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Full Length Research Paper

Polyphenols and antioxidant properties in forced and naturally aged Brazilian beer

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Beer is a beverage obtained through alcoholic fermentation of malt wort, usually made of barley, which could be added of other cereals, such as corn, rice or wheat. Its alcoholic content is between 3 and 8%. It can be considered a good source of polyphenols derived both from malt and hop. Due to its antioxidant capacity and low alcoholic content, its able to improve plasma antioxidant activity, reducing the risk of cardiovascular diseases, without the negative effects of high doses of alcohol. Beer is an unstable product that is involved in a series of chemical, physical and sensorial transformations during its shelf life. This study evaluated the oxidative profile of two different types of Brazilian beer submitted to a forced aging process (6 days at 42°C) and natural aging (4 months at room temperature). The applied tests were: total polyphenol content, hydrogen-donating ability (DPPH), reducing power and copper reducing activity. Results showed no changes in total polyphenol content or antioxidant capacity during forced aging. Beers aged naturally showed a decrease in their polyphenol content and antioxidant capacity.

Key words: Beer, polyphenols, antioxidant capacity, aging, storage.

INTRODUCTION

With the increasing consume and export of beer, shelf-life problems have become a very important issue for most breweries. Beer aging is a complex phenomenon and to understand the reaction mechanisms responsible for those changes in aged beer is of great importance. The biochemical processes which occur during beer storage proceed simultaneously but at different rates. The extent of these reactions depends on the storage conditions and interaction of pathways. Beer contains compounds with antioxidant properties such as reducing sugars, Maillard reaction products, vitamins and phenolic compounds (Oñate-jaen et al., 2006). Polyphenolic compounds are important antioxidants, with mechanisms involving both free radical scavenging and metal chelation. During beer storage, phenolic compounds react with proteins and form high molecular weight species and hazes (Vanderhaegen et al., 2006). Moreover, polyphenols are considered to have a negligible effect on the oxidative stability of beer (Andersen et al., 2000). The brewing industry is concerned with the stability of its final products. During storage, the quality of beer decreases and the formation of haze, browning reaction and production of undesirable flavors occur. The speed of aging depends on the storage conditions and the composition of the beverage (Oñate-jaen et al., 2006).

This study compares the biochemical changes that undergo during forced and natural aging processes of beer, regarding its phenolic content, anti and pro-oxidant properties and reducing power.

MATERIALS AND METHODS

Samples

Two different types of Pilsen Brazilian beer were periodically evaluated during their shelf life, submitted to natural and forced aging. The two types of beer (Samples A and B) differed in the

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raw material (type of hop, malt and yeast strain) used in their process. During forced aging study the beer samples were stored at 42°C for 6 days. At each day the samples were submitted to biochemical tests in order to determine the concentration of total polyphenol and their antioxidant capacity. The same types of beer were also stored at room temperature for 4 months and left to age naturally. The same assays were conducted in order to determine the concentration of total polyphenol and antioxidant capacity and compare them to the results obtained in the forced aging process. Two cans of each type of beer were used for each section of assays, both for forced and natural aging. Each type, individually, was mixed, submitted to 3 min of ultrasound (UltraSonic Cleaner Thornton – Unique) and filtered.

Total phenolic content

The total phenolic content was determined spectrophotometrically using Folin-Ciocalteau reagent (Singleton and Rossi, 1965). An amount of 1 ml of Folin-Ciocalteau reagent (Cromoline) was added to 2 ml of diluted sample and 9 ml of distilled water. After 3 min, 8 ml of Na_2CO_3 solution (7.5%) was added. The absorbance was read after 2 h at 760 nm (espectrophotometer Beckman Coulter DU 640). Total phenolic content was calculated using galic acid as standard.

Free radical scavenging capacity

Free radical scavenging was determined in the presence of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the methodology described by Blois (1958) and Molyneux (2004). DPPH is characterized as a stable free radical by virtue of the delocalization of the spare electron, which gives rise to the deep violet color with absorption at about 520 nm. Diluted samples in different concentrations were added to a methanolic solution (250 ∝mol, 2 ml) of DPPH (Sigma-Aldrich). The total volume was 5 ml. The mixture was shaken and left to stand at room temperature in the dark for 2 h. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. Free radical scavenging was calculated by the discolouration of DPPH expressed as percentage of the control, which contained destilled water instead of the sample. It was expressed as EC50, the amount of sample that is needed for 50% discolouration of DPPH. Lower values of EC50 shows higher activity.

Reducing power

The reducing power was determined according to the methodology applied by Lugasi (2003). Diluted sample (1 ml) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 min and 2.5 ml of trichloracetic acid (10%) was added. A portion of the solution (2.5 ml) was mixed with the same amount of distilled water and 0.5 ml of $FeCl_3$ solution (0.1%). The absorbance was read at 700 nm. Reducing power was expressed as ascorbic acid equivalent (ASE ml^{-1}), which means that 1 ml sample exhibits the same reducing power as the given amount of ascorbic acid (Sigma-Aldrich) expressed in ∝mol.

Copper reducing activity

The antioxidant activity of some phenolic compounds can be determined by their reducing activity over Cu^{2+}. The methodology applied to this study was based on a new approach called CRAI (copper reducing activity index), proposed by Rufián-Henares

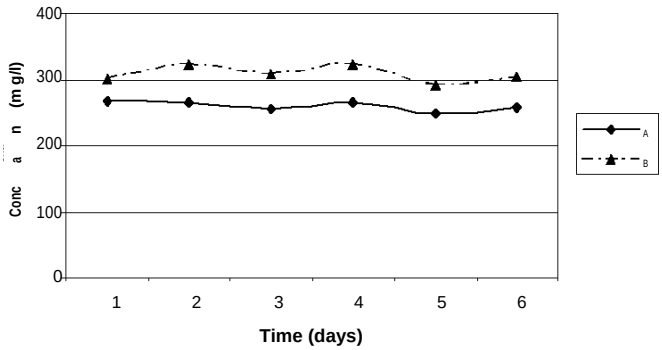

Figure 1. Total phenol content during forced aging.

(2006). This test measures the reduction of Cu^{2+} and formation of Cu^{+} in the presence of antioxidants, 100 µl of a 20 mM $CuSO_4$ aqueous solution, in the presence of 5 ml of diluted sample (1:10). After a reduction time of 10 min, 250 µl of DDTC 1% and 250 µl of a 30% solution of NH_3 were added. The mixture was vortexed for 5 s to allow the formation of the insoluble complex Cu^{2+}-DDTC and finally centrifuged at room temperature for 8 min at 10000 rpm. (Beckman Coulter - Allegra X-22R). Aqueous solution of Trolox® (0.15 to 1.15 mM) was used for calibration. Cu^{+} was quantified as the soluble Cu^{2+}-DDTC complex in the supernadant at 450 nm in a Beckman Coulter DU 640 espectrophotometer. All reagents used in this assay were acquired from Sigma-Aldrich.

Statistical analyses

The biochemical assays were done in duplicate and the results were analyzed through Anova and Tukey test, using the software Statistica (StatSoft, Inc., 2001). $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Total phenolic content

Total phenolic content did not show any significant difference along the forced aging period and it was significantly higher for Sample B (Figure 1). The raw material used to produce this sample might be the main reason for this difference.

Lermusieau et al. (2001) evaluated different hop varieties, finding differences in total phenol content up to 250%. Thus, the use of different hop and barley varieties in beer production may lead to differences in the final phenol concentration, as observed in this study. The samples studied showed total phenol content scores between 250 - 300 mg/l, similar to the values measured by Lugasi (2003) in lager European beer (270 - 470 mg/l) and lower than values found in dark beer (380 - 600 mg/l). The total phenol content found in lager beer is similar to the contents found in white wine (100 - 330 mg/l) and lower to the ones found in red wine (690 - 2500 mg/l). During the natural

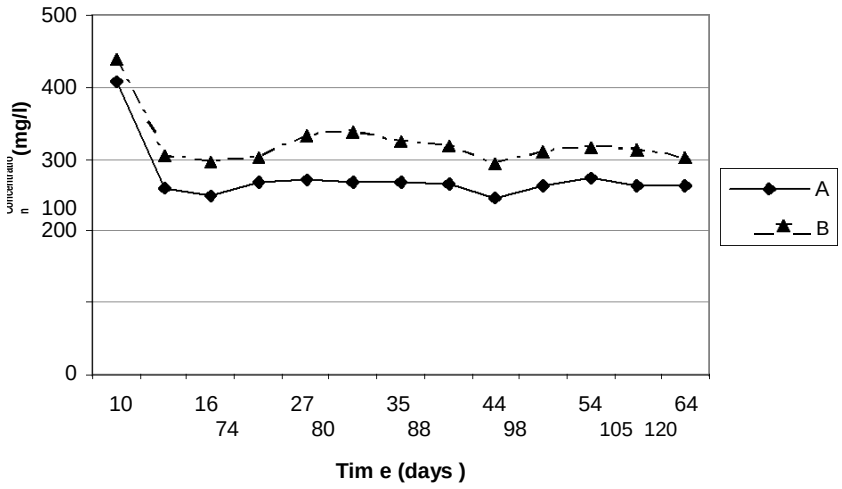

Figure 2. Total phenol content during natural aging.

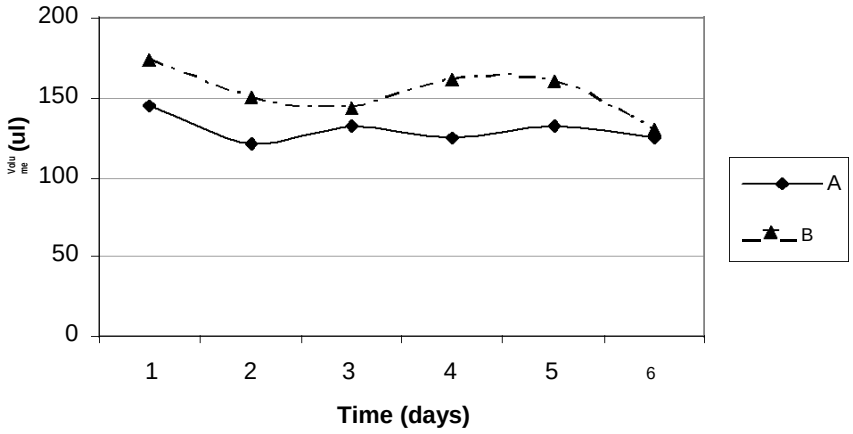

Figure 3. EC50 values during forced aging.

aging the total phenol content decreased after 16 days in both samples, and remained constant after this period until the end of the storage (Figure 2). The sample B showed significantly higher phenol content. Total phenol content ranged from 375 - 439 mg/l at the beginning of the aging period, and decreased significantly after 16 days, remaining constant after this period until the end of the storage. Freitas (2006) determined total phenol content in Brazilian Pilsen beer, reporting values between 250 - 535 mg/l. The total phenol content decreased nearly 35% in the first two weeks of storage, and it can be related to a higher level of phenol oxidation in the beginning of the storage. The structural changes due to oxidation have not been completely elucidated, but it is believed that simple polyphenols polymerize to higher molecular weight species (tannins). These reactions can be induced in the presence of acetaldehyde, formed by yeast or ethanol oxidation (Vanderhaegen et al., 2006).

The Folin-Ciocalteau methodology quantifies the total mass of phenol compounds in a mixture; therefore a polymerization of these compounds would not affect its determination. This fact explains the total phenol content profile observed in this study, which remained constant after two weeks of storage. Although the antioxidant capacity decreases along the shelf life because of phenol compounds polymerization, the total phenol content remains the same. The same phenomenon was observed by Vanderhaegen et al. (2003), who determined total phenol content and antioxidant capacity in beer storage for 180 days at 20 and 40°C.

Free radical scavenging capacity

The free radical scavenging capacity, measured through the reduction of the free radical DPPH did not show any significant differences along the forced aging period (Figure 3). However, during the natural aging, the EC50 values increased significantly in the last days of storage as shown in Figure 4, indicating that a greater volume of

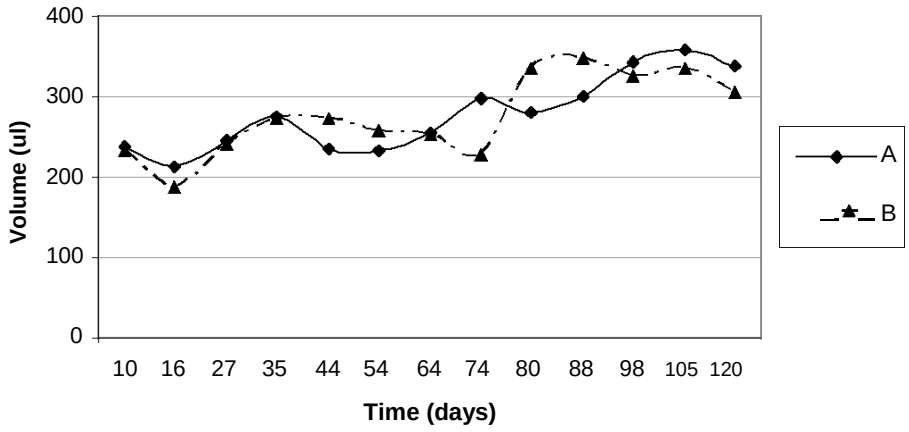

Figure 4. EC50 values during natural aging.

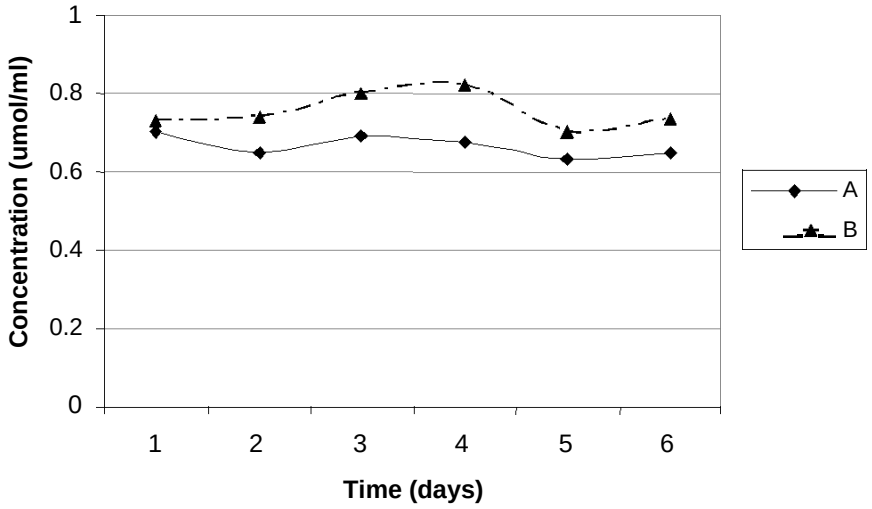

Figure 5. Reducing power during forced aging.

sample is needed in order to obtain a 50% reduction of the free radical. It means that in the end of the storage the samples analyzed showed weaker capacity to scavenge free radicals. The samples did not show significant differences from each other, both for natural and forced aging.

Reducing power

The reducing power is related to the secondary anti-oxidant activity. Secondary or preventive antioxidants can reduce the rate of chain initiation in the lipid peroxidation process or react with the products of peroxidation leading to non-radical stable products (Lugasi, 2003). Results for forced and natural aging are presented in Figures 5 and 6, respectively. During natural aging there is a decrease after 30 days of storage and a new tendency of decrease in the end of the storage, after 100 days. The samples did not present any change along the forced aging period. The Sample B presented a significantly higher reducing power during all storage period.

Copper reducing activity

Both samples showed a significant decrease between the first and last day of forced aging, as indicated in Figure 7. It was observed that there were no significant differences between the samples. The reduction of Cu^{2+} to Cu^{+} in the presence of phenolic compounds represents the pro-oxidant capacity of these compounds, due to the higher oxidant properties of Cu^{+} compared to Cu^{2+}. When copper is in contact with a reducing substance, it is reduced until Cu^{+} in an extent close to the reducing power of the analyzed antioxidant. Thus, the increase on Cu^{+} content is directly proportional to the increase of phenolic content (Rufián-Henares, 2006). The copper ion is

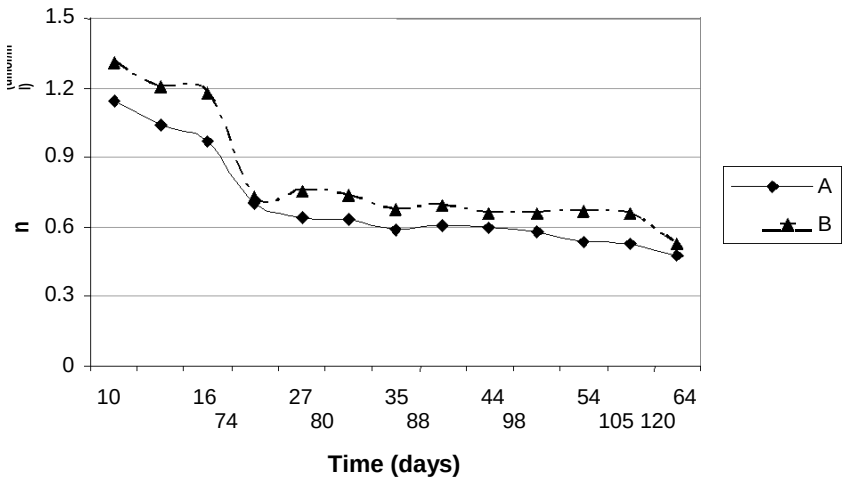

Figure 6. Reducing power during natural aging.

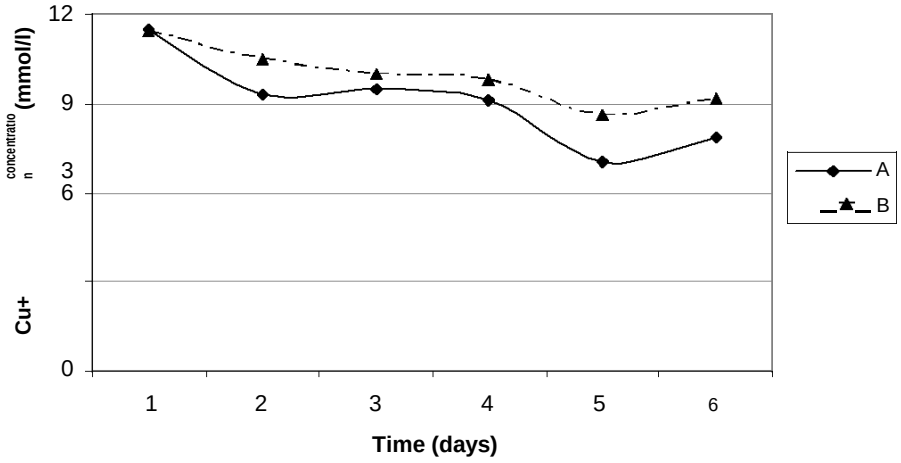

Figure 7. Copper reducing activity during forced aging.

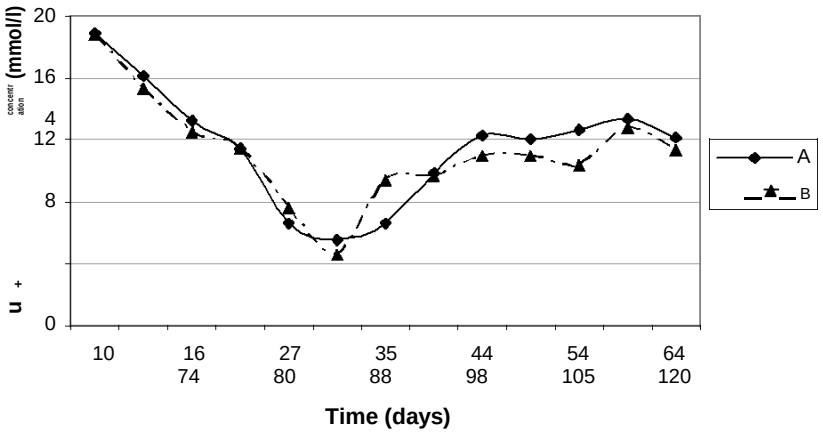

Figure 8. Copper reducing activity during natural aging.

naturally found in food as Cu^{2+}, which can be reduced to Cu^{+} in the presence of antioxidants. During natural aging, a decrease in the reduction of Cu^{2+} was observed until the 50th day of storage (Figure 8). After this period there

was an increase in copper reducing power, as observed by the increase of the reducing rate of Cu^{2+}. This change in the curve pattern may have happened due to the formation of new reducing compounds, such as products from Maillard reaction (Vanderhaegen et al., 2006) and possible changes in the phenolic composition, since it has been reported in literature that monomeric phenolic compounds have lower reducing status than more complex phenolic molecules (Rufián-Henares, 2006).

Conclusions

Beer is a complex mixture of bioactive substances, in which molecules are subjected to many reactions during storage. Different types of beer were differentiated by the total phenolic content and antioxidant activity. Thus, the raw material can indeed influence some characteristics of beer regarding its biochemical pattern. The parameters used in this study to perform the forced aging process are related by the brewing industry to cause undesirable sensorial changes in beer, but as observed it can not substitute the natural aging process in order to estimate the biochemical changes occurring in aged beers.

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