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Polysaccharides influence on the interaction between tannic acid and haze active proteins in beer

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ABSTRACT

Colloidal instability in beer is mainly caused by interactions between proteins and polyphenols. These two combine producing a visible haze that reduces the physical shelf life of beer. The haze active proteins (HAPs) react with tannic acid (TA); therefore, this reaction provides a way to determine HAP concentrations in beer. Beers also contain a number of constituents that may influence the protein–polyphenol haze formation. We used a response surface methodology to predict the influence of total polysaccharides (TPS) and proteins on beer haze. Experiments were carried out using the Central Composite Design (CCD) methodology. Samples of beer were prepared with variable concentrations of TPS and proteins. TPS concentrations ranged between 1.34 and 2.23 g L^{-1} and proteins concentrations between 0.11 and 0.18 g L^{-1}. Results show that the increase in turbidity in response to TPS addition was similar to that in response to protein addition. Our regression analyses indicate a significant dependency and correlation between the observed values and the predicted response values (R^2 = 97.87% and R^2[adj] = 95.75%). Furthermore, these values indicate that our experimental model can explain 95.75% of the total variation. Therefore, using TA as an indicator of the interaction of TPS with proteins, as commonly done, can lead to considerable errors, since the polysaccharides also react with TA, and this reaction actually causes a considerable increase in turbidity.

1. Introduction

Beer after fermentation shows significant turbidity due to the presence of yeast and the cellular tissue comminuted during the malt processing (Benítez, Martinez Amezaga, Sosa, Peruchena, & Lozano, 2013; Fleet & Siebert, 2005; Siebert, 2006; Van der Sman, Vollebregta, Mepschen, & Noordman, 2012). To produce a clear beverage with good visual appearance the colloidal particles must be removed (Fleet & Siebert, 2005; Siebert, 2006; Steiner, Becker, & Gastl, 2010). Beer is typically filtered with diatomaceous earth (Atkinson, 2005; Bamforth, 2009). Subsequently, the stabilization stage continues to prevent the formation of postpackaging haze (Bamforth, 2009; Briggs, Boulton, Brookes, & Stevens, 2004).

The most common cause of postpackaging haze is due to the interaction between proteins and polyphenols, eventually producing variably-sized colloidal particles (Siebert, 2009; Steiner et al., 2010).

Two fundamental types of proteins were identified in beer: those that cause foam, which must be retained, and those responsible for haze formation, which should be eliminated. A number of approaches have been used to determine the amount of HA-proteins in samples. The most successful approach consists of adding a fixed amount of TA to a sample (Bamforth, 2009; Siebert, 2006, 2009). After incubating the sample, any measured increase in turbidity is expected to be proportional to the amount of HA-proteins in the sample. The advantage of this approach is that only substances that are able to form haze with polyphenols will respond (Siebert, 2006, 2009).

Beers also contain a number of constituents, such as alcohol and the hydrogen ion (pH), which can influence the protein–polyphenol haze formation. In this paper, two other factors are studied: ionic strength and polysaccharides concentration.

The ionic strength can modify the turbidity response of the interaction of proteins with TA. Benitez, Genovese, and Lozano (2007a) studied the effect of KCl on the colloidal particles of apple juice. This salt modified the electric potential of the colloidal particles but not its stability. Therefore, as the behavior of the interaction of TA with apple juice is similar to that of TA with beer, KCl was used in this study. Finally, the most inorganic salts usually found in beer are potassium (200–

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450 mg L^{-1}), as cations, and chloride (120–500 mg L^{-1}) and phosphate (170–600 mg L^{-1}), as anions (Buiatti, 2009).

Gelatin has the ability to bind to TA in the same way as do beer proteins, because of their similar composition in proline and degree of denaturation. Oh, Hoff, Armstrong, and Haff (1980) showed that ionic strength influenced the hydrophobic bonding between TA and either gelatin or poly-proline. Different interactions of gelatin and beer proteins, with TA could indicate differences in the composition of the liquid medium surrounding the proteins and, thus, differences in the influence of the ionic strength.

Some researchers argue that haze can be stabilized by reducing either only proteins or only polyphenols, because according to them, polysaccharides do not participate in the mechanism that forms haze, but are instead simply incorporated as haze particles (McMurrough, Madigan, Kelly, & O'Rourke, 1999; Siebert, 1999, 2006, 2009).

Proteins react with TA, making TA useful to determine protein concentrations in beer. To predict the influence of total polysaccharides (TPS) and proteins on beer haze we used a response surface methodology. In addition, we investigated the effect of ionic strength on the nephelometric turbidity response (τ) during haze formation. Finally, we propose an experimental model to predict the influence of TPS and proteins on TA turbidity (τ_{TA}).

2. Materials and methods

2.1. Beer preparation

Mashing was carried out in a 40 L stainless steel container. The procedure started by mixing 7.5 kg of barley malt from Argentina (Cargill Malt Division) with deionized water at 62 °C for 90 min. Subsequently, the wort was boiled for 1 h with the addition of hops for bitterness and flavor. This bitter wort was left to settle for 30 min before it was cooled to 12 °C. The wort was pitched with Lager yeast (Saflager S-23, Fermentis, France). The fermentation was carried out at 12 °C for a period of 15 days, followed by a maturation period of 7 days and a cold rest at 3 °C for another 2 days. Finally, each prepared samples was filtrated using a Buchner funnel (Ø = 50 mm) with a filter bed consisting of a pre-coat of 1 g diatomaceous earth (Standard Super-Cel, mean porosity = 3.5 μm, permeability = 2.8×10^{-13} m^2, Refil, Argentina) over a filter paper Whatman No. 3 under vacuum (−50 kPa). All samples were prepared in triplicate (Benítez et al., 2013).

2.2. Colloidal particles determination

Turbidity depends on the concentration, size, and relative refractive index of its particles in suspension (McClements, 2005). The average diameter ($\overline{D}$) and the nephelometric turbidity (τ) are easy to measure and are widely used as parameters to describe particle size and turbidity of commercial beverages. The expression of Eq. (1) was used to describe the turbidimetric behavior of colloidal particles in apple juice (Benitez, Genovese, & Lozano, 2007b; Benitez, Lozano, & Genovese, 2010) and beers (Benítez et al., 2013) and derives from the following equation proposed by Dobbins and Jizmagian (1966):

$$\tau = \frac{3}{2}\frac{C}{\rho_m}\frac{Q_{av}}{\overline{D}} \quad (1)$$

where ρ_m is the density of the continuous phase, C is the particle concentration, and Q_{av} is the nephelometric average scattering efficiency of the polydispersed and irregular particles.

As predicted by Eq. (1), beer turbidity before filtration was found to be directly proportional to particle concentration. Consequently, experimental data were fitted with straight lines through the origin:

$$\tau = \tau_e C \quad (2)$$

where τ_e is the specific turbidity (Dickinson, 1994).

In the present work, Eq. (2) is used to predict the concentration of the initial colloidal particles of beer (C_0).

2.3. Ionic strength modification

The influence of the ionic strength on the interaction of proteins and TA was evaluated with two assays. The first assay consisted of mixing the beer samples with increasing additions of KCl (0.02, 0.04, 0.06, 0.08 and 0.1 M) and a subsequent addition of a solution with a constant concentration of TA (Sigma-Aldrich, Germany). Turbidity was measured after 30 min. The second assay consisted of using a gelatin (Sigma-Aldrich, Germany) solution with the same concentration as proteins in the beer samples. The alcohol level was fixed with ethanol 96% (Biopack, Argentina) and the pH = 4 was fixed with a buffer solution (Biopack, Argentina). The gelatin solution was added with increasing concentration of KCl (0.02, 0.04, 0.06, 0.08 and 0.1 M) and a subsequent addition of a solution with a constant concentration of TA. Turbidity was measured after 30 min. Due to the difference in ionic strength between the beer sample and gelatin, the electrical conductivity of the beer sample was measured and correlated with the ionic strength of a KCl solution (Benitez et al., 2007a). This correlation provides an estimate for the ionic strength of the beer sample.

2.4. Polysaccharides interference

2.4.1. Preparation of a TPS concentrated solution

HA-proteins from beer were extracted with bentonite (0.5 wt.%/volume commercial sodium bentonite type I; La Elcha; Mendoza, Argentina) (Benítez & Lozano, 2007) and polyvinylpolypyrrolidone (15 g L^{-1}, Polyclar 10, International Specialty Products, Argentina) (Mitchell, Hong, May, Wright, & Bamforth, 2005). Bentonite is used for protein removal (Sadosky et al., 2002; Siebert, 2009). The negative reaction resulting from the Bradford method (Bradford, 1976) for proteins and the Folin–Ciocalteu method for total polyphenols (Singleton, Orthofer, & Lamuela-Raventos, 1999) was used to verify whether haze active precursors had been removed. TPS were extracted by ethanol (80%) precipitation and drying at 40 °C, as described by Segarra, Lao, López-Tamames, and De la Torre-Boronat (1995).

2.4.2. Assay to determine the interaction of TPS on TA turbidity (τ_{TA})

It is well known that TA selectively combines with HA-proteins, but it is unknown whether it interacts with TPS. To explore this interaction TPS solution with increasing concentrations, range from 0.2 ± 0.05 to 3.2 ± 0.4 g L^{-1}, were added to five samples of beer and water, respectively. The solutions were left to settle for 30 min, and turbidity was measured. Subsequently, a TA solution (55 mg L^{-1}) was added to the samples. The solution was left to settle for another 30 min, and turbidity was measured again.

2.4.3. Assay to determine the interaction of TPS on gelatin turbidity (τ_G)

It is well known that gelatin selectively reacts with polyphenols forming haze (Benítez & Lozano, 2007; Siebert, 2006, 2009), but gelatin–polysaccharides interactions are poorly understood. Therefore, TPS solutions with increasing concentrations, range from 0.2 ± 0.05 to 3.2 ± 0.4 g L^{-1}, were added to water and beer samples, respectively. Turbidity was measured after 30 min, and then a gelatin (Sigma-Aldrich, Germany) solution (55 mg L^{-1}) was added (Van Buren, 1989). Solutions were left to settle for 30 min, and turbidity was measured again.

2.4.4. Assay to determine the interaction of TPS and HA-proteins on τ_{TA} and τ_G

The response surface methodology (RSM) was used to study the simultaneous influence of TPS and HA-proteins on τ_{TA}, as it allows us to find the optimal variation and indentify the influence of both factors. The 3D response surface was used to determine the individual and cumulative effect of the factors and the mutual interaction between the factors and the dependent variable.