 24 | 689 \pm 25 | 182 \pm 8 | 680 \pm 31 | 852,351 \pm 47 | 405,679 \pm 23 | 827,064 \pm 196 |
| 50 | 1,145 \pm 38 | 296 \pm 17 | 1,294 \pm 51 | 766 \pm 27 | 207 \pm 8 | 776 \pm 33 | 1240,429 \pm 1 | 589,624 \pm 1 | 1214,094 \pm 13 |

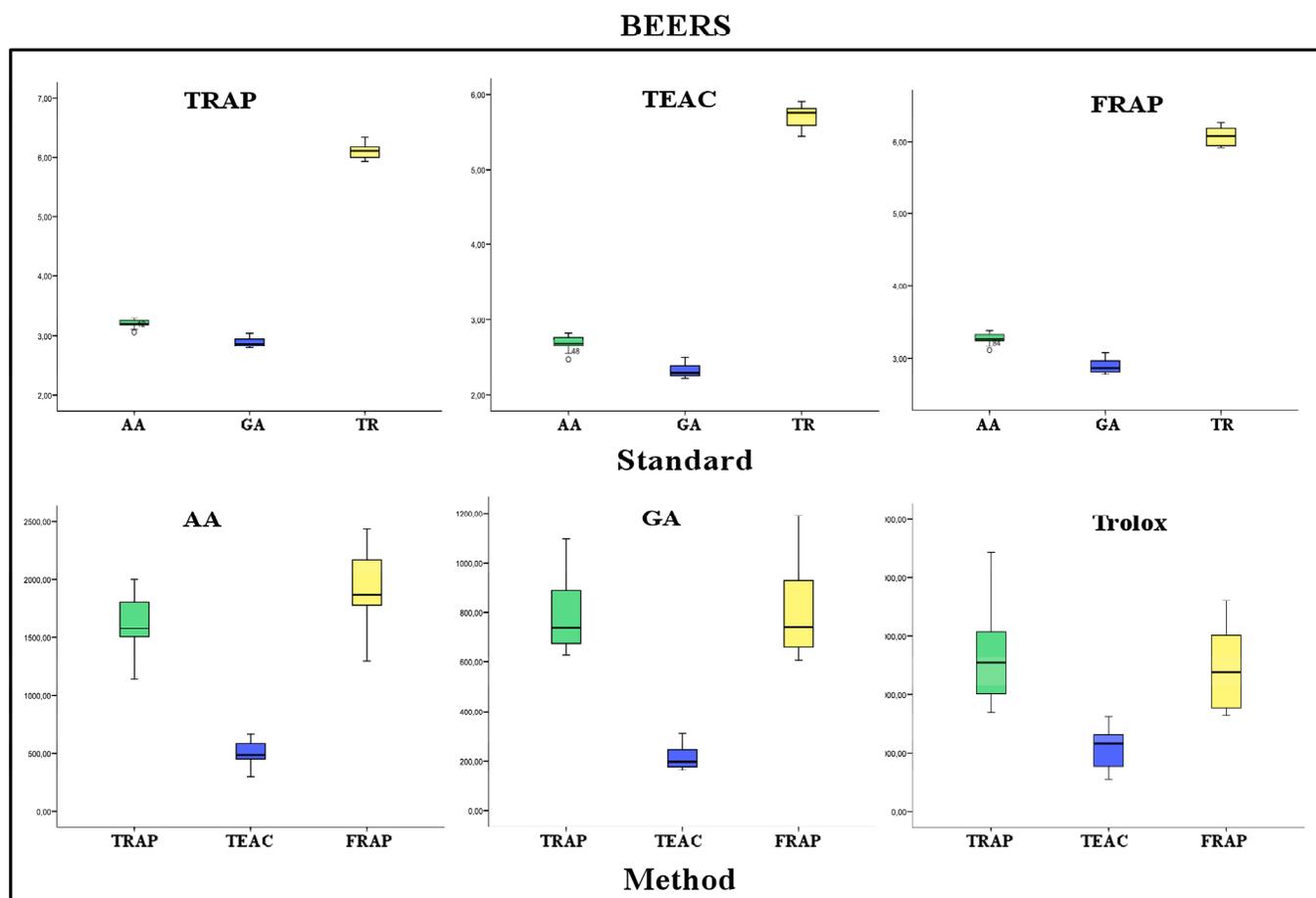


Fig. (4). Effect of standard and method on beers.

the observed results. ACs may vary extensively with the method or the standard in use. Regarding method, FRAP assay leads to much higher ACs, regardless the type of sample. In terms of standard, GA presented the lowest AC values. It is also important to consider, that the different values obtained by applying different methods and using different standards, can be attributed to the interfering effect from co-existing compounds, in each assay and for standard used.

The several marketed beverages displayed very different behaviour in terms of antioxidant properties. Comparing the different kinds of beverage, it was obvious that beers presented the highest values of AC, comparing with the other analyzed drinks. Some parameters may allow controlling the observed AC. For instance, flavoured waters may be managed by changing apple juice flavour and kind of antioxidant.

In general, the reported values revealed that the inclusion of the AC as a commercial indicator for consumer preference requires additional information. Both method and standard must be indicated. As a general recommendation, FRAP method could be employed. It gives much higher analytical values, a feature that may enhance the accuracy for samples with low ACs, widening the range of application. The use of AA as standard could be also recommended, as it is the cheaper compound providing the higher ACs.

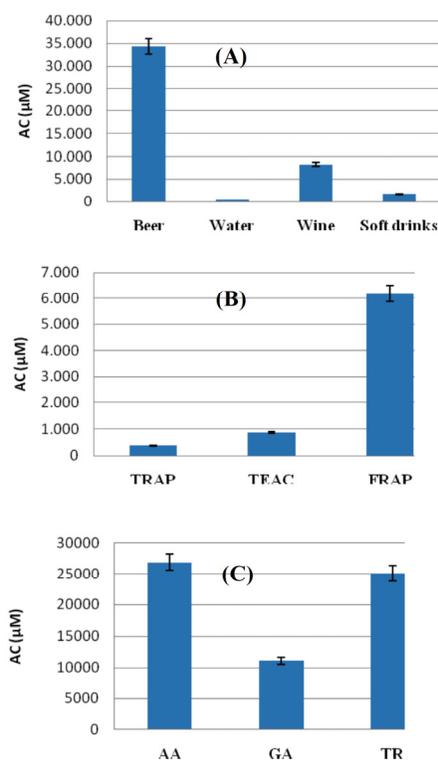


Fig. (5). Values of AC by kind of beverage (A), method (B) and standard (C).

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CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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