

Characterization of Craft Beers and their Bioactive Compounds

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The increasing number of microbreweries in recent years is a success by the variety of craft beers available in the pubs and markets. The beer is a beverage rich in phenolic compounds and antioxidants; however, little information is available about physicochemical quality and bioactive compounds of craft beers. Four styles of craft beers were produced in a microbrewery: American Classic Pilsner, American Pale Ale, Brown Poter and Irish Red Ale. The physicochemical analysis showed the established parameters for beers. In relation to the bioactive compounds, the total phenolic compounds and the great content of caffeic acid should be highlighted. This work traces a profile bioactive compounds and physicochemical analysis of the craft beers produced on small scale basis, a quality product without additives to a differentiated target consumer.

1. Introduction

Beer is a very popular alcoholic beverage (Russo et al., 2013). Craft beers are a differential product in relation to commercial beers; craft beers take as raw material only malt and hops, they are not filtered and do not use additives. Over the past 21 years, there was an increasing number of microbreweries in the United States, jumping from 192 microbreweries in 1994 to 1.871 in 2014 (Brewers Association, 2016).

Phenolic compounds in the beer are important antioxidants, presenting mechanisms involving the elimination of free radicals; however, during beer storage, phenolic compounds react with proteins causing turbidity (Siqueira et al., 2011). The large scale brewing makes use of the clarification process using polyvinylpyrrolidone (PVPP), which consists of removing phenolic compounds (Aron et al, 2010). After the clarification, the beer has to be stabilized with exogenous antioxidants to enhance the flavor; although, the consumers are looking for products with reduced use of additives (Zhao et al., 2010).

The oxidative stress is involved in the pathology of many diseases, such as atherosclerosis, diabetes and cancer; however, a diet rich in antioxidants may protect against oxidative stress. Among the antioxidants present in food, phenolic compounds are the most abundant (Szwajgier, 2009; Nardini et al., 2006). Phenolic compounds present in the beer have high bioavailability, reaching a maximum concentration in the blood plasma after 30 minutes of ingestion (Nardini et al., 2006).

There are several scientific papers quantifying the total phenolic compounds and antioxidant activity in commercial beers (Zhao et al., 2010; Piazzon et al., 2010; Zhao et al., 2013; Steiner et al., 2012) and there are few articles detailing physicochemical characteristics, phenolic acids and antioxidant activity of craft beers. Researches on craft beers are necessary considering that small breweries are expanding worldwide (Ceppi et al., 2010).

Therefore, the aim of this study was to evaluate the physicochemical characteristics and quantify phenolic compounds, phenolic acids and antioxidant activity of craft beers such as American Pale Ale, Brown Poter, American Classic Pilsner, and Irish Red Ale.

2. Material and Methods

2.1 Raw materials

Malts were purchased from Castle Malting®, hops in pellets T-90 were acquired from RW Emmel Company LTDA. The dry yeast Fermentis US-05 was used to produce American Pale Ale, Brown Poter and Irish Red Ale and the dry yeast Fermentis W-34/70, the American Classic Pilsner. The amounts of each raw material are shown in Table 1.

Table 1: Formulation of the beers

| American Pale Ale | | Brown Poter | | Classic American Pilsner | | Irish Red Ale | |
|------------------------|-------|-------------------------------|-------|--------------------------|-------|-------------------------------|-------|
| Raw material* | Kg | Raw material* | kg | Raw material* | kg | Raw material* | kg |
| Château Pale Ale | 2.9 | Château Pale Ale | 2.75 | Château Pilsen | 3.35 | Château Pilsen | 2.00 |
| Chateau Pilsen | 1.3 | Chateau Pilsen | 1.0 | Château Pale Ale | 1.25 | Château Pale Ale | 1.75 |
| Chateau Munich | 0.5 | Chateau Munich | 0.40 | Château Munich | 0.25 | Chateau Melano | 1.25 |
| Chateau Cara Ruby | 0.15 | Chateau Melano | 0.35 | Chateau Melano | 0.15 | Northern Brewer [Boil 55 min] | 0.013 |
| Chateau Biscuit | 0.15 | Chateau Cara Gold | 0.30 | Warrior [Boil 55 min] | 0.012 | Fuggle [Boil 10 min] | 0.004 |
| Columbus [Boil 55 min] | 0.01 | Chateau Chocolat | 0.20 | Sladek [Boil 10 min] | 0.010 | US-05 | 0.011 |
| Chinook [Boil 10 min] | 0.012 | Northern Brewer [Boil 55 min] | 0.012 | Premiant [Boil 10 min] | 0.010 | | |
| Columbus [Boil 10 min] | 0.007 | Fuggle [Boil 10 min] | 0.015 | W - 34/70 | 0.022 | | |
| US-05 | 0.011 | US-05 | 0.011 | | | | |

* 18 liters of bottled beer.

2.2 Beer production

The amounts of each raw material are shown in Table 1. The manufacturing process was carried out following these steps (Ceppi et al., 2010; Linko et al., 1998) malts were crushed on dry basis by two-roll mill and then introduced into the mashing tank with temperature controller and stirring system in the presence of water (18 liters). Mashing was performed by the infusion process at temperatures of 53 °C, 62 °C, 69 °C, 72 °C and 78 °C for 20, 40, 20, 20, 10 minutes, respectively.

The wort filtration was accomplished using a tank with a false bottom groove. The primary wort was separated from the malt residue by conventional filtration under atmospheric pressure, and the residue itself used as a filter. After filtration of the primary wort, the residue cake was washed with 10 kg of water (78 °C) to extract the residual sugar and obtain the secondary wort. The primary and secondary worts were blended in order to initiate the boiling process (100 °C) at atmospheric pressure for 60 minutes. After 5 minutes of boiling, bitterness hops were added and the second hops addition was made after 50 minutes. With boiling completed, the whirlpool operation was performed for 5 minutes with 30 minutes of rest for trub separation by decantation. The clarified wort was cooled to 15 °C and transferred to the fermenter where the brewing yeast was added; then, the process of fermentation began for 7 days at 8 °C and for 3 days at 13 °C for American Classic Pilsner and 7 days at 18 °C for the other beers. At the end of fermentation, the temperature of the fermenter was reduced to 0 °C for two days in order to settle the yeast and so the beer was transferred to the maturation tank.

The beers were aged for 21 days at 0 °C temperature. After that, 6 g/L of inverted sugar was added and the beer was bottled. Sanitized amber bottles of 500 ml were used. The re-fermentation processing was performed for 7 days at 18 °C to carbonate the beer, and then stored at 0 °C.

2.3 Physicochemical analysis

The beers were analyzed to determine specific gravity, original extract, apparent extract real extract, apparent attenuation, real attenuation, alcohol, energy value of beer by calculation, color, bitterness, vicinal diketones, dissolved CO₂, haze in beer, pH and Nibem according to the methods described by Analytica-EBC, (2005).

2.4 Total phenolic compounds

Total phenolic compounds were determined according to the spectrophotometric method Folin-Ciocalteu, (Singleton and Rossi, 1965) with modifications (Zhao et al, 2010).

The method consists of using 0.5 ml of diluted beer with the addition of 2.5 ml of Folin-Ciocalteu diluted 10 times, waiting for 5 minutes of reaction, then add 2 mL of Na₂CO₃ 7.5 % and supplement with deionized water till reaching 10 ml. After 1 hour of resting at room temperature, the reading was held at 760 nm. The measurement was then compared to a standard curve of gallic acid (GAE) and the result was expressed in mg of GAE per liter of beer (mg GAE.L⁻¹).

2.5 Antioxidant activity by the sequestration method of radicals – DPPH

The DPPH content was determined according to the method described by HE et al. (2012), the diluted beer sample (0.1 mL) was added to 3.9 mL of 0.05 mmol/L DPPH solution dissolved in ethanol solution. The solution was incubated in the 37°C water bath and reacted in the dark for 60 min, and then the absorbance was measured at 517 nm. The blank group was distilled water. Equation 1 shows the calculation for reading the content of DPPH.

$$\text{Inhibition (\%)} = (A_b - A_s)/A_b \times 100 \text{ (eq. 1)}$$

Where:

A_b = absorbance control and A_s = absorbance sample.

2.6 Determination of individual phenolic compounds

2.6.1 Samples Preparation

The samples were prepared following the method of Zhao et al., (2010).

For each beer style three bottles were used, homogenized and degassed for 30 minutes. A sample of 50 mL was removed with the addition of 20 g NaCl and 50 mL of ethyl acetate; then the solution was vigorously mixed and centrifuged at 10.000 G for 10 minutes, the supernatant collected and the operation was repeated more two times combining the collected aliquots.

The rotary evaporation was carried out using a combination of the three washes in reduced pressure at 35 °C until complete dryness and the residue was reconstituted with 2 mL of methanol HPLC grade and filtered through 0.45 micron PTFE membrane.

2.6.2 Chromatography conditions

The chromatographic separations was carried out using Chromatographic Workstation (Termo®), equipped with a Chromquest management program containing: reciprocating piston pump with four-way model 240; Rheodyne injection valve model 8096 with a sampling loop of 10 µL and a diode array detector (DAD); chromatographic column C18 (microsorb 150 x 4.6 mm with 5 µm particles). For chromatographic separation, mobile phase A (0.1 % acetic acid in water) and B (0.1 % acetic acid in methanol), in a gradient system with flow of 0.8 mL/min and the injected sample volume was 10 µL. The established gradient program was used: 0 min, 5 % B; 15 min, 20 % B; 35 min, 40 % B; 42 min, 65 % B; 50 min, 80 % B, 52 min, 5 % B; 60 min, 5 % B (Zhao et al., 2010).

Calculation for each phenolic acid concentration was carried out by integrating the areas read at 280 and 240 nm attained using the standard calibration curve of each compound. The result is expressed in mg per liter of beer (mg.L⁻¹).

2.7 Statistical Analysis

The obtained results for bioactive compounds (Table 3) were assessed through analysis of variance (ANOVA), and the averages submitted to Tukey test at 5 % probability using statistical software Assisat.

3. Results and Discussion

3.1 Physicochemical analysis

In each beer, the worting time was standardized to 1 hour and 50 minutes. The results of the physicochemical characteristics of the beers are presented in Table 2.

The beers were different in relation to the original mash, ranging from 12.01 to 13.9 °P; this fact is due to roasting malt level used in Brown Poter and Irish Red Ale beers. This process provides darkness to malt, reduces enzymatic activity and gives worts with less sugar.

The apparent attenuation ranged from 75.77 to 79.51 %, showing that the yeast used was appropriate. The aim to produce different styles of beers was achieved as the alcohol, color and bitterness parameters are according to BJCP, (2015). The craft beer turbidity varied from 18.35 EBC to 25.77 EBC. This range is larger than commercial beers (Steiner et al., 2012) but similar to the ones found in wheat beers (He et al, 2012) thus unfiltered beers characteristics.

Vicinal diketones in beers are responsible for the butter aroma of beer (Liguori et al., 2015) other studying authors analyzed vicinal diketone of 11 American beers and found values of 20 - 100 ppb (Krogerus et al., 2013). The vicinal diketones of this work ranged from 153 to 258 ppb. The higher values can be explained due the re-fermentation process when beer was exposed to the oxygen left inside the bottle (Krogerus et al, 2013). The foam stability is an important characteristic of beers (Depraetere et al., 2004) the results for Nibem and pH are consistent with several studies in the literature (Ceppi et al., 2010; Depraetere et al., 2004; Klose et al., 2011).

Table 2: Physicochemical analysis of craft beers

| Analysis | Unit | American Pale Ale | Brown Poter | Classic American Pilsner | Irish Red Ale |
|----------------------|---------------------|-------------------|----------------|--------------------------|-----------------|
| Specific gravity | g / cm ³ | 1.0098 ± 0.0002 | 1.0101 ± 0.002 | 1.0096 ± 0.0001 | 1.0084 ± 0.0002 |
| Original Extract | ° P | 13.90 ± 0.06 | 12.65 ± 0.05 | 13.60 ± 0.05 | 12.01 ± 0.07 |
| Apparent Extract | ° P | 2.99 ± 0.01 | 3.06 ± 0.02 | 2.92 ± 0.02 | 2.61 ± 0.02 |
| Real Extract | ° P | 5.09 ± 0.02 | 4.91 ± 0.01 | 4.97 ± 0.03 | 4.42 ± 0.02 |
| Apparent attenuation | % | 78.51 ± 0.02 | 75.77 ± 0.03 | 78.51 ± 0.02 | 78.27 ± 0.02 |
| Real attenuation | % | 65.13 ± 0.02 | 62.76 ± 0.03 | 65.08 ± 0.02 | 64.67 ± 0.02 |
| Alcohol by volume | % v/v | 5.88 ± 0.04 | 5.13 ± 0.03 | 5.74 ± 0.03 | 5.00 ± 0.04 |
| Alcohol by weight | %w/w | 4.60 ± 0.02 | 4.01 ± 0.03 | 4.49 ± 0.02 | 3.91 ± 0.02 |
| Calories | Kcal / 100 ml | 50.07 ± 0.6 | 45.68 ± 0.5 | 49.29 ± 0.4 | 43.22 ± 0.6 |
| Color | EBC | 34.20 ± 0.07 | 78.4 ± 0.04 | 19.3 ± 0.05 | 37.7 ± 0.06 |
| Bitterness | BU | 40.90 ± 0.06 | 26.1 ± 0.07 | 32.7 ± 0.06 | 22.0 ± 0.05 |
| Vicinal diketones | ppb | 197 ± 0.08 | 258 ± 0.07 | 153 ± 0.09 | 195 ± 0.08 |
| Carbon dioxide | g / litre | 0.42 ± 0.1 | 0.47 ± 0.1 | 0.74 ± 0.1 | 0.50 ± 0.09 |
| Haze in beer | EBC | 18.35 ± 0.1 | 25.77 ± 0.09 | 19.04 ± 0.08 | 20.51 ± 0.1 |
| pH | - | 4.24 ± 0.03 | 4.15 ± 0.05 | 4.53 ± 0.03 | 4.11 ± 0.04 |
| Nibem | s | 300 ± 5.8 | 217 ± 6.1 | 181 ± 6.1 | 195 ± 5.6 |

3.2 Analysis of phenolic compounds, DPPH and phenolic acids

Phenolic compounds are derived from the beer malt and hops, which are considered a very important source of antioxidants, and they play critical roles in the sensory properties, color and colloidal stability of beer flavor (Aron et al., 2010; Vanderhaegen et al., 2006).

The phenolic compounds of the four beers were examined by the Folin-Ciocalteu assay and the results are shown in Table 3. The samples studied presented considerable levels of phenolics and the values ranged from 448.57 to 531.30 mg GAE.L⁻¹.

The total values of phenolic compounds in this study were superior to those found by Zhao et al., (2010). They found values from 152.01 to 339.12 mg GAE.L⁻¹, and smaller than those found by Piazzon et al., (2010) 875

mg GAE.L⁻¹. This deviation can be explained by beers with high original mash and with dark color, which tends to increase the value of phenolic compounds (Piazzon et al., 2010).

The radical scavenging activity (DPPH) of the four samples are shown in Table 3. The radical scavenging activity of the hydrogen free radicals in particular to hydroperoxide radicals is responsible for lipid oxidation (Zhao et al., 2013). The beers were able to inhibit from 29.4 to 48.5 % of free radicals. These values are in accordance to those reported by Granato et al., (2011), who found values from 4.75 to 59.98 % for Brazilian commercial beers. The beer Brown Poter showed the greatest inhibition ability, as according to Zhao et al., (2010), beers with high caffeic acid content are those with the highest radical scavenging activity, preventing lipid oxidation (Aron et al., 2010).

The identification of phenolic acids (gallic acid, caffeic acid, ferulic acid and p-coumaric acid) were chosen because they were those presenting higher concentrations in several studies on beers (Zhao et al., 2010; Szwajgier, 2009; Piazzon et al., 2010; Zhang et al., 2013).

The results of concentrations of phenolic acids are shown in Table 3. The caffeic acid showed the highest concentration (9.05 mg.L⁻¹) in the beers studied. A study analyzing 34 commercial beers (Zhao et al., 2010) obtained a lower concentration for caffeic acid from 0.08 to 1.22 mg.L⁻¹. The gallic acid ranged from 0.33 to 1.71 mg.L⁻¹, concentrations lower to those obtained by Zhao et al., (2010) from 1.81 to 10.39 mg.L⁻¹.

The values for p-coumaric acid and ferulic acid are in accordance to those of the literature (Szwajgier, 2009). The phenolic acids quantities are different from others works and can be explained by the brewing steps, in particular filtering and clarifying, which affect the composition (Gorjanovic et al., 2010). The raw materials used to manufacture beer also influence the final product such as phenolic acids profile. The malt and hops vary greatly in polyphenols content due to cultivation region, crop handling and processing (Piazzon et al., 2010; Vanderhaegen et al., 2006).

The biological effects and the antioxidant activity of caffeic, ferulic and p-coumaric acids have been the subject of several studies in recent years (Piazzon et al., 2010; Ghiselli et al., 2000). Beer is a beverage with low alcohol content and high concentrations of bioactive compounds. Studies have shown that moderate beer consumption is associated with healthier cardiovascular system in humans who consume 1 to 3 doses per day, that is, 30-40 % reduction of coronary disease when compared to people who does not drink (Piazzon et al., 2010).

Table 3. Concentration of phenolic acids (mg.L⁻¹), total phenolic compounds (mg.L⁻¹) and inhibition (%) of free radicals in four craft beers

| | Samples | | | |
|-----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | Americam Pale Ale | Brown Poter | Classic American Pilsner | Irish Red Ale |
| gallic acid | 1.23 ± 0.03 ^b | 1,71 ± 0,03 ^a | 0,33 ± 0,02 ^d | 0,78 ± 0,05 ^c |
| caffeic acid | 8.49 ± 0.08 ^b | 9.05 ± 0.06 ^a | 3,95 ± 0,10 ^c | 8,22 ± 0,11 ^b |
| ferulic acid | 4.02 ± 0.03 ^a | 2.77 ± 0.04 ^c | 3.07 ± 0.07 ^b | 2,12 ± 0,03 ^d |
| p-coumaric acid | 0.12 ± 0.03 ^b | 0.19 ± 0.05 ^b | 0,39 ± 0,03 ^a | 0.18 ± 0.03 ^b |
| Total phenolic compounds | 520.15 ± 0.32 ^a | 531.30 ± 0.29 ^a | 448.57 ± 0.37 ^b | 475.05 ± 0.25 ^b |
| inhibition of free radicals | 46.7 ± 0.13 ^a | 48.5 ± 0.19 ^b | 29.4 ± 0.21 ^c | 35.7 ± 0.17 ^b |

Averages followed by a single letter in line do not differ statistically. Tukey test was applied at significance level of 5% probability.

4. Conclusion

This study showed the possibility of different manufacturing styles of craft beers in microbreweries that meet the physical and chemical quality standards. The Brown Poter beer presented higher concentrations of bioactive compounds followed by American Pale Ale, Irish Red Ale and American Pilsner. Thus, this work validates the great growth of breweries producing quality beers that reaches consumers in search for a differentiated product.

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