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Antiradical and Reducing Potential of Commercial Beers

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Abstract

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The antioxidant properties of commercial beers and their changes during storage were investigated. The relationship between the antioxidant capacity and total polyphenol contents of a range of commercial beers were evaluated. The results show that the antiradical and reducing potential varies depending on the type of beer and the processing steps involved in its production. Higher antiradical potential and polyphenol content in dark beers than in lager, dealcoholised and wheat beers were determined. A strong relationship was found between the total polyphenol content and both antiradical activity and reducing power, as measured by DPPH and FRAP assays. When any decrease in antioxidant activity as a result of storage was observed, it occurred mainly after the initial 4-week storage period. The total polyphenol content dropped more sharply than the antiradical and reducing ability over the same time periods.

Keywords: antioxidants; beer; DPPH; FRAP; polyphenols

Beer is a beverage prepared from barley malt, hop, water and top- or bottom-fermenting yeast. Barley malt may be replaced partially or completely with wheat malt to obtain wheat beer. When producing lager, kilned (standard) malt is used and wort is fermented with bottom-fermenting yeast strains of *Saccharomyces pastorianus*. For darker beer types, malt blends which consist of dark (roasted) malt contributing to the ultimate colour and to roast aromas as well as pale malt are used. A variety of beer styles, including bock and porter, are classified as dark beers. Bock is sweet, lightly hopped, relatively strong lager. Porter is similar in style to bock, but darker and with higher extract and alcohol. Top-fermented beers differ from bottom-fermented lagers in their special flavour mainly induced by top-fermenting yeast strains of *Saccharomyces cerevisiae*. After fermentation and maturation beer is usually filtered to clearness.

As with the majority of food products, the physical and chemical characteristics of beers may become altered during storage, making them less attractive to consumers. With modern production methods, it is far easier to guarantee microbiological and colloidal stability than to maintain flavour stability. For example, to delay the formation of haze in the final product, haze-forming fractions of proteins and tannins are often removed. Flavour deterioration is connected with oxidative degradation of beer constituents by reactive oxygen species such as oxygen or nitrogen radicals as well as by non-radicals with the ability to oxidise or convert molecules into oxidising radicals. However, beer is rich in substances that can help protect against oxidation, the most important of which are sulphur-containing compounds, bitter hop resins, vitamins, and Maillard reaction products (Aron & Shellhammer 2010). Moreover, beer contains various phenolic compounds from malt or hops, which also exhibit antioxidant properties (Leitao *et al*. 2011, 2012).

The aim of this study was to determine the antiradical and reducing potential of a range of Polish commercial beers. Lager, wheat, bock, porter and alcohol-free beers were analysed using FRAP and DPPH techniques. FRAP evaluates the ability to reduce the Fe(III) complex to Fe(II) (Benzie & Strain 1996), whereas DPPH assesses the reduction by antioxidants of free radical $DPPH^{\bullet+}$ (2,2-diphenyl-1-picrylhydrazyl) (Mishra *et al*. 2012). Since polyphenols are known to inhibit oxidative reactions, the polyphenol content was measured against beer antioxidant activity.

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MATERIAL AND METHODS

Material. Table 1 presents the types, producers, and processing details of the beers tested in this study. All beers were manufactured in Polish breweries and purchased from either local retailer or restaurant brewery. Thirteen beers were filtrated, twelve pasteurised, and eight stabilised.

All sixteen beverages were analysed immediately upon delivery. Eight brands were then chosen, and half the bottles of each brand were stored at 25°C for 4 and 8 weeks, respectively. They were then analysed again.

DPPH$^{\bullet+}$ radical scavenging activity. The DPPH$^{\bullet+}$ assay was used to determine antioxidant capacity. The working solution was prepared by dissolving 0.0025 g of DPPH$^{\bullet+}$ reagent in 100 ml of methanol. A 0.3 ml sample diluted 10-fold for light beers and 20-fold for dark beers was added to 6 ml of DPPH$^{\bullet+}$ working solution and placed in the dark for 30 min at room temperature. The absorbance (A_{30}) was measured at a wavelength of λ = 515 nm against a methanol solution (A_0). The percent of DPPH$^{\bullet+}$ scavenged by the end of the reaction time was calculated according to the equation:

$$\%\text{Red DPPH}^{\bullet+} = \left(1 - \frac{A_{30}}{A_0}\right) \times 100\%$$

A Trolox calibration curve was plotted as a function of the percentage of DPPH$^{\bullet+}$ radical scavenging activity, from which antiradical activity was calculated and expressed as mmol of Trolox equivalents (TE) per litre.

Ferric ion reducing antioxidant power (FRAP). FRAP analysis was carried out according to the method described by Benzie and Strain (1996). Final results of the FRAP assay were expressed in mmol Fe^{2+} per litre.

Total polyphenol content. Total polyphenol content was determined using the EBC 9.11 method (2013).

Statistical analysis. Each sample was examined in three replicates. Mean values and standard deviations were calculated. Student's *t*-test was performed to determine statistical significance. A *P*-value below 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Antioxidant activity of commercial Polish beers. Different beer types were sourced from micro, small and large breweries. From a total number of 16 beers, 9 were lagers, 5 dark beers, and 1 a top-fermented wheat beer. Differences in production processes were also taken into consideration, as 13 of the beers were filtrated, 8 stabilised, and 12 pasteurised. Figure 1 presents the antiradical and reducing potential of the commercial beers, as determined by DPPH and FRAP assays. Dark beers displayed the highest

Table 1. Characteristics of beers

No.	Type	Original gravity (°P)	Alcohol content (%, v/v)	Brewery	Brewery capacity (× 10^3 hl/year)	Fermentation	Stabilisation	Filtration	Pasteurisation
1	alcohol-free	no data	< 0.5	A	5600	bottom	YES	YES	YES
2	wheat	12.1	5.2	F	< 250	top	NO	NO	YES
3	lager	12.2	6.0	G	1200	bottom	YES	YES	YES
4	lager	12.5	5.7	C	800	bottom	YES	YES	NO
5	lager	12.5	5.8	B	3500	bottom	YES	YES	YES
6	lager	11.1	5.7	A	<5600	bottom	YES	YES	YES
7	lager	12.5	5.7	C	800	bottom	YES	YES	YES
8	lager	12.5	5.1	D	0.45	bottom	NO	NO	NO
9	lager	11.0	4.5	I	20	bottom	NO	YES	YES
10	lager	12.2	5.6	H	50	bottom	NO	YES	YES
11	lager	14.5	6.2	F	< 250	bottom	NO	YES	NO
12	bock	16.0	6.5	E	160	bottom	YES	YES	YES
13	porter	22.0	9.5	E	160	bottom	YES	YES	YES
14	bock	15.1	6.5	F	< 250	bottom	NO	YES	YES
15	bock	14.5	6.0	D	0.45	bottom	NO	NO	NO
16	porter	18.1	8.0	F	< 250	bottom	NO	YES	YES

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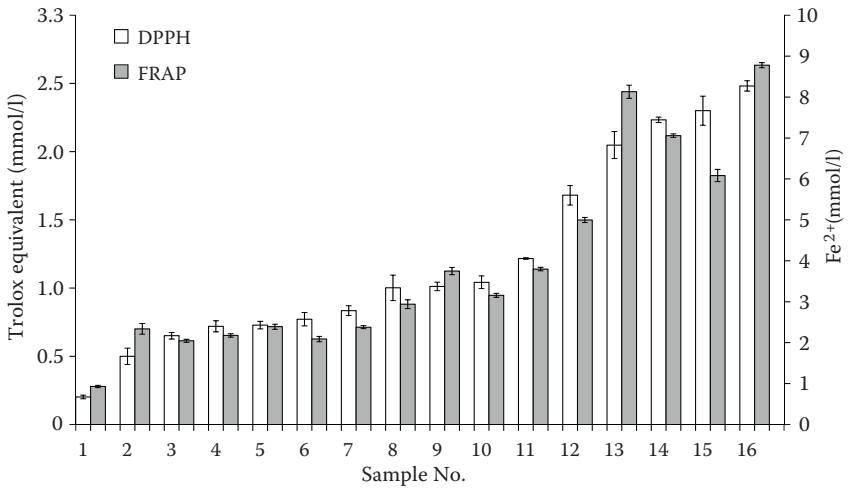

Figure 1. Antiradical and reducing potential of commercial beers determined by DPPH and FRAP assays (for exlanation of sample No. see Table 1)

antioxidant activity in DPPH assays, ranging from 1.68 mmol Trolox equivalent (TE)/l (No. 12) up to 2.48 mmol TE/l (No. 16). The antiradical potentials of lager beers from small breweries ranged from 1.00 mmol TE/l to 1.30 mmol TE/l. Lager beers from large breweries showed antiradical activity ranging from 0.65 mmol TE/l to 0.85 mmol TE/l. Wheat beer (No. 2) exhibited relatively low antiradical activity, at 0.50 mmol TE/l, while the lowest activity measured, at 0.20 mmol TE/l, was in alcohol-free beer (No. 1).

Results obtained from FRAP and DPPH assays revealed the same trend (Figure 1). Dark beers displayed the highest reducing potential, ranging from 5.00 to 8.78 mmol $FeSO_4$/l. The reducing potential of lager beers from small breweries ranged from 2.94 to 3.80 mmol $FeSO_4$/l, while that of lager beers from large breweries ranged from 2.04 to 2.39 mmol $FeSO_4$/l. The lowest reducing potential measured was in alcohol-free beer (No. 1), with 0.93 mmol Fe^{2+}/l. Bock and porter beers are produced from wort of higher gravity. It requires an increased charge of malt, which is a primary source of beer antioxidants. In turn, alcohol-free beers are usually brewed with lower original wort extract. Hence, the higher antiradical and reducing potential determined in bock and porter beers in relation to the other beers can be attributed to higher amounts of naturally occurring antioxidants of barley malt, including polyphenols, thiols, carotenoids, and vitamins. However, the highest antiradical and reducing potential in dark beers may be due to the use of dark malt, which contains heat-induced compounds such as melanoidins and reductones, formed by the Maillard reaction during kilning or roasting (Inns *et al*. 2007). Although there is still a debate whether dark beers have higher antioxidant capacity. Their production requires the use of dark malt, which is exposed to increased heat and oxidative stress during the kilning or roasting process. Cortes *et al*. (2010) argued that using dark malt leads to reduced oxidative stability. In light of our findings, the use of dark malt can be seen not to have an undesirable effect on the antioxidant potential of beer.

Piazzon *et al*. (2010) also associated variations in reducing potential with different beer types, with the lowest reducing potential in dealcoholised beer and the highest in dark beers (bock).

Within all beer types, beverages which had not been stabilised (e.g. lager Nos 8–11) to prevent the formation of haze exhibited higher antiradical and reducing activity than those that had been stabilised (e.g. lager Nos 3–7).

Different beer brands exhibited a wide variation in the antiradical and reducing potential. The results of FRAP and DPPH assays differed by factors 9 and 12, respectively, which is almost double the variation registered by Tafulo *et al*. (2010), who also researched the antiradical and reducing properties of different brands of beer. In lager beers, the DPPH antiradical activity and FRAP reducing potentials varied by up to factor 2. In studies by Zhao *et al*. (2010), who analysed the reducing power and DPPH antiradical activity of 34 lager beers, the differences were up to 4 and 5 fold, respectively. In a similar study of 40 lager beers by Zhao *et al*. (2013), the DPPH antioxidant potentials and reducing powers varied by factors up to 4 and 3, respectively. In comparison with these previous studies, our research shows that the antioxidant capacity of beers can be much more varied, and suggests that there may be a scope for enhancing many brands through the proper choice of raw materials and changes in the production process.

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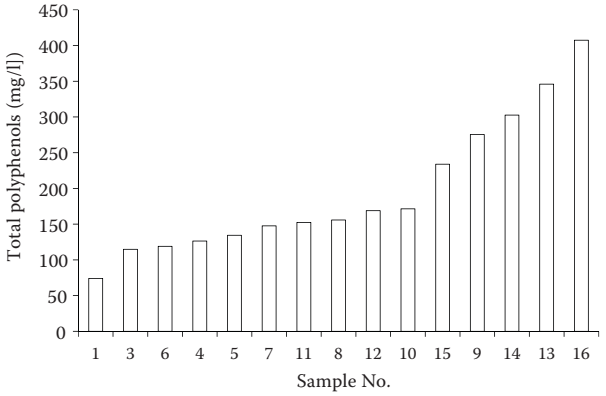

Figure 2. Total polyphenol content in commercial beers (for exlanation of sample No. see Table 1)

We observed a strong relationship between DPPH and FRAP results, with a correlation coefficient R^2 of 0.95 (n = 14, P < 0.05). The same correlation between DPPH radical scavenging activity and reducing power (n = 34, P < 0.05) was found in the study by Zhao *et al*. (2010). However, in the more recent paper by the same authors (Zhao *et al*. 2013), this correlation was only 0.61 (n = 40, P < 0.01). Evaluations of antioxidant activity are based on distinct reaction mechanisms and take into account different proportions of the multiple substances with antioxidant properties in beers. As a result, differences in results obtained using various methods may occur. Nonetheless, in our study the results obtained using each method (DPPH and FRAP) corresponded with the other.

Antioxidant activity was collated with the polyphenol content of the different beers. The highest polyphenol contents measured, ranging from 233.9 mg/l to 407.5 mg/l, were in dark beers (Figure 2). A high polyphenol content of 275.5 mg/l was also found in lager beer No. 9. However, other lager beers produced both by small and large breweries had low polyphenol concentrations, ranging from 114.8 mg/l to 147.6 mg/l. The lowest polyphenol content (74.0 mg/l) was in alcohol-free beer, which can be attributed to reducing a malt charge in its production. It is estimated that malt contributes about 70–80% of beer polyphenols. Hence, higher original gravity tends to increase the polyphenol content in beer. Our findings are in agreement with those of Piazzon *et al*. (2010), who observed a higher polyphenol content in dark beers than in lager, dealcoholised and wheat beers. Within each beer type, the concentration of polyphenols in beverages which were not stabilised (e.g. lager Nos 8–11) to prevent the formation of haze was higher than in those that had been stabilised (e.g. lager Nos 3–7).

A relationship was found between the total polyphenol content and both antiradical activity and reducing power, as determined by DPPH and FRAP assays. The correlation coefficient R^2 (n = 13, P < 0.05) between the total polyphenol content and antioxidant activity measured using the DPPH method was 0.61, while in studies by Zhao *et al*. (2013) this figure was 0.43 (n = 34, P < 0.05) (2010) and 0.83 (n = 40, P < 0.01) (2013). The correlation coefficient R^2 between the total polyphenol content and reducing potential, measured using a FRAP assay, was 0.72 (n = 13, P < 0.05). In the two studies by Zhao *et al*. (2010, 2013), these figures were 0.46 (n = 34, P < 0.01) (2010) and 0.74 (n = 40, P < 0.01) (2013). Piazzon *et al*. (2010) described a stronger relationship between the polyphenol concentration and FRAP reducing potential, with an R^2 of 0.92 (n = 35, P < 0.0001). Andersen *et al*. (2000) argued that polyphenols have little influence on the antioxidant activity of beer. However, in view of the strong relationship between both antiradical activity and reducing power and the total polyphenol content found in this study, it can be hypothesised that excessive polyphenol removal during beer production to enhance beer colloidal stability could have a detrimental effect on flavour stability.

Change in antioxidant activity during storage. Changes in the antiradical and reducing potential of the beers as a result of storage were investigated using DPPH and FRAP assays (Figure 3). In general, when any decrease in antioxidant activity was observed, it occurred mainly after the initial storage period (4 weeks). Over the next 4 weeks, antioxidant activity stabilised. Siqueira *et al*. (2011) also found the highest decrease in antioxidant activity at the beginning of storage, i.e. over the first 35 days. In the case of the four lager beers (Nos 4–7), there was no significant change in antiradical capacity. This may seem surprising, since lager beers are usually considered extremely sensitive to staling. However, the beers had been bought from retail vendors and, although they were analysed at least 3–6 months before the expiry date, it is possible that some had already been stored in the shops for lengthy periods. Therefore, the sharp fall in antioxidant activity that can be supposed to occur in the initial period of storage may have preceded our study.

The greatest decrease in antiradical capacity was observed in restaurant bock No. 15, which was reduced by 27% after 4 weeks of storage and by 48% after 8 weeks. A significant drop was also observed in restaurant lager No. 8, in which antiradical activity

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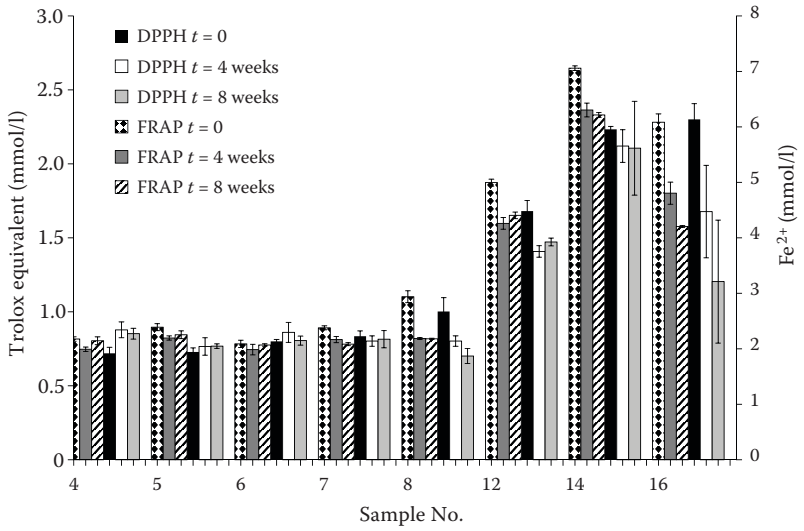

Figure 3. Changes in antiradical and reducing potential as a result of storage determined by DPPH and FRAP methods (for exlanation of sample No. see Table 1)

was reduced by 20% over 4 weeks of storage, and by 30% over 8 weeks. Such results are not unexpected, since while restaurant beers are being produced and dispensed, the level of oxygen exposure is not controlled. These beers do not undergo any treatment to extend their shelf-life and should therefore be consumed within a very short time. Moreover, the restaurant beers analysed in our study were unpasteurised. Similarly, He *et al*. (2012) observed around 10% lower antioxidant activity in fresh cloudy wheat beer over the first 18 days of storage at 20°C. Lund *et al*. (2012) and Hoff *et al*. (2013) showed that the radical formation during storage occurs faster in unpasteurised than in pasteurised beer, and that beer pasteurisation greatly improves oxidative stability. However, Cao *et al*. (2011) observed that if pasteurization intensity is too severe, this may result in lower polyphenol content and reduced oxidative stability.

Similar findings were obtained using FRAP assays. The highest decrease in reducing potential was in restaurant bock No. 15: a reduction of 21% over the first 4 weeks and of 31% over the total 8 weeks of storage. The reducing potential of restaurant lager No. 8 dropped by 26% during the initial storage period, but no further change was observed. Beer Nos 7, 12 and 14 exhibited approximately a 10% drop in reducing capacity, while in the remaining beers no significant changes were noted. There was therefore a strong relationship between the DPPH and FRAP results obtained from stored beer, with a correlation coefficient R^2 of 0.96 (n = 24, P < 0.05).

Changes in the total polyphenol content during storage were also investigated after 4 and 8 weeks. Figure 4 shows the total polyphenol content as a function of storage time. The greatest decrease of 50% was observed in restaurant bock No. 15. However, it is worth noticing that there was also a considerable fall in the total polyphenol content of bock beers. Products Nos 12 and 14 registered drops in polyphenols of 43 and 44%, respectively. Less of a decrease was observed in lager Nos 5 and 7, in which the total polyphenol content was reduced by 34 and 20%, respectively. However, in certain lagers the polyphenol concentration was almost unchanged (Nos 8 and 6) or even slightly increased (No. 4).

As with antioxidant activity, the change in total polyphenol content was greatest during the initial storage period. Siqueira *et al.* (2011) also found that the most significant drop in polyphenol concentration in beer occurred during the first 4 weeks of natural aging. However, we found only a weak relationship between the total polyphenol content and antiradical and reducing potential after storage. The respective correlation coefficients R^2 (n = 24, P < 0.05) were 0.44 and 0.43. In general, the total polyphenol content dropped more sharply than the antiradical and reducing ability over the same time periods. The sharper

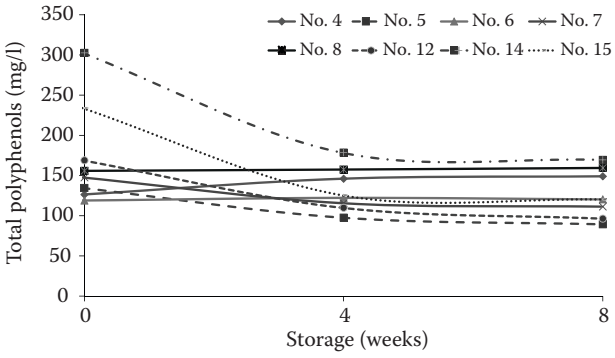

Figure 4. Change of total polyphenol content in different beers during storage

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decrease in polyphenol concentration compared with that of antioxidant capacity suggests that other compounds may also play a significant role in determining the oxidative stability of beer. Of these antioxidants, sulphur dioxide, hop bitter acids, and Maillard reaction products (e.g. reductones) might have a crucial impact. Sulphur dioxide is produced by yeast during fermentation and survives into the final beer. Sulphur dioxide concentration depends on a yeast strain to a large extent. Analysed beers, coming from various breweries, could differ in sulphur dioxide significantly.

References

Andersen M., Outtrup H., Skibsted L. (2000): Potential antioxidants in beer assessed by ESR spin trapping. Journal of Agriculture and Food Chemistry, 48: 3106–3111.

Aron P.M., Shellhammer T.H. (2010): A discussion of polyphenols in beer. Physical and flavour stability. Journal of the Institute of Brewing, 116: 369–380.

Benzie F., Strain J.J. (1996): The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Journal of Analytical Biochemistry, 239: 70–76.

Cao L., Zhou G., Guo P., Li Y. (2011): Influence of pasteurising intensity on beer flavour stability. Journal of the Institute of Brewing, 117: 587–592.

Cortes N., Kunz T., Suarez A.F., Hughes P., Methner F.J. (2010): Development and correlation between the organic radical concentration in different malt types and oxidative beer stability. Journal of the American Society of Brewing Chemists, 68: 107–113.

EBC 9.11 (2013): Total Polyphenols in Beer by Spectrophotometry. In: Analytica EBC, EBC Analysis Commitee. Nürnberg, Fachverlag Hans Carl GmbH.

He G., Du J., Zhang K., Wei G., Wang W. (2012): Antioxidant capability and potableness of fresh cloudy wheat beer stored at different temperatures. Journal of the Institute of Brewing, 118: 386–392.

Hoff S., Lund M.N., Petersen M.A., Frank W., Andersen M.L. (2013): Storage stability of pasteurized non-filtered beer. Journal of the Institute of Brewing, 119: 172–181.

Inns E.L., Buggey L.A., Booer C., Nursten H.E., Ames J.M. (2007): Effect of heat treatment on the antioxidant activity, color and free phenolic acid profile of malt. Journal of Agriculture and Food Chemistry, 55: 6539–6546.

Leitao C., Marchioni E., Bergaentzlé M., Zhao M., Didierjean L., Taidi B., Ennahar S. (2011): Effect of processing steps on the polyphenol content and antioxidant activity of beer. Journal of Agricultural and Food Chemistry, 59: 1249–1255.

Leitao C., Marchioni E., Bergaentzlé M., Zhao M., Didierjean L., Miesch L., Holder E., Miesch M., Ennahar S. (2012): Fate of polyphenols and antioxidant activity of barley throughout malting and brewing. Journal of Cereal Science, 55: 318–322.

Lund M.N., Hoff S., Berner T.S., Lametsch R., Andersen M.L. (2012): Effect of pasteurization on the protein composition and oxidative stability of beer during storage. Journal of Agricultural and Food Chemistry, 60: 12362–12370.

Mishra K., Ojha H., Chaudhury N.K. (2012): Estimation of antiradical properties of antioxidants using DPPH assay: a critical review and results. Food Chemistry, 130: 1036–1043.

Piazzon A., Forte M., Nardini M. (2010): Characterization of polyphenol content and antioxidant activity of different beer types. Journal of Agricultural and Food Chemistry, 58: 10677–10683.

Siqueira P.B., Bolini H.M.A., Macedo G.A. (2011): Polyphenols and antioxidant properties in forced and naturally aged Brazilian beer. Journal of Brewing and Distilling, 2: 45–50.

Tafulo P.A.R., Queirós R.B., Delerue-Matos C.M., Sales M.G.F. (2010): Control and comparison of the antioxidant capacity of beers. Food Research International, 43: 1702–1709.

Zhao H., Chen W., Lu J., Zhao M. (2010): Polyphenol profiles and antioxidant activities of commercial beers. Food Chemistry, 119: 1150–1158.

Zhao H., Li H., Sun G., Yang B., Zhao M. (2013): Assessment of endogenous antioxidant compounds and antioxidant activities of lager beers. Journal of the Science of Food and Agriculture, 93: 910–917.

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