



# Effect of crossflow microfiltration on the clarification and stability of beer from 100% low- $\beta$ -glucan barley or malt



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## ARTICLE INFO

### Article history:

Received 30 April 2017

Received in revised form

21 July 2017

Accepted 21 July 2017

Available online 24 July 2017

### Keywords:

Beer clarification

Ceramic hollow-fiber membrane

Exogenous enzymes

Low  $\beta$ -glucan barley grains

Permeation flux

## ABSTRACT

The clarifying effect of crossflow microfiltration (CFMF) on two types of lager beers made of 100% low  $\beta$ -glucan barley (LBGB), as such using the commercial Ondea<sup>®</sup>Pro enzyme preparation or pre-malted, was assessed and compared to that obtained using a commercial Pils Malt beer. All rough beers resulted to be quite similar with some differences in viscosity, color, and  $\beta$ -glucan, arabinoxylan, and polyphenol contents. The use of malted LBGB grains gave rise to a beer with low  $\beta$ -glucan ( $13 \pm 4$  mg/L) and arabinoxylan ( $568 \pm 40$  mg/L) concentrations, but high and stable colloidal turbidity ( $43.9 \pm 0.2$  EBC unit), with a consequent reduction in both permeation flux [ $23 \pm 2$  L/(m<sup>2</sup> h)] and clarified beer quality. The best results in terms of filterability and permeated beer quality were obtained using 100% LBGB beer. Despite its high  $\beta$ -glucan ( $233 \pm 4$  mg/L) and arabinoxylan ( $917 \pm 39$  mg/L) contents, the limiting permeation flux approached  $147 \pm 24$  L/(m<sup>2</sup> h), about three times higher than obtained with the conventional all malted beer. The commercial Ondea<sup>®</sup>Pro enzyme preparation and CFMF clarification of the resulting rough beer might represent a valid option to produce a 100% barley beer, even if the raw materials used are not well suited for malting.

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## 1. Introduction

Barley is the basic component of conventional beer, contributing to its aroma, flavor and body. The germination of barley grains activates several cereal-derived enzymes that are essential to convert the constitutive barley starch molecules, as well as those present in other adjuncts, into simple sugars. This step has so far constrained brewers to use a high proportion of malted cereals, which in some cases are supplemented with exogenous amylases, such as salivary amylases (e.g., to produce the South American corn beer named “Chicha”), and/or simple carbohydrates from sweet agave or honey (Buglass, 2011, ch. 2). At the same time, some natural characteristics of barley grains (mainly, high protein,  $\beta$ -glucan and polyphenol contents) affect the extract yield, process efficiency, as well as visual and flavor quality of the resulting beer (Marconi et al., 2011; O'Rourke, 2002).

The  $\beta$ -glucan content ranges from 2 to 6 g/100 g in malt and barley cell walls, and from about 0 to 3.95 g/L (with an average content of 100–300 mg/L) in beer (Leedham, Savage, Crabb, &

Morgan, 1975). If malt contains a high proportion of  $\beta$ -glucans or is poorly modified, or the brewing process employs a significant proportion of unmalted barley, the  $\beta$ -glucan fraction can survive unmodified into the beer due, in part, to the temperature-labile nature of native malt  $\beta$ -glucanases (active up to a maximum temperature of 60 °C). High  $\beta$ -glucan levels have generally been associated with many brewing problems, such as low extract yields (Bamforth, 1994; Edmunds, Allan, Fincher, & Stuart, 1994; Evans, Stenholm, Vilpola, Home, & Hughes, 1998), high wort and beer viscosity (Adamic, 1977, chp. 6), slow wort separation and beer filtration rate, especially in the case of membrane filtration, as well as a reduced beer colloidal stability (Cimini & Moresi, 2014; Gans & Denk, 1995; Oonsivilai, Patelakis, Speers, & Paulson, 1999). Despite the aforementioned great variation in malt  $\beta$ -glucan content, most brewers are of the opinion that the  $\beta$ -glucan content should be less than 4 g/100 g and preferably less than 1 g/100 g (Berg, 2002).

Many of such brewing problems may be minimized by resorting to selected barley varieties (Beaven, 1947; Fenwick, 1998). Barley is one of the most genetically diverse cereals, its amylose and  $\beta$ -glucan contents are primarily under genetic control. Barley genetic diversity provides many opportunities to identify and breed barley

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varieties for specific end uses, and also fulfill specific requirements by maltsters and brewers (Briggs, 1998, chp. 6). Applying improved processing technologies represents another solution. In particular, Berg (2002) suggested that the mashing and germination steps can be performed at higher temperatures and/or humidity to enhance the activity of naturally occurring  $\beta$ -glucanases without overstimulating  $\beta$ -glucan solubilase. This procedure resulted in a beer with too dark a color for many pale beer formulation and a higher protein content. Thus, an adsorbent (i.e., clay, magnesium montmorillonite) was needed to remove most of the undesirable constituents formed during malting from the wort or beer. Decreased mash aeration, thicker mashes, coarser malt milling, and gentler stirring generally decrease the  $\beta$ -glucan content in finished wort. These methods may however decrease the production efficiency or rate. Addition of exogenous  $\beta$ -glucanases to the mash or beer is a relatively recent option, and its main negative effects are extra operating costs and sometimes ineffectiveness if used in a beer with high turbidity (Cimini & Moresi, 2015).

The contribution of the so-called white biotech sector to the brewing process includes a wide range of applications that are specified in the electronic supplement (Table S1), even if the first stimulating idea dealt with the one-step malting and mashing process. Nielsen (1971) showed that beer could be made from 100% barley or very under-modified malt in combination with commercial enzymes (Astrup & Hannemann, 2000). Bamforth (2009) discussed a "brave new world" for alcohol factories in the future.

Today, a commercial enzyme preparation called Ondea<sup>®</sup>Pro enables brewers to produce beers with unmalted barley as the only raw material for extract. The synergistic action among the endogenous barley enzymes (i.e.,  $\beta$ -amylases to degrade starch into maltose, exo-proteases to release free amino nitrogen, FAN, etc.), and exogenous microbial enzymes (such as pullulanase, proteases, endo- and exo-peptidases) would result in a good starch and protein degradation. Good lautering with a clear wort being obtained by combining well-adjusted mills with an enzyme pool consisting of  $\beta$ -glucanase, xylanase, and lipase. Thus, it would be possible to produce a final beer quite similar to that attainable with malt (Kløverpris, Baltzer, & Nielsen, 2009; Steiner, Auer, Becker, & Gastl, 2012).

In all probability, the future more sustainable, smart and fast brewing process would rely on cleaner and smarter technologies, such as enzyme pretreatments, one-step clarification and removal of beer-spoiling organisms with no thermal treatment, improved barley and hop varieties, and yeast immobilization, so as to ensure or increase both beer quality, environmental sustainability, and system efficiency. In the circumstances, cross flow microfiltration (CFMF) might exert a key role (Cimini & Moresi, 2014) even if such a process still suffers from average permeation fluxes [50–100 L/(m<sup>2</sup> h)] quite lower than those achievable [250–500 L/(m<sup>2</sup> h)] with conventional powder filters (Buttrick, 2007; Fillaudeau, Blanpain-Avet, & Daufin, 2006). By adding exogenous  $\beta$ -glucanases during the maturation step of an industrial pale lager, Cimini, Marconi, Perretti, and Moresi (2014) and Cimini and Moresi (2015) were able to reduce the concentration of the viscosifying compounds and thus improve the CFMF performance and make such process not only economically viable, but also environmentally safe.

The main objectives of this work were to assess the effect of CFMF using a novel ceramic hollow-fiber membrane module on the clarification and stabilization of three different lager beers, by accounting for the main chemico-physical characteristics of raw materials used and final permeated beers. In particular, one beer was made from a conventional Pils malt while the other two ones were from previously selected low  $\beta$ -glucan barley grains (LBGB), as conventionally malted or in conjunction with the aforementioned Ondea<sup>®</sup>Pro commercial enzyme preparation.

## 2. Materials and methods

### 2.1. Raw materials

The three pale lager beers produced in this work made use of the following raw materials: i) a commercial Pils Malt, provided by Durst-Malz (Bruchsal-Heidelsheim, Bruchsal, Germany) to produce the all malt beer A; ii) low- $\beta$ -glucan barley (LBGB) grains of the Quench variety, previously selected by the Italian Brewing Research Centre (CERB, Casalina di Deruta, Perugia, Italy). LBGB grains were malted and then used to prepare the 100% LBGB malt beer B. Alternatively, they were used as such in conjunction with the commercial Ondea<sup>®</sup>Pro enzyme solution (Novozymes A/S, Bagsvaerd, Denmark) to obtain the 100% LBGB beer C.

### 2.2. Malting

The malting process for LBGB grains was carried out using a micromalting plant (Custom Lab, Milton Keynes, UK) equipped with automatic control of temperature and air flow. The malting program used was as follows:

- Steeping was performed as follows: barley grains were immersed in water at 18 °C for 5 h by injecting 25 L/min of purified compressed wet air every 5 min. Grains were then drained for 16 h, and finally submerged in water at 16 °C for 4 h by injecting 25 L/min of purified compressed wet air every 5 min.
- Wet grain germination was carried out at 16 °C for 6 d by injecting purified compressed wet air every 10 min to remove the carbon dioxide produced during grain germination
- Grain kilning was performed in three consecutive steps: the first step at 55 °C for 15 h, the second step at 72 °C for 4 h using an air recycle ratio of about 20%, and the last step at 82 °C for 3 h using an air recycle of about 70%.

### 2.3. Wort production

It was carried out in the 25-L pilot plant (Braumeister, Speidel, Ofterdingen, Germany) available at CERB (Casalina di Deruta, Perugia, Italy). Five kg of each raw material used, as such or malted, were milled using smooth rollers at different gap distances (Table 1). The resulting grist was mixed with a specific amount of water at 52 °C (Table 1) in the mash tun. When using 100% LBGB, 25 ± 7 mL of the aforementioned enzyme preparation were added together with 5 mL of an aqueous solution containing 80 g/100 g lactic acid (Vinoferm, Beverlo, Belgium) in order to adjust the pH of the mash to about 5.7. Table 1 shows the mashing program applied to prepare the worts to obtain the aforementioned beers A-C. After mashing, the mash extract was withdrawn from the lautertun and collected in a 30-L stainless-steel kettle tank, and integrated with the liquor recovered after sparging the solid residues with 15 L of pretreated water at 82 °C. The final wort having a density of about 1.048 kg/L was heated up to boiling, hopped with 15 g of hop pellets (Hallertau Magnum) with a bitterness capacity of 14 International Bitterness Unit (IBU), and then boiled for 90 min.

### 2.4. Beer fermentation

Fermentation was conducted in the same manner for all the three beers being studied by using 21 L of hopped and clarified wort at 12 °Plato and adding 11.5 g of lager dry yeast (Saflager W-34/70, Fermentis, Marcq-en-Barœul, France). The fermentation was carried out at ~12 °C for 10 d. The fermentation temperature was then

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