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# Effect of the mashing process on the performance of a lipophilic hop extract to reduce the primary gushing of beer

Zahra Shokribousjein[a,*], An Philippaerts[b], David G. Riveros[a], Sylvie M. Deckers[a], Mohammadreza Khalesi[a], Christiaan Michiels[a], Jan A. Delcour[a], Kurt Gebruers[a], Hubert Verachtert[a], Guy Derdelinckx[a], Bert Sels[b]

[a] KULeuven, Department of Microbial and Molecular Systems (M2S), and Leuven Food Science and Nutrition Research Centre (LFoRCe-MaltBeerSci), Heverlee, Belgium
[b] KULeuven, Centre for Surface Chemistry and Catalysis, Heverlee, Belgium

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ABSTRACT

The presence of Class II hydrophobins produced by fungi on barley results in primary gushing of beer. Gushing is the spontaneous overfoaming of carbonated beverages by opening of bottles. Solving gushing problems caused by brewing raw materials has received much scientific attention. Lipophilic extract of hops are introduced to brewers as foam suppressor in fermenters. We studied the effects of hop extract on gushing and found that lipophilic hop extract could reduce gushing. The effects are different when hop extract is added before mashing than when it is added after mashing. Hop extract contains fats and waxes and the effects on gushing are explained by a change in the physical state of its components during mashing which are due to temperature effects. Especially the effect of saturated fatty acids and waxes becomes apparent which are known as gushing inducers. This indicates that with respect to gushing potential of the hop extract's components, it is better to be added to cold wort (after mashing and filtration). Our study also showed an important effect of the filtration step on the amount of gushing.



## Introduction

Hydrophobins are small proteins produced by filamentous fungi such as *Fusarium* sp., *Nigrospora* sp. and *Trichoderma* sp. These proteins are very important in fungal life. They reduce water surface tension and form a protective coating on fungal structures, which allows aerial growth of hyphae, protects aerial conidia against desiccation and wetting, and also helps the aerial dispersal of spores (Wösten and Wessels, 1997). Based on solubility and sequence comparison, they are divided into Class I and Class II (Linder et al., 2005; Sarlin et al., 2005). Whereas Class I hydrophobins assemblages are only soluble in strong acids (trifluoroacetic acid (TFA), formic acid), Class II hydrophobins assemblages are more soluble (60% ethanol or 2% hot sodium dodecyl sulfate (SDS)) (Linder et al., 2005; Hektor and Scholtmeijer, 2005; Lumsdon et al., 2005). Hydrophobins contain hydrophobic and hydrophilic amino acids and are amphiphilic strong surface active molecules (Linder et al., 2005). As a consequence hydrophobins can be found in different forms: as monomers, dimers, tetramers in hydrophilic solutions or as self-assemblies forming membranes at hydrophilic–hydrophobic interfaces (Szilvay et al., 2006). They seem to oligomerize through the hydrophobic surface areas in order to hide their hydrophobic areas from the solvent (Kallio et al., 2007). In carbonated beverages self-assemblies occur around hydrophobic $CO_2$ gaseous molecules and this is now recognized as a major reaction involved in primary gushing of beer, a phenomenon long time associated with the nature of barley (Gjertsen et al., 1963; Pellaud, 2002). Gushing of beer causes economic losses and beer reputation damage. Many attempts have been made to prevent or cure primary gushing. Suggested preventive methods are mostly biological control approaches. Lowe and Arendt (2004) published a literature review on the effects of using lactic acid bacteria in malting and brewing. They cited different studies on the correlations between LAB and the formation of mycotoxins and gushing factors produced by filamentous fungi. Addition of lactic acid starter cultures during the steeping of barley delays *Fusarium* contaminations (Laitila et al., 1997). LAB preserve barley and malt from mould growth by providing a low pH in addition to bacteriocins, hydrogen peroxide, formic acid, propionic acid, acetoin and diacetyl (Lindgren and Dobrogosv, 1990; Stiles, 1996).

* Corresponding author at: Centr. Levensmidd.- & Microb. Technol., Kasteelpark arenberg 20, bus 2463, 3001 Heverlee, Belgium. Tel.: +32 16 328535; fax: +32 16 321997.
*E-mail address:* Zahra.shokribousjein@biw.kuleuven.be (Z. Shokribousjein).

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Further developments of curative methods for primary gushing may rely on a better understanding of the basic mechanisms of gushing. According to Casey (1996), Drager (1996) and Fischer et al. (1997) the mechanism can rely on the formation and stabilization of large amounts of micro-bubbles in beer. Later it was reported by several authors that nano-bubbles are covered by hydrophobin molecules and that by opening of the beverage container, the pressure drops and the nano-bubbles explode. The explosion gives the required energy for the formation of many nucleation sites in the beverage, allowing the dissolved $CO_2$ molecules to escape under gaseous bubble form which grow and rise to the surface and result in gushing (Sahu et al., 2006; Deckers et al., 2010; Christian et al., 2010). Many researchers used physical approaches to solve the gushing problem. Sarlin et al. (2007) reported that the fungal ability to produce hydrophobins during malting is reduced by prolonged storage of barley. Pasteurization can cure gushing temporarily by increasing the internal pressure of beer which tends to destabilization of coatings formed by hydrophobins around nanobubbles and breaking of nano bubbles (Pellaud, 2002; Briggs et al., 2004; Valant, 2005; Gastl et al., 2009; Ilberg et al., 2009; Deckers et al., 2010).

Aastrup (2003) reduced the gushing tendency of malt by adding proteolytic enzymes. In another investigation, Laibl and Geiger in 2003 mentioned that polar lipids produced by *Fusarium* sp. have gushing reducing effects. In an investigation by Hanke et al. (2009) gushing was suppressed by the addition of the hop components like linalool and humulones.

As hops are one of the most important components of beer many investigations were carried out regarding the impact of different hop extracts on primary gushing. Muller et al. (2010) reported that different hop varieties have different effects on beer gushing. Higher polyphenol content resulted in higher amounts of overfoaming. The mechanism of this effect was assumed to be the formation of critical nuclei or the presence of inhibiting gushing substances in beer. Kastner (1909) demonstrated that a specific hop dosage has suppressing effects on primary gushing. Curtis et al. (1961) confirmed this finding and stated that lower concentrations of hop in beer favours higher gushing tendency. Hops contain different compounds with different impacts on primary gushing. Gardner (1973) determined that 1 ppm hop oil in beer inhibits gushing. He ascribed this property to the terpene fraction and to β-caryophyllene. However a 1 ppm hop oil cannot be used because of negative effects on beer taste.

Nevertheless our investigation will further focus on the effects of a kind of hop extract that is applied now as foam suppressor in the brewing process. This so called lipophilic hop extract contains hop fats and waxes. Results on its gushing reducing effect shall be reported (Shokribousjein et al., 2013). In the current study, we report the effects of the mashing process on the properties of the lipophilic hop extract regarding a reduction of the gushing potential induced by hydrophobin HFBI from *Trichoderma reesei*.

## Materials and methods

### *Production and extraction of HFBI*

*T. reesei* MUCL 44908 was cultivated in *Trichoderma* medium for 7 days on a rotary shaker (150 rpm at 25 °C) and Class II hydrophobin HFBI was extracted from mycelium according to Deckers et al. (2011). The extract was applied to a reverse phase HPLC (RP-HPLC) 15RPC column (6.4 mm × 100 mm; GE Healthcare). The column was eluted with a gradient solution from 0.1% TFA in MilliQ water (A) to 0.1% TFA in acetonitrile (B) and monitored by UV detection at 214 nm. Fractions of 1 mL collected between 40 and 50% mobile phase B, were collected and examined by MALDI-TOF analysis. All fractions containing 7.5 kDa proteins were used for N-terminal amino acid sequencing for further identification of HFBI. After confirmation that the purified hydrophobin is HFBI, a crude mycelium extract was further used for convenience in the present study.

### *Gas chromatography (GC) and GC–MS analyses of hop extract antifoam*

#### *Extraction and preparation of hop extract samples for GC and GC–MS analysis*

The lipid compounds of hop extract (1 mL) were extracted with chloroform (2 mL) prior to GC and GC–MS analyses. The extract was evaporated to dryness under $N_2$ followed by converting of tri-, di- and mono-glycerides, as well as the free fatty acids, to fatty acid methyl esters (FAME). This was done by dissolution of dry extract in 1 mL of mixture of Boron trifluoride-methanol solution ($BF_3$ in methanol (1.5 M, Acros)). After incubation at 70 °C for 1 h, the reaction was stopped by addition of 2 mL distilled water. The esters were extracted two times with 2 mL octane and used for chromatographic analysis.

In addition to esterification silylation is the most widely used derivatization method for sample analysis by GC. In silylation, active hydrogen is replaced by an alkylsilyl group, such as trimethylsilyl (TMS). Compared to their parent compounds, silyl derivatives are more volatile, less polar, and more thermally stable. Therefore GC separation is improved and detection is enhanced. By silylation, since triglycerides contain no free —OH groups only the mono- and di-glycerides and free fatty acids are silylated. This aids GC analysis in adequate separation and quantification of the compounds.

In these experiments GC analysis was carried out with two different columns and temperature levels: in one set of experiments, analysis was performed at high temperature (320 °C) and with non-polar column (CP-SIL 5 capillary column (60 m × 0.25 mm i.d., 0.32 μm)) to detect all the compounds present in the hop extract and in another set of GC analysis the quantification of FAMEs was analysed by polar column (CP-SIL 88 highly polar column (0.25 mm i.d. × 0.2 μm)) at lower temperature (225 °C). In addition, with GC–MS equipment, the lipophilic fraction was analysed. The procedures are explained in more details below.

#### *GC analysis of lipophilic fraction*

A Shimadzu GC-210 gas chromatograph equipped with a split injector, a flame ionization detector (FID) and a CP-SIL 5 capillary column (60 m × 0.25 mm i.d., 0.32 μm) was used for GC analysis of the lipophilic compounds. $N_2$ was used as the carrier gas. The injector and the detector temperatures were set at 280 and 320 °C, respectively. The oven was programmed at 180 °C for 20 min. Then the temperature was increased to 225 °C at 10 °C/min, followed by a 15 min hold at 225 °C. Finally the temperature was raised at 10 °C/min to 320 °C and held there for 45 min. Peaks were identified using a mixture of standards (FAMEs (Supelco, Sigma–Aldrich) and alkane standard solution C21–C40 (Fluka)) and with information provided by GC–MS analysis (see below). Quantification was based on area percentage.

#### *GC analysis of FAMEs*

FAMEs, obtained after derivatization with $BF_3$ in methanol, were precisely analysed and quantified with a Hewlett Packard HP 6890 gas chromatograph with a split injection system (split ratio = 100:1) and $N_2$ as the carrier gas. A 100 m CP-SIL 88 highly polar column (0.25 mm i.d. × 0.2 μm) was used for separation. Initially, the column temperature was maintained at 180 °C for 50 min and then raised at 10 °C/min to 225 °C and held there for 25 min. The FID detector was maintained at 280 °C. FAMEs were identified based