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

Zahra Shokribousjein [a,*], David Riveros Galan [a], Chris Michiels [a], Kurt Gebruers [a], Hubert Verachtert [a], Johan Martens [b], Christine Peeters [c], Guy Derdelinckx [a]

[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
[c] KULeuven, Expertise Unit on Educational Provision, Faculty of Bioscience Engineering, Heverlee, Belgium

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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.

* Corresponding author. Postal address: Centre for Food and Microbial Technology, Kasteelpark Arenberg 20 – box 2463, 3001 Heverlee, Leuven, Belgium. Tel.: +32 16 37 68 15.

E-mail addresses: Zahra.shokribousjein@biw.kuleuven.be, zahrashokri@gmail.com (Z. Shokribousjein).

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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-*

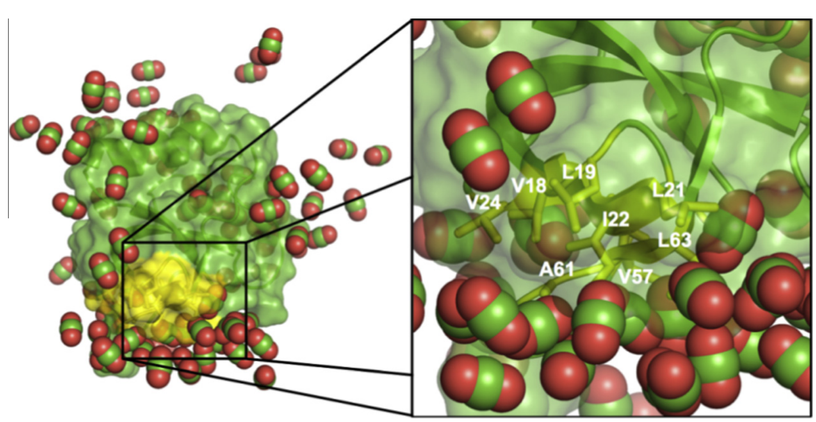

Fig. 1. Gaseous CO_2 clustering around hydrophobic patch of class II hydrophobin HFBII (Deckers et al., 2012).