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# Control and comparison of the antioxidant capacity of beers

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ABSTRACT

The purpose of the present work is to determine the antioxidant capacity (AC) of 27 commercial beers. The AC indicates the degree of protection of a certain organism against oxidative damage provoked by reactive oxygen and nitrogen species.

Assays were carried out by the following methods: (i) total radical trapping antioxidant parameter (TRAP); (ii) trolox equivalent antioxidant capacity (TEAC); (iii) trolox equivalent antioxidant capacity (DPPH); (iv) ferric-ion reducing antioxidant parameter (FRAP); (v) cupric reducing antioxidant capacity (CUPRAC); (vi) oxygen radical absorbance capacity (ORAC). Ascorbic acid (AA), gallic acid (GA) and trolox (TR) were used as standards.

All beers showed antioxidant power, but a wide range of ACs was observed. The effect of several factors upon these differences was studied. Statistical differences were found between ACs of beers of different colours. ORAC method provided always higher experimental ACs, of significant statistical differences to other assays.



## 1. Introduction

Beers raw materials are water, malt, non-malted cereals, hops and yeast. Beers can be classified as ale or lager considering the kind of fermentation they are subjected to. Ale beers are produced after a "high" fermentation process, meaning that the yeast stays on top, with fermentation temperature ranging from 15 to 25 °C. These kinds of beers have a pronounced taste of hops and have alcohol contents between 4% and 8%. Lager beers have a deep or "low" fermentation, the yeast stays in the bottom, and the fermentation is carried out at 5–10 °C.

Beer is a worldwide traditional natural beverage, with low calories and no fat, with organic acids and vitamins (coming from malt), proteins, hop (a mild sedative and an appetite stimulant) and water. Beer has a higher nutritional value than other alcoholic beverages, because of its minerals and essential nutrients such as potassium, magnesium, calcium and sodium. The use of cereals and malt to produce beer may also contribute for the ingestion of naturally occurring antioxidant (AO) compounds, such as polyphenols. Therefore, a possible benefit from beer consumption, not yet studied, may derive from its AO proprieties (Ghiselli et al., 2000b; Girotti, Bolelli, Fini, Budini, & Arfelli, 2002; Wei, Mura, & Shibamoto, 2001). AOs are "any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" (Halliwell, 2007). AOs act in various ways, which include complexation of redox-catalytic metal ions, scavenging of free radicals, and decomposition of peroxides.

The intensity of this effect depends on the chemical structure and concentration of the AO present.

The antioxidant capacity (AC) is the measurement of moles of a given free radical scavenged by a test solution, independently of the antioxidant present in the mixture (Mello & Kubota, 2007). There are various assays described in literature for AC determination. Oxygen radical absorbance capacity (ORAC) and total radical trapping antioxidant parameter (TRAP) are based on hydrogen atoms transfer (HAT) that monitor competitive kinetic reactions ($M(n) + e\,(from\ AO) \rightarrow AO^{\bullet} + M(n-1)$) (Huang, Ou, & Prior, 2005). Trolox equivalent antioxidant capacity (TEAC), ferric-ion reducing antioxidant parameter (FRAP), trolox equivalent antioxidant capacity (DPPH), and cupric reducing antioxidant capacity (CUPRAC) assays are based on the electron transfer (ET) of a reduction reaction ($ROO^{\bullet} + AO \rightarrow ROOH + A^{\bullet}$) (Huang et al., 2005).

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TRAP method was developed by Wayner, Burton, Ingold, and Locke (1985). It is based on the generation of peroxyl radical by the attack of an azo compound to a substrate. Typically, these radicals have enough energy to extract hydrogen from a substrate. When 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate acid) (ABTS) is added to the azo compound 2,2

sodium acetate buffer (50 mM, pH of 4.3) was incubated at 45 °C for 60 min. The solution was brought to room temperature. A volume of 2400 μL of the above described solution was mixed with 800 μL of sample in a cell. The absorbance was read at 734 nm, 15 min after the reaction started. The standard concentrations ranged 1.3–35 μM for both AA and TR, and 1.3–10 μM for GA. Linear regression of each curve was used to determine the AC of samples, expressed in μM of each standard (Campos et al., 1996).

For TEAC assay a solution of 7 mM of ABTS and 2.45 mM of sodium persulphate was prepared in a phosphate buffer (pH of 7) and incubated over night in the dark to give a very intense greenish blue solution. 12.5 mL of this solution were diluted in 500.0 mL of phosphate buffer (pH of 7). Two-thousand microliters of this solution were placed in a cell and mixed with 900 μL of water and 100 μL of the sample. The absorbance was read at 734 nm, 15 min after the reaction started. For both standards AA and TR the range of concentration used was 1.7–46.7 μM and 0.7–13.3 for GA (Huang et al., 2005).

On FRAP assay, 200.0 mL of acetate buffer (23 mM pH of 3.6) were mixed with 20.0 mL of TPTZ (20.0 mM), 20.0 mL of $FeCl_3{\cdot}6H_2O$ 20 mM, and 12 mL of deionised water. TPTZ solution was dissolved in HCl (40 mM) at 50 °C. One thousand five hundred microliters of the previously described reagent was mixed with 1300 μL of acetate buffer, 30 μL of sample, and 170 μL of deionised water in a cuvette cell. This mixture was kept at 37 °C. Absorbance readings were made at 593 nm every 30 min till absorbance stabilization. The range of concentration used for AA and TR standards was 0.8–33.2 μM and 0.8–16.6 μM to GA standard.

For DPPH method, a solution of DPPH (0.19 mM) was prepared in 2:1 (v/v) of ethanol and sodium acetate (0.1 M); 2800 μL of this solution and 200 μL of sample were mixed in a cell. The decolouration of the DPPH radical was measured at 525 nm, 10 min after the reaction started. The concentration range for AA and TR was 1.7–50 μM and 3.3–66.7 μM to GA.

CUPRAC assay required three different solutions: copper (II) chloride ($1.0 \times 10^{-2}$ M), ammonium buffer (1 M, pH of 7) and neocuproine ($7.5 \times 10^{-3}$ M) in 96% ethanol. One microliter of Cu(II), 1 mL of buffer and 0.1 mL of sample were mixed in a cell, and the absorbance was read at 450 nm, 30 min after the reaction started. The range of concentration for GA was 0.6–18.3 μM and 1.2–61.0 μM to AA and TR standards.

On ORAC assay a solution of flouresceine (1.4 nM) and other of AAPH (4.8 mM) in phosphate buffer (75 mM, pH 7.4) were prepared. 1.7 mL of AAPH, 1 mL of flouresceine and 300 μL of diluted sample (3000×) were mixed in the cell. Readings were made every 30 min at the wavelengths of excitation and emission of 485 and 523 nm, respectively, until fluorescence became 0.5% of the initial value. The analytical signal for each sample was determined through the AUC by (Huang et al., 2002),

$$\mathrm{AUC} = 1 + \frac{f_1}{f_0} + \frac{f_2}{f_0} + \cdots \frac{f_n}{f_0},$$

where $f_0$ is the initial fluorescence and $f_n$ is fluorescence in time $n$. The AC was determined by (Huang et al., 2002),

$$\mathrm{AC} = \frac{\mathrm{AUC_{Sample}} - \mathrm{AUC_{Blank}}}{\mathrm{AUC_{Standard}} - \mathrm{AUC_{Blank}}} \times \frac{\text{Standard Concentration}}{\text{Sample Concentration}}.$$

### 2.5. Statistical anal sis

All AC values were analyzed by the Statistical Package for Social Science (SPSS, version 15.0). The one-way ANOVA test was used to identify the homogenous sub-groups between groups of samples. All observations were made independently. The application of this test is possible when the AC values obtained: (I) behave as a normal distribution, and (II) with homogeneous variances between each group (homocedasticity). The confidence interval was 99%. Normality was verified thought the Shapiro–Wilk test. In some cases, ACs were transformed to reach normality. Homocedasticity was tested by Levene test. The descriptive analysis of each variable includes several statistical parameters, such as average, variance, standard deviation, minimum and maximum value, graphic representation in box whiskers (Pestana & Gageiro, 2005), and identification of outliers.

## 3. Results and discussion

The AOs in food and beverages that we ingest daily are of the most importance once the human being does not have the ability of eliminating some of the free radicals produced at a biological level. Since beers are produced from natural products, they have naturally occurring AOs that may constitute a barrier to radical damage. By testing AC of beers it is possible to estimate a relative degree of protection after AOs ingestion, but the results obtained vary with method and standard making their interpretation very difficult. In addition, since not all beers are equal, and in order to identify the ACs of beers and correlate these with their main components, it is essential to measure the effect of several factors of the chemical system. The identification of these factors is made by a qualitative characterization of the concerned samples.

### 3.1. Qualitative characteri ation of samples

From the chemical point of view, beers may contain naturally occurring compounds extracted from cereals, by-products of the fermentation process (typically *ale* or *lager*), and food additives. The former group may include colourants, flavours, AOs, sweeteners, alcohol, colour, acidity regulators, and juice, among other additives. The analyzed samples are distributed and/or composed as indicated in (Table 1); brand names were omitted and named A–H.

Each compound in beers behaving as an AO may contribute to enhance their AC. Regarding food additives, only AOs, flavours, juices and citric acid are expected to increase in a more or less extent the AC, due to their ability of avoiding the oxidation of coexisting compounds by means of their own oxidation.

### 3.2. Determination of AC

The AC of beers was tested by TRAP, TEAC, DPPH, FRAP, CUPRAC, and ORAC assays and evaluated against the standards AA, GA and TR (Table 2). In general, the results pointed out that all beers displayed AC properties, although the values varied a lot with the sample, method and standard. ACs were higher for ORAC assay despite the standard used. ORAC was the only method measuring fluorescence decay, which is technically much more sensitive to small variations in concentration. Thus, ORAC calibrations were established for much smaller concentration ranges of AOs, which corresponded to higher ACs in the samples.

In general, ACs were lower when GA was used as standard and higher when TR was used. These differences were a consequence of the different sensitivities and linear concentration ranges observed within calibrations obtained with different AO standards, which in turn resulted from the different kinetics observed for each AO. In this case, GA reacted lesser than other AOs. ACs for AA, GA and TR standards ranged from 30, 33, and 122–2,097, 14,672 and 29,106 μM, respectively. Samples number 8 and 2 displayed, in almost all methods and standards, the highest and the smallest ACs, respectively. In order to assess if these differences have statistical significance, ANOVA test was applied to these results.

**Table 1**
Distribution by constitution parameter of the analyze samples.

| Brand | Name | Fermentation type | Origin | Colouring | Flavour | Antioxidant | Sweetner | Acidifier | Alcohol (%) | Colour | Juice | Other additives |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 1 | Lager | Portuguese | Caramel III (E150) | With | Without | With | Without | 4 | Red | Without | With |
| | 2 | Lager | Portuguese | Without | With | Without | With | Without | 4 | Gold | With | Without |
| | 3 | Lager | Portuguese | Without | With | Without | With | With (Citric acid) | 0 | Gold | With | Without |
| | 4 | Lager | Portuguese | Without | Without | Without | Without | Without | 0 | Gold | Without | Without |
| | 5 | Lager | Portuguese | Without | With | Without | Without | Without | 0 | Gold | With | With |
| | 6 | Lager | Portuguese | Without | Without | Without | Without | Without | 6 | Gold | Without | With |
| | 7 | Ale | Portuguese | Without | Without | Without | Without | Without | 6 | Red | Without | Without |
| | 8 | Ale | Portuguese | Caramel III (E150) | Without | Without | Without | Without | 5 | Black | Without | Without |
| | 9 | Ale | Portuguese | Caramel III (E150) | With | Without | Without | Without | 0 | Black | Without | Without |
| B | 10 | Lager | Portuguese | Without | Without | Without | Without | Without | 5 | Gold | Without | Without |
| | 11 | Lager | Portuguese | Without | Without | Without | Without | Without | 4 | Gold | Without | Without |
| | 12 | Lager | Portuguese | Without | Without | Without | Without | Without | 6 | Red | Without | Without |
| | 13 | Lager | Portuguese | Caramel III (E150) | Without | Without | Without | Without | 7 | Black | Without | Without |
| | 14 | Lager | Portuguese | Caramel III (E150) | Without | Without | Without | Without | 0 | Black | Without | Without |
| C | 15 | Ale | Belgian | Without | Without | Without | Without | Without | 8 | Amber | Without | Without |
| | 16 | Ale | Belgian | Without | Without | Without | Without | Without | 10 | Reddish brown | Without | Without |
| | 17 | Ale | Belgian | Without | Without | With (ascorbic acid) | Without | Without | 9 | Amber | Without | Without |
| | 18 | Ale | Belgian | Without | Without | With (ascorbic acid) | Without | Without | 7 | Reddish brown | Without | Without |
| | 19 | Ale | Belgian | Caramel III (E150) | Without | With (ascorbic acid) | Without | Without | 7 | Amber | Without | With |
| D | 20 | Ale | Belgian | Without | Without | Without | Without | Without | 7 | Reddish brown | Without | Without |
| | 21 | Ale | Belgian | Without | Without | Without | Without | Without | 8 | Reddish brown | Without | Without |
| | 22 | Ale | Belgian | Without | Without | Without | Without | Without | 7 | Reddish brown | Without | Without |
| | 23 | Ale | Belgian | Without | Without | Without | Without | Without | 8 | Amber | Without | Without |
| E | 24 | Lager | Portuguese | Without | Without | Without | Without | Without | 5 | Gold | Without | Without |
| F | 25 | Lager | Portuguese | Without | Without | Without | Without | Without | 5 | Gold | Without | Without |
| G | 26 | Lager | Portuguese | Without | Without | Without | Without | Without | 5 | Gold | Without | Without |
| H | 27 | Lager | Portuguese | Without | Without | Without | Without | Without | 5 | Gold | Without | Without |

**Table 2a**
AC mean (μM) and standard deviation values for all methods and standards studied.

| Sample | TRAP | | | TEAC | | | DPPH | | |
|---|---|---|---|---|---|---|---|---|---|
| | AA | GA | TR | AA | GA | TR | AA | GA | TR |
| 1 | 1489.5 ± 24.8 | 446.5 ± 10.8 | 1754.3 ± 33.2 | 734.3 ± 45.8 | 196.4 ± 14.7 | 736.2 ± 57.0 | 1048.9 ± 28.9 | 372.5 ± 38.3 | 1283.8 ± 133.1 |
| 2 | 1126.2 ± 78.9 | 287.5 ± 34.5 | 1268.1 ± 105.5 | 606.4 ± 0.0 | 155.5 ± 0.0 | 577.1 ± 0.0 | 823.8 ± 36.8 | 419.7 ± 62.5 | 1447.8 ± 217.1 |
| 3 | 1532.5 ± 27.0 | 465.3 ± 11.8 | 1811.8 ± 36.2 | 812.2 ± 3.2 | 221.3 ± 1.0 | 833.1 ± 4.0 | 1360.6 ± 182.9 | 573.0 ± 48.4 | 1980.1 ± 168.0 |
| 4 | 1435.3 ± 60.9 | 422.8 ± 26.6 | 1681.8 ± 81.4 | 714.4 ± 80.4 | 190.1 ± 25.7 | 711.4 ± 100.0 | 1168.0 ± 0.0 | 326.2 ± 0.6 | 1123.0 ± 2.0 |
| 5 | 1374.8 ± 20.3 | 396.3 ± 8.9 | 1600.7 ± 27.1 | 649.1 ± 5.6 | 169.2 ± 1.8 | 630.1 ± 7.0 | 1138.2 ± 121.0 | 597.1 ± 373.4 | 608.1 ± 1296.6 |
| 6 | 1502.3 ± 11.3 | 452.1 ± 4.9 | 1771.3 ± 15.1 | 712.1 ± 82.0 | 189.3 ± 26.2 | 708.6 ± 102.0 | 876.8 ± 9.2 | 271.7 ± 40.7 | 934.0 ± 141.3 |
| 7 | 1829.0 ± 117.2 | 595.1 ± 51.3 | 2208.4 ± 156.8 | 906.5 ± 14.5 | 251.5 ± 4.6 | 950.5 ± 18.0 | 1520.6 ± 90.8 | 437.5 ± 60.8 | 1509.7 ± 211.0 |
| 8 | 1999.5 ± 6.8 | 669.7 ± 3.0 | 2436.6 ± 9.0 | 1098.6 ± 30.5 | 313.0 ± 9.8 | 1189.5 ± 38.0 | 2184.0 ± 15.8 | 594.2 ± 9.4 | 2053.9 ± 32.8 |
| 9 | 1631.3 ± 45.1 | 508.6 ± 19.7 | 1944.0 ± 60.3 | 726.9 ± 1.6 | 194.1 ± 0.5 | 727.0 ± 2.0 | 1015.4 ± 73.7 | 292.4 ± 50.1 | 1005.7 ± 174.1 |
| 10 | 1602.7 ± 22.5 | 496.0 ± 9.9 | 1905.7 ± 30.2 | 629.7 ± 111.7 | 163.0 ± 35.7 | 606.1 ± 139.0 | 1068.5 ± 82.9 | 301.1 ± 30.1 | 1036.1 ± 104.5 |
| 11 | 1782.7 ± 56.3 | 574.8 ± 24.7 | 2146.6 ± 75.4 | 840.0 ± 57.1 | 230.2 ± 18.3 | 867.7 ± 71.0 | 1607.1 ± 97.4 | 477.2 ± 8.3 | 1647.7 ± 28.7 |
| 12 | 1567.6 ± 58.6 | 480.7 ± 25.6 | 1858.7 ± 78.4 | 736.0 ± 11.3 | 197.0 ± 3.6 | 738.3 ± 14.0 | 1181.0 ± 57.9 | 329.0 ± 83.8 | 1132.8 ± 290.9 |
| 13 | 1915.0 ± 0.0 | 632.7 ± 0.0 | 2323.6 ± 0.0 | 963.3 ± 12.9 | 269.7 ± 4.1 | 1021.2 ± 16.0 | 1627.6 ± 100.0 | 444.7 ± 49.5 | 1534.7 ± 172.1 |
| 14 | 1766.8 ± 11.3 | 567.9 ± 4.9 | 2125.3 ± 15.1 | 799.1 ± 15.3 | 217.2 ± 4.9 | 816.8 ± 19.0 | 1274.1 ± 13.2 | 366.8 ± 7.7 | 1264.2 ± 26.6 |
| 15 | 1836.9 ± 2.3 | 598.6 ± 1.0 | 2219.1 ± 3.0 | 813.3 ± 46.6 | 221.7 ± 14.9 | 834.5 ± 58.0 | 1494.9 ± 23.3 | 712.6 ± 11.1 | 1470.5 ± 23.2 |
| 16 | 1970.8 ± 15.8 | 657.1 ± 6.9 | 2398.2 ± 21.1 | 883.2 ± 37.8 | 244.1 ± 12.1 | 921.5 ± 47.0 | 1580,4 ± 6.7 | 753.2 ± 3.2 | 1555.7 ± 6.6 |
| 17 | 1722.2 ± 56.3 | 548.3 ± 24.7 | 2065.6 ± 75.4 | 827.5 ± 36.2 | 226.2 ± 11.6 | 852.2 ± 45.0 | 1442.3 ± 95.4 | 687.7 ± 45.3 | 1418.1 ± 95.1 |
| 18 | 1930.9 ± 22.5 | 639.7 ± 9.9 | 2344.9 ± 30.2 | 936.1 ± 40.2 | 261.0 ± 12.9 | 987.3 ± 50.0 | 1511.3 ± 86.6 | 720.4 ± 41.0 | 1486.9 ± 86.3 |
| 19 | 1554.8 ± 9.0 | 475.1 ± 3.9 | 1841.7 ± 12.1 | 659.3 ± 13.7 | 172.4 ± 4.4 | 642.8 ± 17.0 | 1145.7 ± 71.0 | 547.0 ± 33.7 | 1122.4 ± 70.8 |
| 20 | 1835.3 ± 54.1 | 597.9 ± 23.7 | 2217.0 ± 72.4 | 894.0 ± 17.7 | 247.5 ± 5.7 | 934.9 ± 22.0 | 1392.1 ± 182.0 | 663.9 ± 86.3 | 1368.0 ± 181.4 |
| 21 | 1997.9 ± 18.0 | 669.0 ± 7.9 | 2434.5 ± 24.1 | 1009.4 ± 39.4 | 284.4 ± 12.6 | 1078.5 ± 49.0 | 1679.3 ± 13.3 | 800.1 ± 6.3 | 1654.3 ± 13.3 |
| 22 | 1727.0 ± 247.9 | 550.4 ± 108.5 | 2072.0 ± 331.7 | 982.1 ± 47.4 | 275.7 ± 15.2 | 1044.6 ± 59.0 | 1638.5 ± 24.4 | 780.7 ± 11.6 | 1613.6 ± 24.3 |
| 23 | 1962.8 ± 18.0 | 653.6 ± 7.9 | 2387.6 ± 24.1 | 1009.9 ± 9.6 | 284.6 ± 3.1 | 1079.2 ± 12.0 | 1619.6 ± 84.3 | 771.8 ± 40.0 | 1594.8 ± 84.1 |
| 24 | 1510.2 ± 94.7 | 455.6 ± 41.4 | 1782.0 ± 126.7 | 650.8 ± 64.3 | 169.7 ± 20.6 | 632.2 ± 80.0 | 920.5 ± 76.6 | 440.2 ± 36.3 | 898.0 ± 76.3 |
| 25 | 1280.7 ± 4.5 | 355.1 ± 2.0 | 1474.9 ± 6.0 | 709.3 ± 52.2 | 188.4 ± 16.7 | 705.1 ± 65.0 | 747.1 ± 37.7 | 358.0 ± 17.9 | 725.1 ± 37.6 |
| 26 | 1542.1 ± 18.0 | 469.5 ± 7.9 | 1824.6 ± 24.1 | 688.8 ± 24.9 | 181.9 ± 8.0 | 679.6 ± 31.0 | 753.3 ± 139.8 | 361.0 ± 66.3 | 731.4 ± 139.4 |
| 27 | 1145.3 ± 38.3 | 295.9 ± 16.8 | 1293.7 ± 51.3 | 766.1 ± 26.5 | 206.6 ± 8.5 | 775.8 ± 33.0 | 1180.2 ± 11.1 | 563.4 ± 5.3 | 1156.8 ± 11.1 |

### 3.3. Effect of the method

The effect of the method was tested individually for the three standards. The corresponding descriptive statistic data is presented in Table 3. The relative order of AC per method was always DPPH < TEAC < FRAP < CUPRAC < TRAP < ORAC for each standard.

Comparing all methods, then AC was about seven times higher for ORAC assays, as can be seen from the mean and standard errors plotted in Fig. 1. This observation may result from the different nature of the optical method used in this assay, the only one measuring emission of energy instead of absorbance. The box-plots in Fig. 1 confirm that ORAC is the assay with higher ACs in all stan-

**Table 2b**
AC mean (μM) and standard deviation values for all methods and standards studied.

| Sample | FRAP | | | CUPRAC | | | ORAC | | |
|---|---|---|---|---|---|---|---|---|---|
| | AA | GA | TR | AA | GA | TR | AA | GA | TR |
| 1 | 108.4 ± 0.0 | 272.6 ± 0.0 | 285.1 ± 0.0 | 1131.4 ± 17.1 | 278.0 ± 4.7 | 1387.5 ± 21.3 | 2985.3 ± 225.7 | 3131.5 ± 136.3 | 7585.5 ± 254.1 |
| 2 | 31.9 ± 0.0 | 120.9 ± 0.0 | 181.5 ± 0.0 | 800.8 ± 48.9 | 186.8 ± 13.5 | 977.5 ± 60.7 | ND | 1046.4 ± 764.1 | 3697.3 ± 1424.9 |
| 3 | 121.1 ± 0.0 | 297.8 ± 0.0 | 302.4 ± 0.0 | 1200.6 ± 2.4 | 297.1 ± 0.7 | 1473.4 ± 3.0 | 2477.2 ± 821.3 | 2824.7 ± 495.9 | 7013.3 ± 924.8 |
| 4 | 155.1 ± 0.0 | 365.3 ± 0.0 | 348.4 ± 0.0 | 1005.0 ± 39.2 | 243.2 ± 10.8 | 1230.8 ± 48.6 | 2643.7 ± 1566.4 | 2925.2 ± 945.9 | 7200.9 ± 1763.8 |
| 5 | 98.6 ± 0.6 | 253.2 ± 1.2 | 271.9 ± 0.8 | 1107.1 ± 17.1 | 271.4 ± 4.7 | 1357.5 ± 21.3 | 5753.1 ± 1977.1 | 4802.9 ± 1193.9 | 10702.2 ± 2226.2 |
| 6 | 101.6 ± 0.0 | 259.1 ± 0.0 | 275.9 ± 0.0 | 1053.5 ± 4.9 | 256.6 ± 1.4 | 1290.9 ± 6.1 | 5408.2 ± 672.7 | 4594.6 ± 406.2 | 10313.8 ± 757.4 |
| 7 | 179.3 ± 0.6 | 413.3 ± 1.2 | 381.3 ± 0.8 | 1666.1 ± 19.6 | 425.6 ± 5.4 | 2051.0 ± 24.3 | 13220.2 ± 172.4 | 9312.0 ± 104.1 | 19.110.3 ± 194.2 |
| 8 | 306.3 ± 0.0 | 665.3 ± 0.0 | 553.4 ± 0.0 | 2771.9 ± 7.3 | 730.7 ± 2.0 | 3423.0 ± 9.1 | 22096.7 ± 3436.6 | 14672.2 ± 2075.2 | 29105.6 ± 3869.7 |
| 9 | 152.5 ± 0.0 | 360.2 ± 0.0 | 345.0 ± 0.0 | 1214.4 ± 7.3 | 301.0 ± 2.0 | 1490.6 ± 9.1 | 5705.4 ± 2207.9 | 4774.0 ± 1333.2 | 10648.4 ± 2486.1 |
| 10 | 186.1 ± 1.8 | 426.8 ± 3.6 | 390.5 ± 2.4 | 918.5 ± 9.8 | 219.3 ± 2.7 | 1123.5 ± 12.1 | 2441.1 ± 2609.4 | 2802.9 ± 1575.7 | 6972.7 ± 2938.2 |
| 11 | 188.2 ± 86.5 | 431.0 ± 171.6 | 393.4 ± 117.3 | 1489.6 ± 48.9 | 376.9 ± 13.5 | 1832.0 ± 60.7 | 5342.1 ± 12.4 | 4554.7 ± 7.5 | 10239.3 ± 14.0 |
| 12 | 172.5 ± 49.9 | 399.8 ± 98.9 | 372.1 ± 67.6 | 1005.0 ± 34.3 | 243.2 ± 9.5 | 1230.8 ± 42.5 | 2995.6 ± 2808.1 | 3137.7 ± 1695.7 | 7597.1 ± 3162.1 |
| 13 | 244.3 ± 18.0 | 542.2 ± 35.8 | 469.4 ± 24.4 | 1564.0 ± 56.3 | 397.4 ± 15.5 | 1924.3 ± 69.8 | 9384.7 ± 410.5 | 6995.8 ± 247.9 | 14791.4 ± 462.2 |
| 14 | ND | 33.2 ± 73.9 | 121.6 ± 50.5 | 1340.8 ± 166.4 | 335.8 ± 45.9 | 1647.3 ± 206.5 | 4175.6 ± 265.9 | 3850.3 ± 160.6 | 8925.8 ± 299.5 |
| 15 | 217.9 ± 50.5 | 490.0 ± 100.1 | 433.7 ± 68.4 | 1741.8 ± 17.6 | 439.7 ± 4.9 | 2141.7 ± 21.9 | 20773.8 ± 1635.7 | 13873.3 ± 987.7 | 27615.8 ± 1841.8 |

**Table 3**
Descriptive statistics of AC methods by method.

| Method | Standard | Number of samples | Mean | Standard deviation | Confidence interval 95% | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Upper limit | Lower limit | | |
| TRAP | AA | 27 | 1650.8 | 250.8 | 1551.6 | 1750.0 | 1126.2 | 1999.5 |
| | AG | 27 | 517.1 | 109.7 | 473.7 | 560.5 | 287.5 | 669.7 |
| | TR | 27 | 1970.1 | 335.5 | 1837.4 | 2102.8 | 1268.1 | 2436.6 |
| | Total | 81 | 1379.3 | 674.2 | 1230.3 | 1528.4 | 287.5 | 2436.6 |
| TEAC | AA | 27 | 805.9 | 133.9 | 752.9 | 858.8 | 606.4 | 1098.6 |
| | AG | 27 | 219.3 | 42.8 | 202.4 | 236.3 | 155.5 | 313.0 |
| | TR | 27 | 825.3 | 166.6 | 759.3 | 891.2 | 577.1 | 1189.5 |
| | Total | 81 | 616.8 | 309.0 | 548.5 | 685.1 | 155.5 | 1189.5 |
| DPPH | AA | 26 | 159.2 | 69.0 | 131.3 | 187.1 | 30.2 | 306.3 |
| | AG | 27 | 360.9 | 149.4 | 301.8 | 420.0 | 33.2 | 665.3 |
| | TR | 27 | 345.5 | 102.1 | 305.1 | 385.8 | 121.6 | 553.4 |
| | Total | 80 | 290.1 | 143.8 | 258.1 | 322.1 | 30.2 | 665.3 |
| FRAP | AA | 27 | 1296.3 | 341.6 | 1161.1 | 1431.4 | 747.1 | 2184.0 |
| | AG | 27 | 517.2 | 172.1 | 449.1 | 585.2 | 271.7 | 800.1 |
| | TR | 27 | 1363.4 | 362.9 | 1219.9 | 1507.0 | 725.1 | 2063.7 |
| | Total | 81 | 1059.0 | 489.6 | 950.7 | 1167.2 | 271.7 | 2184.0 |
| CUPRAC | AA | 27 | 1425.1 | 510.6 | 1223.1 | 1627.1 | 800.8 | 2771.9 |
| | AG | 27 | 358.1 | 137.9 | 303.5 | 412.6 | 186.8 | 730.7 |
| | TR | 27 | 1751.5 | 632.1 | 1501.4 | 2001.5 | 977.5 | 3423.0 |
| | Total | 81 | 1178.2 | 761.1 | 1009.9 | 1346.5 | 186.8 | 3423.0 |
| ORAC | AA | 26 | 8964.4 | 6852.3 | 6196.7 | 11732.1 | 209.9 | 22096.7 |
| | AG | 27 | 6531.1 | 4203.0 | 4868.5 | 8193.7 | 1046.4 | 14672.2 |
| | TR | 27 | 13924.8 | 7837.3 | 10824.4 | 17025.1 | 3697.3 | 29105.6 |
| | Total | 80 | 9817.3 | 7112.3 | 8234.5 | 11400.1 | 209.9 | 29105.6 |

dards, showing significant statistical differences to the other studied methods. Their corresponding ACs ranged from 210 to 29,106 μM.

The method displaying the lower ACs in all standards was always DPPH. The mean values of CUPRAC and TRAP methods are very similar, lying within 272–2064 and 187–3423, respectively.

The ANOVA test was carried out for the hypothesis: (i) $H_0$: $\mu_{FRAP} = \mu_{TEAC} = \mu_{TRAP} = \mu_{DPPH} = \mu_{CUPRAC} = \mu_{ORAC}$ and (ii) $H_1$: $\mu_{FRAP} \neq \mu_{TEAC} \neq \mu_{TRAP} \neq \mu_{DPPH} \neq \mu_{CUPRAC} \neq \mu_{ORAC}$. The $F$ test gave $p < 0.01$, thus rejecting the null hypothesis ($H_0$). Post-hoc test showed only two homogeneous sub-groups: one with DPPH, TEAC, FRAP, CUPRAC, and TRAP, and the other with ORAC. This confirmed the statistical difference between ORAC and the other AC assays.

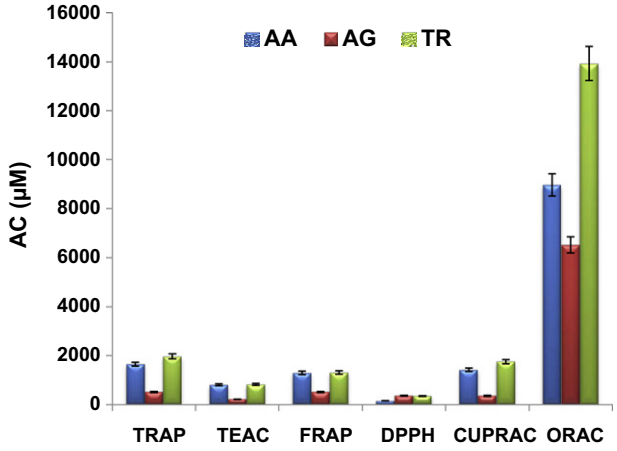


**Fig. 1.** Antioxidant capacity for the various assays and standards.

### 3.4. Effect of standard

The antioxidant "power" of each AO varies with its chemical nature, for which the mean ACs for each standard were also found different, as may be seen in Table 1 and Fig. 2. All assays showed lower AC when GA was used as standard, with the exception of the DPPH assay in which the lower AC value was obtained for AA. For all the studied assays (once again with the exception of the DPPH assay) the higher ACs were obtained having TR as standard. For the DPPH assay the higher value of AC was obtained for the AG standard (Table 4).

The statistical significance of these differences was evaluated by ANOVA test, considering separately the results of each method. The hypotheses were: (i) $H_0$: $\mu_{AA} = \mu_{AG} = \mu_{TR}$ and (ii) $H_1$: $\mu_{AA} \neq \mu_{AG} \neq \mu_{TR}$. The $F$ test gave $p < 0.01$, thus rejecting the null hypothesis ($H_0$).

Post-hoc tests showed at least one standard different than the others for all methods. For TEAC, FRAP, and DPPH methods, GA showed significant statistical differences from AA and TR.

For ORAC method, TR was different from the other standards and for TRAP and CUPRAC methods all standards were different. Therefore, there are significant statistical differences between standards, but the observed pattern depends on the way the AC is assessed.

### 3.5. Effect of the sample

ANOVA was used to evaluate the effect of each parameter described in sample characterization. Normality and homocedasticity of the observations were assured in every test. This study was made separately for each factor and grouping results according to the assay used. The factors commercial brand, fermentation, origin, alcohol and colour presented no statistical differences displaying always homogenous sub-groups.

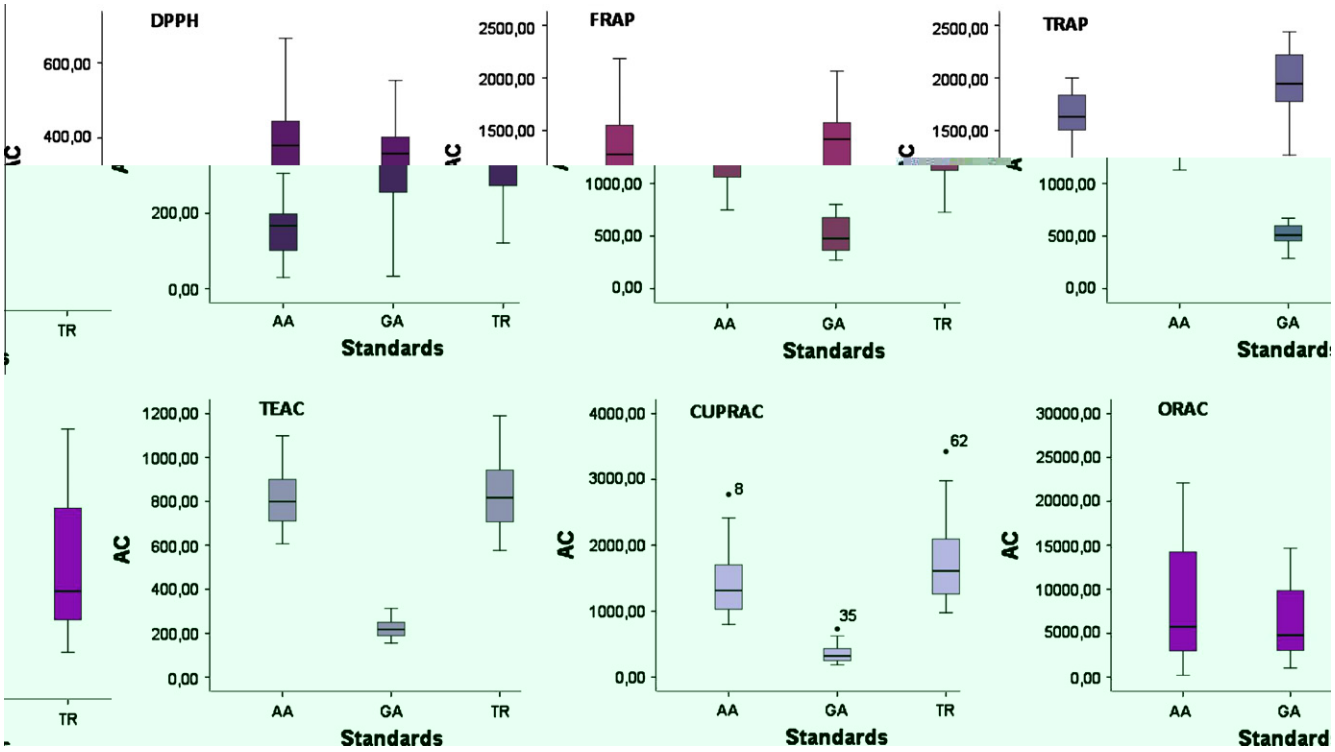


**Fig. 2.** Effect of the standard statistical analysis according the method used.

Commercial brand D showed the highest AC values, followed by C. While brands A and B displayed very similar ACs for TRAP, TEAC, DPPH and FRAP methods, the brand A had higher ACs then B for CUPRAC method and lower for ORAC method. Samples E, F, G and H had the lowest values of AC.

Ale beers had a slightly higher AC than lager samples for all methods and standards studied. Portuguese beers showed a slightly lower AC than Belgian samples, regardless of the method or standard.

The samples with flavourings had a higher AC than those without this component for all methods, except ORAC that gave the opposite result. The samples with and without colouring had very similar values for all methods and standards.

The samples with sweeteners, juice and other additives have higher ACs then those samples that did not have these compounds, although there were no significant statistical differences between the observed groups.

The reddish brown samples had higher ACs than the other colours. This was followed by the beers of ambar, red, black and gold colours. Beers with 7% and 8% of alcohol had higher AC then de samples of 0%, 4%, 5% and 6%.

Globally, most methods suggested statistical differences between beers of different colours. The commercial brand, origin of samples, fermentation, and alcohol content did not seem determinant factors because only some or few methods showed significant statistical differences between the observed samples. Flavouring, AO, sweeteners, juice and other additives were not statistically relevant for the observed AC differences.

## 4. Conclusions

The beers were from different brands, fermentation (ale or lager), origins (Portuguese and Belgium), food colouring, flavours, sweeteners, antioxidants, juice content, acidity regulator, alcohol content and colour. Their ACs were assayed by TRAP, TEAC, DPPH, FRAP, CUPRAC and ORAC, against three different standards (AA, GA, TR).

The highest AC values were obtained using ORAC and the lowest values were obtained by DPPH. TR was the standard, regardless of the assay, that achieved highest ACs. GA was on the other hand, for all assays except for DPPH, the standard that obtained the lowest AC. The different commercial brands tested showed statistical differences between themselves only on FRAP method. Lager type beers had lower AC than the ale ones, and Portuguese beers showed slightly lower ACs then Belgian ones. The reddish-brown beers displayed a higher AC followed by the amber, red, black and gold beers. Results showed that higher alcohol content provides higher AC values. The samples with food colouring had a higher AC then the ones without it, for every method except the ORAC, in this method the results were reverse. Beers with sweeteners, flavours, antioxidants and other additives had a slightly higher AC than the samples without it.

Statistical analysis pointed out that some intrinsic aspects of the samples and the experimental procedure could provide some statistical differences in ACs. The method and the colour of the sample (on most methods) affected significantly the AC.

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