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Effect of a magnetic field on dispersion of a hop extract and the influence on gushing of beer

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ABSTRACT

Hydrophobins are surface active molecules that cause gushing of beer. Gushing is vigorous overfoaming of carbonated beverages without any shaking. A hop extract was used to decrease gushing of wort induced by hydrophobin HFBI. The influence of a magnetic field on dispersion of the hop extract was used to decrease gushing by HFBI. The results indicate that when a magnetic field exerted on hop extract, this compound is dispersed more and smaller particles are formed. Therefore, the specific surface areas of the particles are increased and interact with larger numbers of hydrophobins. This resulted in less gushing by HFBI. When hydrophobins and hop extract together were submitted to magnetic field more gushing was obtained than in the absence of magnet. This is due to the extensive dispersion of the combination by the magnet and can be limited by using less amount of hop extract.

1. Introduction

Filamentous fungi are distributed all over the world and may infect many plants including grains due to their amazing ability to adapt to a wide variety of environmental conditions and ecosystems (Gow and Gadd, 1994). They spread through their hyphae in the substrates that they grow on and they can grow upward into the air and spread spores. These fungi form hydrophobins which are specific proteins needed in such development and growth (Hakanpää et al., 2004). The hydrophobins are needed to attach to solid surfaces such as plant leaves or insect cuticles and help fungi to cause plant diseases such as Head blight of barley, Dutch elm disease, rice blast, and chestnut blight (Sarlin et al., 2005; Tucker and Talbot, 2001). These proteins are involved in the formation of aerial structures of fungi by decreasing the surface tension of water and forming a protective layer on the aerial structures or spores (Talbot, 1997, 1999; Wösten et al., 1999).

Hydrophobins are among the most surface-active molecules known and are stable proteins with a size of about 100 amino acids which resist temperatures of boiling water (Wösten and de Vocht, 2000). Based on their specific characters, they have potential for several applications. They can function as adhesion enhancing molecules for the immobilization of other molecules to solid supports (Linder et al., 2002; Scholtmeijer et al., 2002) and as tags in fusion proteins for affinity purification (Collen et al., 2002). Since these molecules are surface active, they interact highly with non-polar gaseous molecules and form foam. These molecules are also able to self-assemble and form nucleation sites for gaseous CO_2. Thus by addition of these proteins to bottles of carbonated beverages and opening of the bottle after some days of shaking, the energy for nucleation is provided and overfoaming occurs. This is one of the easily detected characteristic of hydrophobins which is called primary gushing. Gushing is observed in many carbonated beverages such as beer (Sarlin et al., 2007). The major reason of primary gushing in beer found to be hydrophobins.

The mechanism of this phenomenon is explained by Deckers et al., 2010 and 2012. They explained that since hydrophobins have at least one exposed hydrophobic patch, they form di- tetra- and oligomers in a solvent to shield their hydrophobic patch. If an interface exists they move to the interface and form a monolayer. In a closed container of a carbonated beverage, gaseous CO_2 molecules are in equilibrium with the solvent form of CO_2. These gaseous molecules form an interface with which hydrophobins interact. Deckers et al. (2012) used molecular dynamics simulations to show the interaction of a class II hydrophobin with CO_2.

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The hydrophobic CO_2 molecules cluster near the hydrophobic patch of hydrophobins (Fig. 1). This effect is enhanced by self-assembled hydrophobins. These findings led to study the possibility to inactivate the hydrophobic patch of hydrophobins as an approach to inhibit gushing.

Preventing or reducing primary gushing continued to be challenging for industry. As mentioned before, this phenomenon is related to the poor microbiological quality of barley and malt, resulting in extraction of hydrophobins into the finished beer. The addition of non-polar molecules to hydrophobins may affect their binding with gaseous CO_2 and gushing. This was shown in a research with saturated and non-saturated hydrocarbons as well as unsaturated fatty acids, binding to hydrophobins and prohibiting gushing (data not published yet). Although inhibition of gushing with the model substances is successful, their addition to the beverages may not be the most appropriate. These molecules not only influence the taste and aroma of the finished beer, but also according to the European food safety authority, the amount of hydrocarbons in food is very limited. Therefore, an acceptable method to inhibit gushing would be the addition of extracts (components) related to the beverage in question. Hop extracts may be appropriate in the context regarding beer gushing.

Several methods to prevent and to solve gushing have been proposed, such as the addition of proteolytic enzymes or adsorbents such as charcoal, activated alumina during the beer filtration step and addition of a mixture of different calcium-enriched silicates (Aastrup et al., 1996; Sarlin et al., 2005; Evans and Bamforth, 2009; Besier et al., 2013). The addition of hop components like free linalool (50 μg/L) and humulones (5 mg/L) to the beer was one of the gushing decreasing methods (Hanke et al., 2009). The amount of total linalool (free and cell wall bound which will be used by yeast) in beer is 500 μg/L and humulones is 5–20 mg/L, which are much higher in the final beer but not free to decrease gushing (Briggs et al., 2010; Hanke, 2009; Hanke et al., 2008).

Membrane filtration of beer (0.1 μm pore size) (Christian et al., 2009), and addition of unsaturated fatty acids has been claimed to have gushing decreasing properties (Hanke et al., 2009).

Among the curative methods of gushing, one study on hop oils demonstrated that the hydrophobic characteristic of hop oils leads to their accumulation on hydrophobic–hydrophilic interfaces. This would result in non-stable nuclei by making gaps between the molecules of gushing promoting surfactants, therefore, reducing the gushing tendency (Hanke et al., 2009).

Hydrophobins are not the only molecules with gushing ability. In a study by Christian et al., 2011 on the gushing provoking tendency of hydrocarbons, they found a relation between structure (either carbon chain length or degree of saturation/unsaturation) and gushing potential of fatty acids. By increasing the carbon length of fatty acids from C10 to C16, overfoaming increased, due to the fact that a specific hydrophobic interaction is needed to initiate gushing. Unsaturated fatty acids were not gushing inducers and even suppressed gushing by saturated molecules.

Some hop extracts are used frequently in brewing to control the foam in boiling kettles and fermenters and to increase their capacity for the process. Such extract is a suitable alternative in order to decrease gushing and the result showed that it decreases gushing of beer when it is added before carbonation (Shokribousjein et al., 2014). The extract creates an interface in wort with which hydrophobins interact and further contact with gaseous CO_2 is inhibited. In finished gushing beer, the hydrophobins and CO_2 were in contact together and form nanobubbles. These nanobubbles are very stable and addition of the hop extract did not destabilize them as much. Therefore, addition of the hop extract to the finished beer is not a useful method to decrease gushing (Shokribousjein et al., 2014) and the extract should be used before carbonation to inhibit gushing. In a research it was shown that destabilization of the formed nanobubbles are possible through dropping of the crates of gushing beers (data not published yet) or pasteurisation (Garbe et al., 2009).

The intensity of effects of hop extract on gushing is related to its specific surface area. Aggregation of the particles of this extract causes a problem for their interaction with hydrophobins. Better dispersion of the extract, increases specific surface area and interaction with hydrophobins and consequently reduces gushing. For a better dispersion, the high temperature during mashing was considered but that approach was not successful. This is due to the extensive dispersion of the hop extract at high temperature which changed it to become a gushing inducer (Shokribousjein et al., 2013). Therefore, it is advised to add the extract in a so called "cold wort" which means after wort cooling. Since this extract is used as a foam control in fermenters, the best step to add it to the wort is before fermentation.

Another method to disperse the hop extract is the use of a magnetic field. The idea of using this method came from the studies of Stuyven et al. (2009) who found that the aggregates of silica particles are dispersed in a magnetohydrodynamic device. Thereafter, Kerkhofs et al. (2011) used a magnetic field for emulsification of mayonnaise. Based on these, an orthogonally magnetic field was investigated on dispersion of the hop extract, and the effect on gushing of hydrophobin HFBI.

2. Materials and methods

2.1. Production and purification of hydrophobin HFBI

Class II hydrophobin HFBI was extracted from *Trichoderma reesei* MUCL 44,908. This fungi was cultivated for 7 days in *Tricho-*

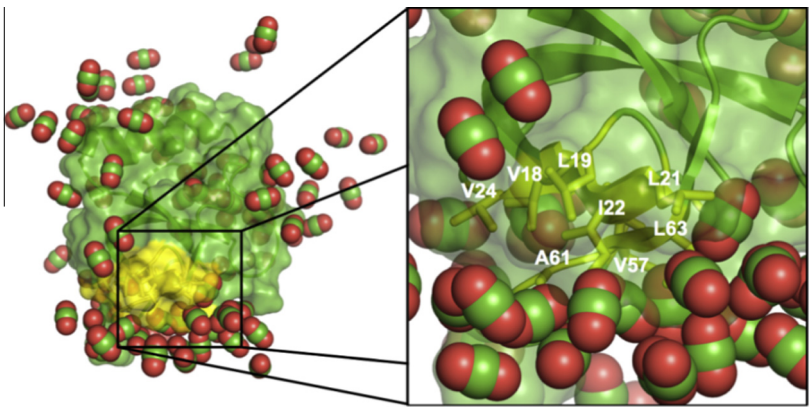

Fig. 1. Gaseous CO_2 clustering around hydrophobic patch of class II hydrophobin HFBII (Deckers et al., 2012).

derma medium containing glucose (40.0 g/L), peptone (4.0 g/L), yeast extract (1.0 g/L), KH_2PO_4 (4.0 g/L), $(NH_4)_2SO_4$ (2.8 g/L), "$MgSO_4 \cdot 7\ H_2O$" (0.6 g/L), $CaCl_2 \cdot 2\ H_2O$ (0.8 g/L) and 2 ml per litre of a trace elements solution. This solution contained "$FeSO_4 \cdot 7\ H_2O$" (5 g/L), "$CoCl_2 \cdot 6\ H_2O$" (3.7 g/L), "$MnSO_4 \cdot H_2O$" (1.6 g/L), "$ZnSO_4 \cdot 7\ H_2O$" (1.4 g/L). The pH of the medium culture was adjusted to 4.5–5.0 with HCl (1 M). After 30 h, 25 ml of glucose solution (240 g/L) was added to 1 L of medium culture. After 7 days shaking (25 °C, 120 rpm), the mycelium was separated from medium culture by centrifugation (8000×*g* for 20 min at 4 °C, Beckman model J2–21). Hydrophobin HFBI was extracted from mycelium by addition of 25 mL of a 170 mM Tris/HCl buffer (pH 9) containing 1% SDS and shaking for 1 h at room temperature. The mycelium was then separated by centrifugation at the same time and speed as above. After obtaining the liquid extract, SDS was precipitated as water-insoluble potassium dodecyl sulphate by addition of 10 mL of a 2 M KCl solution and further centrifugation (8000×*g* for 10 min at 4 °C, Beckman model J2–21). In this step a crude mycelium extract containing HFBI is obtained.

HFBI was further purified by reverse phase high performance liquid chromatography (RP-HPLC) on a 15 RPC column (6.4 × 100 mm, GE Healthcare) with a linear gradient elution from 0.1% TFA in MilliQ water (A) to 0.1% TFA in acetonitrile (B). The elute was monitored by UV detection at 214 nm. The fractions between 40% and 50% of acetonitrile were collected.

Fractions containing hydrophobin HFBI were detected by matrix-assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometry with an Ultraflex II instrument in linear mode and α-cyano-4-hydroxy cinnamic acid matrix (Brüker Daltonics GmbH). Fractions containing 7.5 kDa molecular weight peptides were confirmed to be hydrophobin HFBI by automated Edman degradation on a capillary protein sequencer (N-terminal amino acid sequence) (Procise 491cLC, Applied Biosystems).

The concentration of the purified HFBI was measured with NanoDrop® ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and was 0.15 mg/mL.

After confirmation that the purified hydrophobin is HFBI, a crude mycelium extract was further used in the present study. During purification, there are some losses of the molecules, therefore, the concentration in the crude mycelium is higher than 0.15 mg/mL.

2.2. Gas chromatography (GC) and GC–MS analyses of hop extract antifoam

2.2.1. Sample preparation

Prior to GC and GC–MS analyses, the lipid compounds of the antifoam sample were extracted with chloroform. Part of the chloroform extract was analysed as such, while one other part was further processed in order to prepare derivatives. Before derivatisation, the chloroform extract was evaporated to dryness under N_2. A methanolysation procedure was used as a derivetisation method. During the methanolyzation, the tri-, di- and monoglycerides, as well as the free fatty acids were converted to fatty acid methyl esters (FAME). Therefore, the dry extract was dissolved in a mixture of boron triflouride methanol complex (BF_3) in methanol (1.5 M, Acros). After incubation at 70 °C for 1 h, the derivatisation reaction was stopped by the addition of distilled water and the lipids were extracted with octane. The octane extracts were used for chromatographic analysis of the lipophilic fraction.

2.2.2. GC analysis of lipophilic fraction

A Shimadzu GC-210 gas chromatograph equipped with a split injector, a flame ionisation 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 °C 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 with a mixture of standards (FAMEs, Supelco, Sigma–Aldrich, Belgium), alkane standard solution C21-C40 (Fluka, Sigma–Aldrich, Belgium) and with information provided by GC–MS analysis (see below). Quantification was based on area percentage.

2.2.3. GC–MS analysis of lipophilic fraction

GC–MS analyses were performed on an Agilent 6890 N gas chromatograph coupled to a quadrupole mass spectrometer detector (Agilent). The GC was equipped with a split injector (split ratio = 10:1) and a 30 m × 0.25 mm i.d. × 0.25 μm HP-5 capillary column. The injector temperature was set at 325 °C. After an initial hold for 20 min at 180 °C, the oven was heated from 180 to 225 °C at 10 °C/min and held there for 15 min. Then the temperature was increased to 325 °C at 10 °C/min, followed by a 40 min hold at this temperature. Compounds were identified by comparing their mass spectra with mass spectra in the NIST Mass Spectral Database, by mass fragmentography, and, when possible, by comparison with authentic standards (FAMEs and hydrocarbons).

2.2.4. GC analysis of FAMEs

FAMEs, obtained after derivatization with BF_3 in methanol, were analysed 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 used was maintained at 280 °C. FAMEs were identified based on retention times, using references (Supelco, Sigma–Aldrich). For quantification, heptadecane (Sigma–Aldrich, Belgium) was used as external standard.

2.3. Magnetic field setup in laboratory scale

The experimental magnet setup was carried out in stainless tubes and the pump was Riotherm circulation pump C 12/15T. Fig. 2 shows the system that was used for creating a magnetic field and circulation. An orthogonal magnetic field was applied by mounting 0.31 T magnet on the circulating system with the inner diameter of 1 cm. The whole volume for filling the system was 200 mL. Filling of the system with wort took place at point 1 in Fig. 2. Addition of hydrophobin and hop extract was carried out at point 2 (Fig. 2). After circulation, the sample was taken from point 1 for further analysis. After each run, the system was washed with 2% NaOH for 1 min and rinsed with water (at least 5 times) which was filled from point 5 and removed from point 1.

As the first experiment, 1 mL and 2.5 mL of the hop extract were circulated (1.5 L/min) in 200 mL wort for 1 min and 30 min with and without magnet. The treated wort was mixed with 1 mL hydrophobin extract and gushing and particle sizes were measured. In another experiment, 10 mL hydrophobin extract was circulated in 200 mL wort with and without magnet for 1 min and 30 min. The particle sizes as well as gushing were measured for this sample. To adapt the experiments with the real situation in beer, 10 mL hydrophobin extract was mixed with 1 mL hop extract and the mixture was added to 200 mL of the wort. The same was carried out with 2.5 mL of hop extract. The prepared mixtures were circulated for the same time as above with and without magnet. The gushing analysis as well as particle sizes was measured. All the mentioned experiments are shown schematically below in Fig. 3.

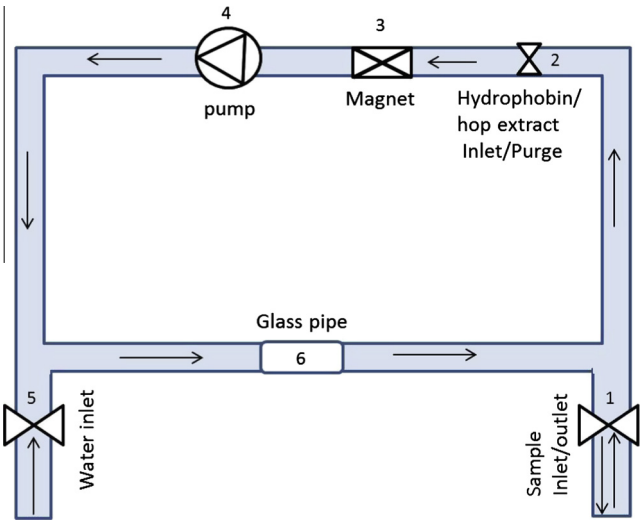

Fig. 2. Schematic illustration of magnet setup with stainless steel tubes.

2.4. Gushing measurement

The gushing test was performed according to the Doubly Modified Carlsberg Test (M^2CT) for the samples in wort (Garbe et al., 2009). Twenty millilitres of samples, prepared as explained above, were added to 1 L sparkling waters which were stored at 2 °C for at least 24 h. In order to have the same amount of liquid in all bottles, prior to the experiments, 20 mL of sparkling water was removed and then the sample was added. The samples were shaken on a horizontal rotating shaker at 150 rpm (Bühler shaker SM30, Germany) for 3 days at 25 °C (Bühler TH30 incubator hoods, Germany). After 3 days, the samples were allowed to stand for 10 min and then turned upside down three times (180°) by 10 s intervals. After the last turning, the bottles were stand for 30 s and then opened. The amount of overfoaming was calculated from the difference of weight after opening. All gushing experiments were carried out with at least five repeats.

The statistical analysis was carried out using IBM SPSS Statistics 22, Shapiro–Wilk method. Based on the normality results, differences of variances were analysed by Independent samples *T*-test or one-way ANOVA.

2.5. Particle size distribution

The particle size of the samples was determined by DLS with a 90 Plus Particle Size Analyzer (Brookhaven Instruments Corporation). Ten repeats for each sample were carried out for 1 min and for better comparison, the mean diameter was calculated and probed. For the particle sizes of samples in industrial scale, the intensity sum distribution was probed versus particle sizes. This representation is easier for comparison of the samples. Statistical analysis was carried out the same as for gushing measurements.

2.6. Magnetic field influence on dispersion of hop extract in brewery scale

In order to study the influence of a magnetic field on the dispersion of hop extract at brewery scale, experiments were carried out in a brewery in Belgium. In this brewery, hop extract is added to the wort after cooling and before transferring to the fermenter. Intention is to decrease the foam in fermenters and to maximise the vessel capacity. For the experiment, 800 g hop extract was mixed with 1 L of cooled wort in a small vessel. The mixture was passed through the magnet (0.31 T) by means of a pump (membrane pump, Prominent 99011124, 3.4 L/min) to be distributed in 27,000 L of wort.

Before mixing with 27,000 L wort, the samples with and without magnet were taken and particle sizes were analysed by DLS (Nanoflex 180° from Microtrac, Germany).

3. Results and discussions

3.1. Gas chromatography/mass spectrometry

The hop extract antifoam was methanolyzed and then analysed with GC and GC–MS. The GC–MS chromatogram of the methanolyzed extract is shown in Fig. 4 and the composition of the main compounds is summarized in Table 1. The main lipid classes identified in the sample were (partial) glycerides, *n*-alkanes, steroid

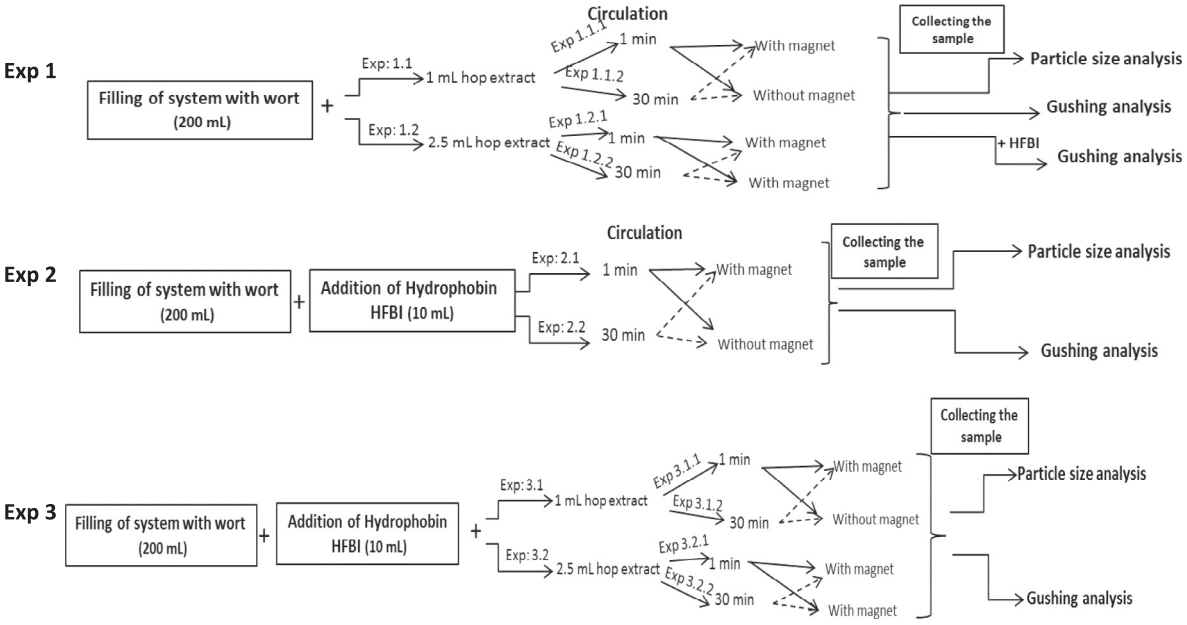

Fig. 3. The schematic representation of different experiments carried out in this study. Filled and dashed arrows show the number of samples which were prepared for each experiment. Each preparation was repeated 4 times and samples were analysed for gushing and particle sizes.

and triterpenoid compounds and wax esters. The most dominant lipid compounds were found in the group of the *n*-alkanes, namely *n*-hentriacontane (C31) (18%) and *n*-nonacosane (C29) (14%). These compounds are labelled A5 and A3, respectively (Fig. 4 A). Other major *n*-alkanes were in the range from C27 to C32. By methanolyzation, free fatty acids and mono- di- and triglycerides are converted to fatty acid methyl esters (FAMEs). Within the group of the FAMEs, the most predominant was linolenic acid (C18:2), comprising 50 wt% of the total FAMEs identified in the derivatized extract (Table 2). Steroid compounds comprise also an important fraction of the extract, the most important is sitosterol (S1 in Fig. 4A). Finally longer wax esters were identified and were found in the range from C38 to C50.

The hop extract contains molecules which behave differently regarding gushing. In another research different non-polar molecules including saturated and unsaturated hydrocarbons as well as fatty acids were studied whether they cause gushing (data not published yet). The results show that long chain saturated hydrocarbons could provide enough hydrophobic interaction with gaseous CO_2 and cause gushing. Unsaturated fatty acids such as linoleic and oleic acid did not induce gushing (data not published yet).

The major components of the hop extract are waxes (C29 and C31) which are strong gushing inducers (Shokribousjein et al., 2014) while some unsaturated fatty acids were gushing inhibitors. Based on the total amount of individual molecules in the hop

Table 1
Composition of lipids from lipophilic fraction of hop extract (based on area percentages of GC–MS analysis).

Compound	%	Compound	%	Compound	%
Fatty acid methyl esters		*n*-alkanes		Wax Esters	3.1
C16:0 (F1)	2.8	C27 (A1)	1.5	Others[a]	40.4
Unsaturated C18 (F2)	9.1	C28 (A2)	0.8		
C18:0 (F3)	1.0	C29 (A3)	14.3		
C20:0 (F4)	1.2	C30 (A4)	1.8		
C22:0 (F5)	2.2	C31 (A5)	18.8		
C24:0 (F6)	0.8	C32 (A6)	2.2		

[a] Mainly steroid and triterpenoid compounds.

Table 2
Composition of the fatty acid fraction obtained by quantitative GC analysis of the methanolyzed extract.

Fatty acid	wt%
C16:0	12.9
C18:0	3.6
C18:1	19.5
C18:2	50.2
C18:3	7.6
C20:0	1.6
C22:0	6.1
C24:0	2.0

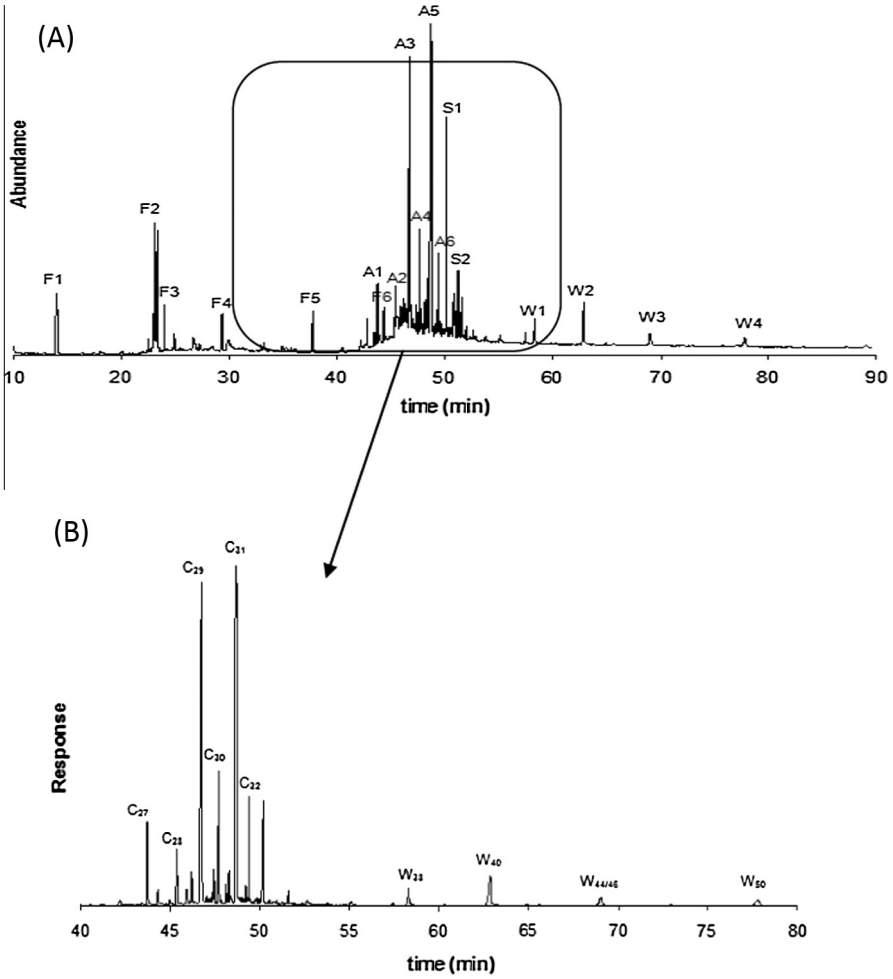

Fig. 4. GC/MS chromatogram of the methanolyzed lipid extracts: F = fatty acid methyl ester, A = *n*-alkane, W = wax ester, S = steroid compounds (A). Ion extraction of GC–MS spectrum ($m/z = 57$), characteristic for alkanes and wax esters (B).

extract, it is found that the majority of the molecules are gushing inducers.

3.2. Particle size and gushing analysis of hydrophobin HFBI and hop extract with/without magnetic field

Figures below show the results of a magnetic field on particle sizes of hop extract antifoam (Fig. 5), of hydrophobin (Fig. 6) and of a mixture of both (Fig. 7), and the effects on gushing are presented. As a control sample, non-gushing wort was circulated with and without magnet for 1 and 30 min. After circulation, no gushing was detected. The particle sizes of the control sample were analysed after 30 min circulation with and without magnet. Particles smaller than 2000 nm did not change by 30 min circulation with magnet, while the particles between 2000 and 5000 nm had a mean decrease of 428 nm.

Fig. 5 (Exp 1) shows the effects of a magnetic field on hop extract antifoam particles and gushing by hydrophobin HFBI. The following experimental condition was used. At first the hop extract antifoam was circulated in wort with and without magnet for 1 min and 30 min. After circulation the particle sizes of the sample and gushing were measured. The prepared sample was mixed with 10 mL mycelium extract (containing HFBI) and gushing was analysed. The obtained data from particle size analysis and gushing measurements were all statistically analysed. The normal distribution was tested with Shapiro–Wilk method. The data distribution were normal, therefore, Independent samples *T*-test was used for analysis of differences of variances (Table 3).

The results show that the hop extract antifoam is dispersed in the presence of a magnetic field. The magnetic field decreased the particle sizes of hop extract antifoam significantly (Table 3). The dispersion is due to the hydrodynamic forces of turbulent flow and Lorentz force. Lorentz force is a combination of an electric and magnetic force which is applied to a particle with a charge which is moving in a magnetic field. Since there are polar molecules in the hop extract antifoam such as fatty acids, Lorentz force is present for this product. This force enhances shear stresses which cause

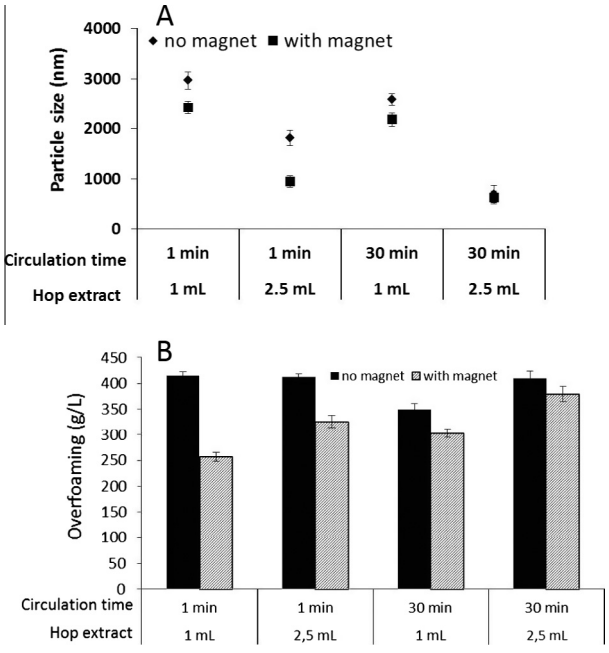

Fig. 5. (A) The particle sizes of hop extract antifoam after circulation with and without magnet for 1 min and 30 min. (B) The gushing amount of HFBI in the presence of the hop extract antifoam which was circulated with and without magnet. The bars indicate standard deviation and the number of repeats was 5.

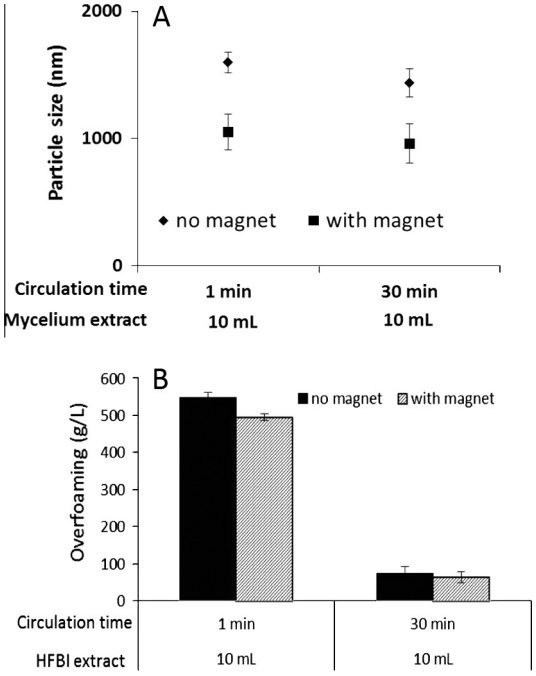

Fig. 6. (A) The particle sizes of the mixture of HFBI and hop extract antifoam after circulation with and without magnet for 1 min and 30 min. (B) The gushing amount of the mixture of HFBI and hop extract antifoam circulated with and without magnet. The bars indicate standard deviation. The number of repeats was 5.

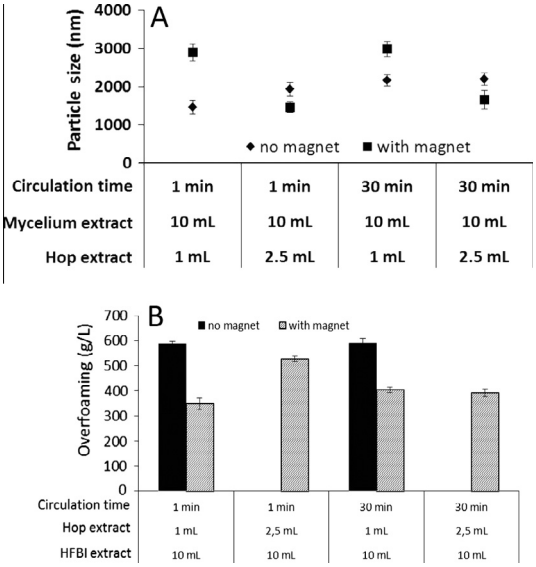

Fig. 7. (A) The particle sizes of the mixture of HFBI and hop extract antifoam after circulation with and without magnet for 1 min and 30 min. (B) The gushing amount of the mixture of HFBI and hop extract antifoam circulated with and without magnet. The bars indicate standard deviation. The number of repeats was 5.

segregation of the particles. This result is in accordance with the results of Stuyven et al. (2009) who studied the dispersion of silica suspension in the magnetic field.

By using the magnet, less overfoaming by HFBI is observed in the presence of smaller particles of hop extract antifoam. The magnetic field disperses hop extract antifoam, increases the specific surface area and allows more reaction with HFBI molecules. Therefore, gushing is decreased. The statistical analysis of the gushing measurements carried out with Independent samples *T*-test method and the results show that magnet could significantly

Table 3
Sample descriptive are analysed *t*-test equality to compare the differences of circulation with and without magnet. The *t*-values for gushing amounts and particle sizes are mentioned in the table. The amount of hop extract antifoam which is circulated in 200 mL wort is indicated.

	Circulation	*t*-Test for	
		Gushing	Particle sizes
1 mL	1 min	−10*	−22*
1 mL	30 min	−21*	12*
2.5 mL	1 min	−6*	−33*
2.5 mL	30 min	ns	−7*

ns = Not significant.
* $p < 0.05$.

decrease gushing by HFBI (Table 3). Gushing by 2.5 mL hop extract antifoam which was circulated for 30 min was not significantly decreased ($t = -1.828$, $d_f = 8$, $P > 0.05$).

Fig. 6 shows the effects of a magnetic field on hydrophobin particles. The following experimental condition was used for this analysis. Ten mililiter of mycelium extract (containing HFBI) was added to wort and circulated with and without magnet. The particle sizes and overfoaming amounts of the sample were measured (Exp 2). This analysis was carried out to find out whether, without any addition of hop extract antifoam gushing could be decreased just by applying a magnetic field on circulating wort. The objective was to see if the magnetic field is able to destabilize the crystalline structures of class II hydrophobins. The crystal structure of class II hydrophobins forms nucleous sites for gaseous CO_2 and causes gushing.

The statistical analysis carried out firstly for normal distribution of data of either particle sizes or gushing amounts and thereafter, for the analysis of differences of variances. The data of particle sizes and gushing were analysed by Shapiro–Wilk method for distribution and they were all normally distributed. For the differences of variances, Independent samples *T*-test method was used and the results show that there is a significant difference in particle sizes of HFBI when they are circulated with magnet (Table 4). Gushing measurements were statistically analysed the same as particle sizes and the results demonstrated that gushing by HFBI is significantly decreased when it is circulated for 1 min with magnet (Table 4). The circulation by magnet for 30 min did not decrease gushing by HFBI significantly ($t = -1.069$, $d_f = 8$, $P > 0.05$). Gushing by HFBI when it was circulated with or without magnetic field for 30 min was significantly less than 1 min (F: 1221, $P < 0.05$).

The results demonstrate that circulation of HFBI in magnetic field decreases the sizes of the particles and also gushing (especially circulation for 30 min). This is probably due to shear stresses by circulation. The circulation with 1.5 L/min for 30 min can affect the other properties of wort. Therefore, it is not possible to use it as a method to decrease gushing in big scale.

Comparing the gushing by 1 min circulation of HFBI (Fig. 6B) with the experiment where the hop extract antifoam was influenced by magnet and then HFBI was added (Fig. 5B), indicates that the amount of gushing which was decreased with hop extract antifoam in magnetic field was significantly higher (100 g) than here (50 g). This shows that using hop extract antifoam along with magnet is more effective to decrease gushing by hydrophobin.

In order to approach the real situation in breweries, hydrophobin and hop extract antifoam were mixed in wort and then circulated with and without magnet for 1 min and 30 min. This is essential to study if hop extract antifoam needs pre-treatment with magnet before addition to wort or it can be added to the gushing wort and then passes through magnetic field. For this analysis the following experimental condition was used. Ten mililiter of mycelium extract (containing HFBI) and 1 mL and 2.5 mL of hop extract antifoam were added to the wort and circulated with and without magnet for 1 min and 30 min (Exp 3). Fig. 7 shows the effects of a magnetic field on a mixture of hop extract antifoam and hydrophobin particles.

The results (Fig. 7B) were very complex but clearly showed that when hop extract antifoam is used in low amount, magnet could decrease gushing, but high amount of the extract did not work as well. The particle sizes and gushing measurements were statistically analysed for normal distribution and thereafter, for differences of variances. In both cases, the data were normal. Therefore, the Independent samples *T*-test method was used for analysing the differences of variances. Either particle sizes or gushing amounts were significantly different when there was the magnetic field (Table 5).

The results demonstrate that by circulation (1 min and 30 min) of the mixture of hop extract antifoam (2.5 mL) and mycelium extract (10 mL), gushing disappears. This is an indication for the best situation for gushing inhibition. This means that the amount of hop extract antifoam in combination with the circulation was sufficient to inhibit gushing. The result with magnet was compared with the results without magnet and it is clear that magnetic field did not inhibit gushing. This is due to the changes obtained by the magnetic forces in addition to the circulation forces (turbulent flow). To complete this analysis, 1 mL and 2.5 mL hop extract antifoam were circulated in wort for 1 min and 30 min (Exp 1) with and without magnet and the gushing was measured. The results show that in presence of the magnetic field, 1 mL hop extract antifoam did not show any gushing, but 2.5 mL of hop extract antifoam overfoamed 51 g/L and 142 g/L after 1 min and 30 min circulation, respectively. This gushing is of the secondary type in which the long chain molecules form nucleous sites for gaseous CO_2 and gushing happens. Based on the particle sizes, it is clear that in the presence of magnetic field, dispersion happens and since there are many gushing inducers in the hop extract antifoam, it shows gushing. The same results were obtained in chapter 5 for extensive dispersion by mashing.

Since hop extract antifoam is composed of mainly gushing inducer molecules, by extensive dispersion, they are released from the matrix and gushing appears. The reason that this phenomenon

Table 4
Sample descriptive are analysed using *t*-test to compare the differences of circulation with and without magnet. Ten mililitre HFBI was circulated in 200 mL wort. The *t*-values for gushing and particle sizes are mentioned.

	Circulation (with and without magnet)	*t*-Test for	
		Gushing	Particle sizes
10 mL HFBI	1 min	−2*	54*
10 mL HFBI	30 min	ns	29*

ns = Not significant.
* $p < 0.05$.

Table 5
Sample descriptive analysed by *t*-test for equality to compare the differences of circulation with and without magnet. The amount of hop extract antifoam and HFBI which are circulated in 200 mL wort is mentioned.

	Circulation (with and without magnet)	*t*-Test for	
		Gushing	Particle sizes
1 mL hop extract + 1 mL HFBI	1 min	−33*	−184*
1 mL hop extract + 1 mL HFBI	30 min	−74*	−10*
2.5 mL hop extract + 1 mL HFBI	1 min	229*	16*
2.5 mL hop extract + 1 mL HFBI	30 min	207*	12*

* $p < 0.05$.

did not happen with 1 mL hop extract antifoam could be due to the less amount of gushing inducers that are released after dispersion.

3.3. Investigation of effects of a magnetic field on the dispersion of hop extract at brewery scale

The analysis of the effect of magnetic field on dispersion of hop extract antifoam in wort was carried out in large scale at an industrial brewing site. This analysis was carried out in laboratory scale (in wort) with some differences including the pump (membrane pump in brewery scale and circulation pump in laboratory scale) and the pump speed (3.4 L/min in brewery scale [turbulent flow] and 1.5 L/min in laboratory scale [turbulent flow]). Unlike the differences, the results were the same. The results of magnetic field on sizes of hop extract antifoam in wort in brewery scale are shown in Fig. 8. According to the results, the particle sizes of the hop extract antifoam were decreased in wort with magnetic field and the same result was obtained in laboratory scale. The big different between the analysis in brewing scale and laboratory scale is the number of times which hop extract would pass through the magnet. In industrial scale, only one time the hop extract particles passed through magnet while in laboratory scale 7 times for 1 min circulation and 225 times for 30 min of circulations.

Statistical analysis was carried out for the particle sizes. The data was tested for normality distribution using Shapiro–Wilk method. The distribution of particles between 0.95 and 289 nm was not normal, therefore, for comparison of the variances, ANOVA was used. Data showed that there was no significant difference between the particle sizes of this range ($p > 0.05$). The distribution of particles between 344 and 6500 nm was normal and the differences of variances were tested using Independent samples *T*-test. The data are shown in Table 6.

This brewery installed a magnet since 2011 for better dispersion of hop extract antifoam and more benefit from this extract

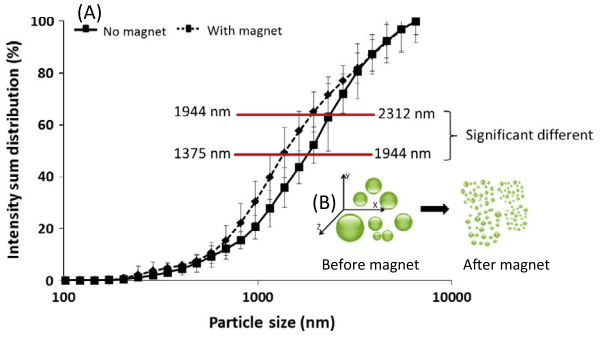

Fig. 8. (A): Particle sizes of hop extract antifoam mixed in wort passing through magnet and no magnet. The standard deviations are shown. The particle sizes which are significantly different because of magnet are mentioned in the graph. (B): Schematic representation of dispersion of particles is shown. This representation is clearly showing that dispersion of the particles by means of magnet is a 3-D effect. Therefore, the specific surface area of the particles are increased by dispersion.

Table 6
Samples descriptive analysis for the particles at the range of 344–6500 nm using Independent samples *T*-test.

Particle size ranges (nm) after treatment with magnet	*t*-Test
344–1156	ns
1375	−2.6*
1635	−2.9*
1944	−2.7*
2312–6500	ns

ns = Not significant.
* $P < 0.05$.

regarding foam control in fermenters. According to the hop extract antifoam supplier (Barth-Haas, England), the hop extract antifoam should be added at a dose range of 10–30 g/hL wort while at this brewery, using a magnetic field, 800 g of this extract is added to 27,000 L of wort. This is 1900 g less than the minimum amount which is advised by the supplier. The benefit is probably due to the magnetic field which provides more active surface area to interact with foaming molecules.

The changes of average particle diameters by magnet are calculated at the identified intensity sum distribution in Table 7.

The decreased diameter increases the specific surface area of the particles. The increase of specific surface area by magnetic field is calculated based on the decreased sizes of particles.

The particles are assumed to be spherical and the differences of Specific Surface Area (SSA) for the particles with the significant difference by magnet are calculated.

$$\mathrm{SSA} = \frac{\text{surface area of a particle}}{\text{volume of the particle}} = \frac{4\pi r^2}{4/3\pi r^3}$$

The changes of specific surface area for the particles which have significant differences after magnet are calculated and mentioned in Table 8:

According to the above calculations, the average of SSA changes because of magnet for the selected points will be: $4 \times 10^{-4}\ \mathrm{nm}^{-1}$.

Through this change, the amount of hop extract antifoam that needs to inhibit foaming is decreased while no overfoaming in fermenters is detected and no gushing.

3.4. Determination of the surface area of hydrophobins that needs to be inactivated: mathematical approach

In order to establish a mathematical measure for the hydrophobic patch of hydrophobins which is needed to be inactivated or covered by non-polar molecules, the calculations were conducted for 10 mL of HFBI with a concentration of 0.15 mg/mL. This calculation was carried out to have an idea about the size of the surface which is needed to inactivate hydrophobins.

$$1\mathrm{HFBI} = 7.5\ \mathrm{kDa} = 7500\ \mathrm{g/mole}$$

$$10\ \mathrm{mL} \times 0.15\ \mathrm{mg/mL} = 1.5\ \mathrm{mg\ HFBI}$$

$$x = \frac{6.02 \times 10^{23}(\mathrm{molecules}) \times 1.5\ \mathrm{mg\ HFBI}}{7500 \times 1000\ \mathrm{mg}}$$

Table 7
Comparison of particle diameters (nm) by magnet at the identified intensity of distribution. Values obtained from measurements made on two different brews (30 runs at each measurement by Microtrac). Values in bold have statistically significant difference.

Intensity sum distribution (%)	Average of particle diameter (nm)		Difference of average particle diameters
	Without magnet	With magnet	
0–20	663	453	210
20–40	1396	1002	394
40–60	**2149**	**1624**	**525**
60–80	3003	2762	341
80–100	4879	5100	−221

Table 8
Changes of SSA for the significant differences of particles by magnet.

Diameter before magnet (nm)	Diameter after magnet (nm)	Changes of SSA
1944	1375	0.00064
2312	1944	0.00025

$x = 1.2 \times 10^{17}$ molecules of HFBI

Based on the fact that the area of hydrophobic patch of one HFBI is 4 nm^2 (Szilvay et al., 2006):

$1.2 \times 10^{17}(\text{molecules}) \times 4\ nm^2 = 4.8 \times 10^{17}\ nm^2 = 0.48\ m^2$

Therefore, at least 0.48 m^2 of a surface is needed to interact with 1.5 mg hydrophobins and inhibit their interaction with gaseous CO_2.

This calculation was carried out for 1.5 mg HFBI for 1 L of wort, while for 100 hL of wort which is even less than 1 day brewing, 4800 m^2 is needed to be inactivated.

4. Conclusion

In this article the dispersion and interaction of HFBI molecules with the hop extract antifoam in the presence of a magnetic field are described. Addition of hop extract antifoam, composed of non-polar molecules, to HFBI decreases the interaction of these molecules with CO_2 and therefore, decreases gushing. The influence of a magnetic field was investigated on the dispersion of hop extract antifoam and HFBI, either individually or in mixture. Gushing by HFBI is used as a measurement for the behaviour of this molecule in the presence of a hop extract antifoam with and without a magnetic field. When hop extract antifoam and HFBI are individually exposed to magnetic field, their particles disperse and smaller sizes are formed. The dispersed hop extract antifoam has more specific surface area with which hydrophobins interact and therefore, gushing is decreased.

The particles formed by HFBI, disperse in the magnetic field and later in the sparkling water, they could make less nucleation sites for interaction with gaseous CO_2 and therefore, gushing is decreased. The dispersion of the particles is governed by the magnetic Lorentz force combined with the turbulent flow.

When HFBI and 2.5 mL hop extract antifoam are mixed and then submitted to the magnetic field, the particles segregate and smaller sizes are detected. They showed higher amounts of gushing compared to the treatment without magnet. This phenomenon shows the extensive dispersion of the hop extract antifoam which releases the gushing inducers.

According to all the results, using a magnetic field and addition of hop extract antifoam is an effective method to decrease gushing but the best method is passing the hop extract antifoam through magnetic field before mixing with the wort. In addition, the concentration of the hop extract antifoam should be as low as possible, since this extract can be dispersed extensively by magnet and shows gushing. In the brewery scale, magnetic field is used for dispersion of hop extract antifoam and very good foaming control was obtained with small amounts of the extract and no gushing was observed.

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