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Fundamental study on the impact of silica gel and tannic acid on hordein levels in beer

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ABSTRACT

Demand for gluten-free foods has been increasing and although gluten-free beers are available, the range of styles is limited. In this study beer made from barley malt was treated with either silica gel or tannic acid and compared to unstabilised beer.

Hordein levels in the beers were analysed using Western blot and *competitive* ELISA. Beer quality parameters such as foam, colour and various flavours were also determined. There was no significant impact on beer quality when using silica gel to stabilise the beer and hordein levels were significantly reduced, the highest dose reducing the beer below 4 ppm.

Stabilisation with tannic acid reduced the hordein content significantly, the lowest dose reduced hordein to below 21 ppm without significant impact on beer quality. Although beer stabilised with the highest dose of tannic acid had a large reduction in hordein content (<6 ppm), the quality of the beer was seriously affected.

Industrial relevance: Existing food stabilisation methods were used to significantly reduce hordein levels in beer. This is an alternative approach for reducing hordein, combining existing technologies making it easy to implement and use in the brewing environment. These stabilisers are readily available from suppliers well known to the food and brewing industry.

The global gluten-free market is increasing and these treatments are all widely accepted and commonly used as food processing aids. This hordein reduction strategy could add value to a product at minimal cost increase, and also increase the types of beer available to the gluten-free consumer.

1. Introduction

In recent years gluten-free foods have seen an enormous surge in popularity, with 60% growth worldwide between the years 2008 and 2013. In 2013 alone, sales of gluten-free food accounted for over $US 2 billion worldwide (source: Euromonitor Passport accessed 20-8-2014). These gluten sensitive consumers range from those suffering with medically diagnosed gluten sensitive conditions, to self-diagnosed individuals and those who believe the gluten-free diet is healthier.

Patients who are obliged to follow a gluten-free diet can suffer from a number of diagnosable conditions. Celiac disease (CD) affects up to 1% of the population worldwide (Tack et al., in review) and the only effective treatment is strict adherence to a gluten-free diet (Van De Kamer & Weijers, 1955). The disease is caused by an inappropriate immune response to ingested gluten proteins (Sollid & Jabri, 2013). This immune response results in damage to the intestine and can ultimately stop adsorption of essential nutrients, causing malnutrition and even cancer in untreated patients (Meresse, Ripoche, Heyman, & Cerf-Bensussan, 2009).

Recently there has been a lot of research done on the pathogenesis and epidemiology of non-celiac gluten sensitivity (NCGS). This term is used for patients who do not suffer from the villus atrophy of CD or the abnormal levels of IgE antibodies associated with wheat allergy, but do have symptoms which are reduced when they adhere to a gluten-free diet (Sapone et al., 2012). The frequency of NCGS is still unclear due to varying definitions for the disease and possible cross-overs with other diseases like irritable bowel syndrome (IBS) (Biesiekierski et al., 2011) but frequencies of NCGS of up to 6% are being reported (Catassi et al., 2013).

All of these conditions are aggravated by dietary gluten. Gluten is a general term for alcohol soluble prolamin storage proteins found in wheat, barley, rye and oats (toxicity of oat prolamins to gluten sensitive consumers is less common). Gluten proteins from wheat are also composed of glutenin which are not soluble in alcohol. Prolamin proteins in wheat are gliadins, in barley they are hordeins and in rye and oats they are secalins and avenins respectively. These prolamin proteins can be found in foods prepared using the aforementioned grains.

As with other allergens the Codex Alimentarius Commission has determined maximum safe levels of gluten allowed in gluten-free

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products (CODEX STAN 118–1979). The Codex has determined that 20 mg/kg (ppm) gluten is the maximum level permitted in gluten-free products. The levels of prolamins are determined by ELISA, and compared to a prolamin standard (Thompson & Mendez, 2008).

Gluten-free beers are now widely available in many countries and are produced by a variety of methods. The most common method is to use ingredients that do not contain gluten, and alternative cereals like sorghum, buckwheat, maize and rice are used directly in the brewing process often with additions of thermostable amylolytic enzymes (Goode, Halbert, & Arendt, 2003; Hager, Taylor, Waters, & Arendt, 2014; Wijngaard & Arendt, 2006).

Another effective method for production of gluten-free beer is by application of enzymes. These enzymes can work by detoxifying gluten proteins by protease action (Lopez & Edens, 2005), or on the other hand enzymes can be used to create covalent bonds between gluten proteins allowing removal by filtration (Wieser & Koehler, 2012). These methods allow use of traditional ingredients to produce beers which are low enough in prolamins to be labelled gluten-free.

A third option, which we used in this study, is to use standard stabilisation process methods to reduce hordein levels in beers. Stabilisation works by removing haze-active proteins, polyphenols or both (Siebert, Carrasco, & Lynn, 1996). Without stabilisation, over time these haze-active precursors interact and form colloidal haze. By reducing either haze-active proteins or polyphenols stability is improved (Bamforth, 1999). Haze-active proteins tend to be very rich in the amino acid proline, much like hordein proteins. Removal of these proline rich proteins is an effective stabilisation method.

In this study we wanted to focus on stabilising agents which target these haze-active proteins. Silica gel and tannic acid were chosen, they are both in common use within the brewing industry and previous research suggested their efficacy (Dostalek, Hochel, Mendez, Hernando, & Gabrovska, 2006; Lewis & Bamforth, 2006; Van Landschoot, 2011). Gluten content of unstabilised beer was compared with beer stabilised with different concentrations of each stabilising agent.

2. Materials and methods

2.1. Wort production

Beer was produced using the 10 hL pilot scale brewing facility in University College Cork. Propino ale malt was purchased from the Malting Company of Ireland Ltd, Cork. The extract content of the malt, dry matter (d.m.), was 82.2% (w/w). Total nitrogen content of the malt was 1.56% (d.m.) and soluble nitrogen was 0.62% (d.m.) which provided a soluble nitrogen ratio of 40%.

Malt (133.5 kg) was mixed with 400 L water and mashed at 50 °C for 20 min, 62 °C for 40 min and 72 °C for 30 min. Lautering was performed for 90 min and 880 L wort was collected prior to boiling. Hop pellets (T-90) were added at 10 min after start of boiling (400 g Hallertauer Tradition, 7.4% α-acid, 510 g Spalter Select, 5.6% α-acid Hopsteiner, Mainburg, Germany) and at the end of boiling (623 g Spalter Select from Hopsteiner and 267 g Cascade, 7.6% α-acid, Simply Hops, Kent, UK). The boiled wort was rested 20 min in the whirlpool prior to cooling and aeration. A volume of 880 L of wort with an initial extract of 9.81% (w/w) was achieved.

2.2. Fermentation and filtration

Fermentation took place at 12 °C for 15 days before maturation at 1 °C. After maturation the beer was filtered using kieselguhr (FP-2 Celatom, EP minerals, Nevada, USA). The filtered beer was then added to kegs containing silica gel (Daraclar 920 from Grace) or tannic acid (Biotannin CS from Kerry). The recommended dose and 10 times the recommended dose of each was used. Silica gel was added at a rate of 50 g/hL and 500 g/hL. Tannic acid was added at 2 g/hL and 20 g/hL.

The beers were then held at 1 °C for 15 min before filtration through 1.5 μm candle filter (ULTIPOR N66 1.5 μm, Pall Corporation, New York, USA). A control unstabilised beer was filtered in the same manner. Each beer treatment was produced in duplicate and the beers were held in cold storage 1 °C prior to bottling and pasteurisation (14 PU).

2.3. Western blotting

Beer samples from each treatment were separated using SDS-PAGE prior to Western blotting. SDS-PAGE was carried out according to a modified Laemmli (1970) procedure using 4–20% precast TGX gradient gel (BioRad, California, USA). Beer samples were de-gassed and mixed (75 μL) with SDS sample buffer (25 μL) resulting in final concentrations of 62.5 mM Tris–HCl at pH 6.8, 10% (v/v) glycerol, 0.1% (w/v) bromophenol blue, 2% (w/v) SDS and 100 mM DTT. Each sample was heated to 100 °C for 5 min before centrifugation (20,000 × *g*) for 30 min. Samples (30 μL) were then loaded onto the SDS-PAGE gel and it was run at 100 V until the dye front reached the bottom of the gel.

The proteins were then transferred to a 0.45 μm nitrocellulose membrane (GE healthcare, UK) at 58 V and 4 °C for 1 h as previously described (Kanerva, Sontag-Strohm, & Lehtonen, 2005). After transfer the membrane was rinsed in TBST (Tris Buffered Saline with Tween) (20 mM Tris pH 7.5, 150 mM NaCl, 0.1% Tween 20) before blocking membrane overnight at 4 °C in 5% BSA (bovine serum albumin) in TBST. The membrane was then rinsed again in TBST before incubating 2 h, shaking with 1:2000 anti-gliadin antibody conjugated to peroxidase (Sigma, Missouri, USA) diluted in 5% BSA in TBST. The membrane was then rinsed in TBST before performing 3 × 5 min washes in TBST prior to application of peroxidase substrate (SuperSignal West Pico Chemiluminescent Substrate, Thermo Scientific, Illinois, USA). The membrane was then exposed to X-Ray film (Kodak Omat LS) and developed in a dark room. Band signal intensity of the films was analysed using Licor's Image Studio Lite software.

2.4. Hordein determination

The level of hordein in each treated beer was determined using a RIDASCREEN Gliadin *competitive* ELISA from R-Biopharm (Darmstadt, Germany). The samples were prepared and analysed according to MEBAK method 2.6.5. Prolamins were extracted from 1 mL beer by adding 9 mL 60% (v/v) ethanol containing 10% (w/v) of fish gelatine (Sigma G7765). Samples were then vortexed and shaken for 10 min prior to centrifugation. The supernatant was diluted and used for hordein determination according to the instructions of the manufacturer. The results from the assay were calculated based on a prolamin standard curve. The prolamin standard is composed of equal parts of gliadin, hordein and secalin (Haas-Lauterbach, Immer, Richter, & Koehler, 2012). The results here are presented as ppm hordein, and are not converted into gluten equivalents.

2.5. Beer analyses

All standard analyses were carried out according to recognised methods published by Mitteleuropäische Brautechnische Analysenkommision (MEBAK, 2011).

Anton Paar density meter (Alcolyzer DMA 4500M with a Beer ME module, Anton Paar, Graz, Austria) was used to determine the extract and alcohol of the wort and beer.

Foam stability of the beers (MEBAK 2.18.4), shown in Fig. 3 panel a, was assessed and measured as a half-life time in seconds, using the Steinfurth Foam Stability Tester (Steinfurth Mess-Systeme GmbH, Essen, Germany).

2.5.1. Beer flavour

Common beer flavours were analysed using several methods, higher alcohols and esters were determined by gas chromatography (GC)