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Review

Hydrophobins, beer foaming and gushing

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ABSTRACT

Hydrophobins belong to the most important proteins produced by filamentous fungi. They are surface active and their foaming potential is due to the presence of particular spatial arrangements of hydrophobic and hydrophilic amino acids. However, their presence eventually leads to overfoaming of beers. In beers and other liquids hydrophobin molecules aggregate around hydrophobic carbon dioxide molecules and form nano-structures, containing entrapped carbon dioxide. By pressure release at opening a bottle of beer, the nano-structures behave as nano-bombs. This explosion causes a sudden release of gaseous carbon dioxide, which is gushing. Several solutions to avoid or to reduce gushing, have been proposed, among which beer pasteurization and the effects of hop components have been studied. This review discusses the nature of hydrophobins, the foaming phenomenon and gushing.

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1. Introduction

Hydrophobins are extracellular (Garbe et al., 2009) surface active proteins produced by filamentous fungi (Linder et al., 2005). They are not toxic nor cytotoxic or immunogenic for humans upon consumption of mushrooms. They are small proteins with a molecular weight mostly between 7 and 15 kDa (Scholtmeijer et al., 2001). They are globular in shape with a 2–3 nm diameter (Linder et al., 2005; Linder, 2009).

Filamentous fungi and dimorphic yeasts (Ascomycetes and Basidiomycetes and Zygomycetes (Scholtmeijer et al., 2001)) produce hydrophobins. At different stages of fungal life (vegetative hyphae, sporulating, fruiting body) different hydrophobins are expressed (Linder et al., 2005). They are often extra-cellular but are also found inside fungal structures such as fruiting bodies or mycelium (Linder, 2009). The first relation between the presence of *Fusarium* sp. on barley and gushing of beer was established by Japanese researchers at EBC Congress of Salzburg (De Clerck, 1973).

Hydrophobins can also be used as emulsifiers in food processing because they are safe for humans (Linder et al., 2005; Cox et al., 2009). Cox et al. (2009) reported that 0.1 wt% HFBII forms exceptional stable foams across a wide range of solution pH conditions in simple solutions. However their presence in beer is undesirable because they are responsible for gushing (Sarlin et al., 2005, 2007). Gushing is the spontaneous and wild overfoaming of overcarbonated beverages that occurs at the opening of the container without any shaking. Gushing is the result of hydrophobins produced by contaminated barleys with moulds and unfortunately, it can only be observed at the end of the production process, after the opening of the container, and thus can cause significant economic losses to the brewer. Because of their importance in brewing, hydrophobins and their relation to foaming and gushing of beer will be discussed.

2. Hydrophobins

2.1. The function of hydrophobins

Hydrophobins have important functions in fungi as they are needed for aerial growth and adhering to solid surfaces. A model for the formation of fungal aerial structures was proposed by Wösten (2001) (Fig. 1). After a submerged feeding mycelium has been formed, the fungus secreted monomeric hydrophobin into the medium. These monomers self-assemble at the medium-air interface into an amphipathic membrane resulting in a decrease in water surface tension. It has not yet been established what happens when hyphae are confronted with the amphipathic protein layer. The hypha may stretch the hydrophobin film enabling intercalation of newly secreted hydrophobin monomers without

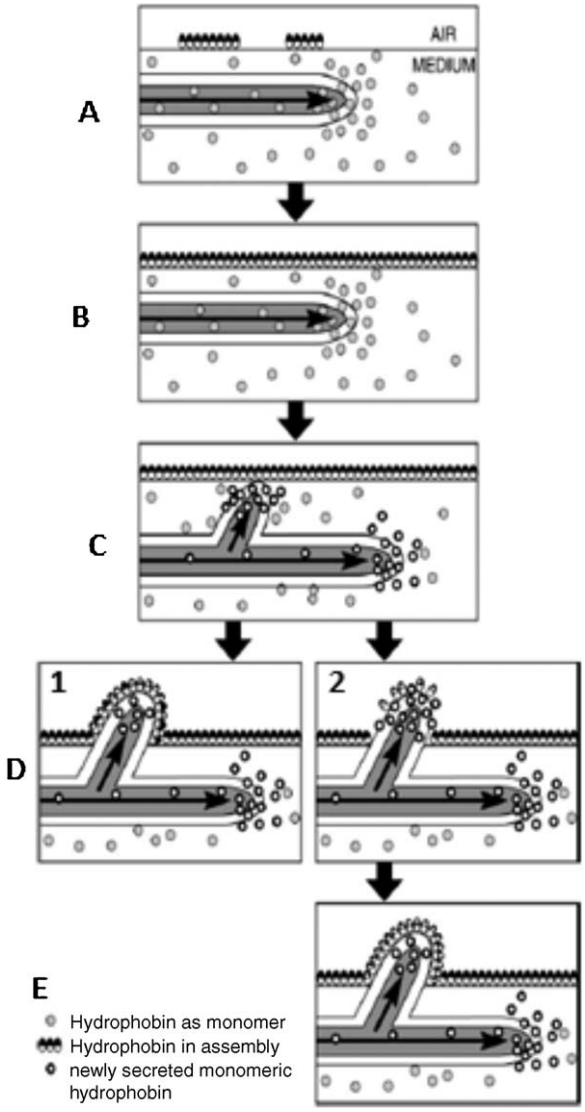

Fig. 1. Representation of the role of hydrophobin in escape of hyphae from aqueous environment (Wösten, 2001). (A) Secretion of hydrophobin monomers, (B) formation of monolayer at the interface, (C) germination of a new hypha, (D1) without rupturing the monolayer the hypha remains in the medium, (D2) rupturing of the monolayer results in exiting of hypha from medium and (E) formation of new monolayer on the surface of exited hypha (E).

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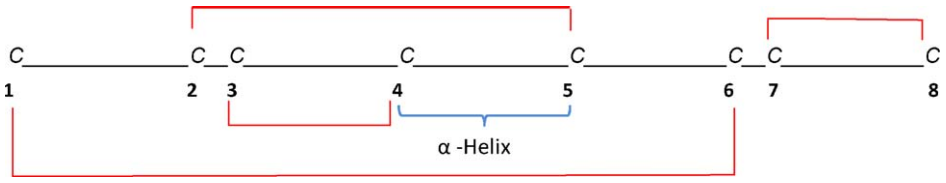

Fig. 2. The order of the eight Cys residues in the hydrophobin, four disulfide bonds are in red and α-Helix between Cys 4 and 5 is in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

rupturing the membrane (Fig. 1D left side) so that the hypha would never leave the aqueous environment. Alternatively, the hyphae break the hydrophobin membrane and the cell wall contacts the air (Fig. 1D right side). Hydrophobins secreted by such hyphae will self-assemble at the cell wall–air interface. The hydrophilic side of the hydrophobin film faces the hydrophilic cell wall, while its hydrophobic side is exposed to the air. The hydrophobin films covering the hyphae and the aqueous environment may fuse (Wösten, 2001) (Fig. 1).

They may protect parts of fungi against wetting and desiccation, by making the surface of conidia, spores and caps of mushrooms hydrophobic. They cause dispersal of spores and water mediated dispersal of conidia. Upon dispersal, fungal parts maybe involved in respiratory problems in people (Linder, 2009). They are useful for fungal nutrition: the hydrophobin layer which covers fungal aerial structures may allow nutrients in the environment to be taken into the cells and also prevent their escape from cells. Hydrophobins at the water-oil interface permit unidirectional passage of small molecules (up to 10,000 Da) from the hydrophobic side to hydrophilic side and prevent passage of molecules ranging from 300 to 10,000 Da from the hydrophilic side. This might then be significant for fungal growth (Linder et al., 2005). Pathogenic fungi relay on the surface-active properties of hydrophobins for attachment to their hosts, for example, on insect cuticles or plant leaves (Paananen et al., 2003).

2.2. The structure of hydrophobins

Hydrophobins are proteins having special spatial arrangements of hydrophobic, hydrophilic and neutral amino acids including 8 cysteine residues. Cysteins 2,3 and 6,7 are neighbors (Linder et al., 2005; Kisko, 2008).

Disulfide bridges are formed between Cystein residues as (1–6), (2–5), (3–4), (7–8). The linkage between residue 3 and 4 (in HFBII of *T. reesei*) and between 7 and 8 form a first and a second β-hairpin loop, and the amino acid residues between Cys-4 and 5 makes a α-Helix structure (Kallio et al., 2007; Kisko, 2008; Linder et al., 2005). For clarity, this is shown in Fig. 2.

It is important to note here that although hydrophobins are proteins, they are very resistant to denaturation at high temperatures (even at 90 °C) (Linder, 2009).

2.3. Classification of hydrophobins

Based on solubility and sequence comparison, hydrophobins are divided into two classes: Class I and Class II (Linder et al., 2005; Sarlin et al., 2005). Class I hydrophobins are soluble in strong acids, e.g. trifluoro acetic acid (TFA), formic acid and after removing the acid, they are functional again (Linder et al., 2005; Szilvay et al., 2007a).

Class II hydrophobins are soluble in organic solvents, e.g. ethanol (60%) (Kisko, 2008) or hot sodium dodecyl sulfate (SDS 2%) (Hektor and Scholtmeijer, 2005; Lumsdon et al., 2005; Scholtmeijer et al., 2001). In some papers, dissolution of assemblies of class II hydrophobins such as cerato-ulmin (CU) through pressure or cooling is reported (Hektor and Scholtmeijer, 2005; Scholtmeijer et al., 2001).

Differences in behavior are due to differences in tertiary structure. Class II hydrophobins have a hydrophobic part (12%) (green in Fig. 3) which is made of 2 β-hairpins including only aliphatic amino acids (Linder, 2009) and a hydrophilic part (yellow in Fig. 3) which includes one α-helix.

Class I hydrophobins are similar to Class II but without α-helix (Kallio et al., 2007) and with more amino acids and diversity in amino acid sequences (Linder et al., 2005).

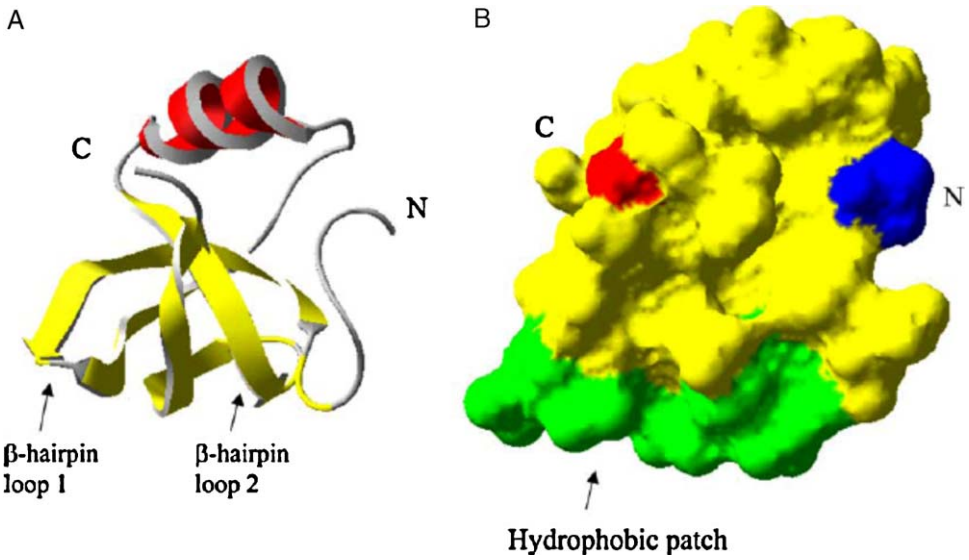

Fig. 3. Three dimensional structure of hydrophobin HFBII from *T. reesei* (Linder et al., 2005).

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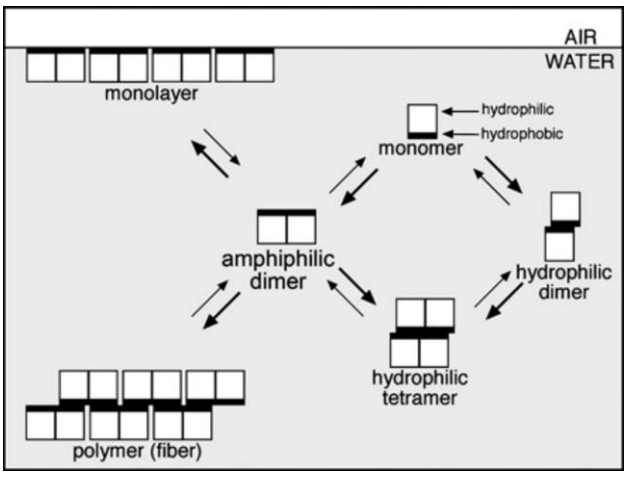

Fig. 4. Multimerisation of hydrophobins in solution and production of surface films on water/air interface (Kallio et al., 2007).

2.4. Comparison of Class I and Class II hydrophobins

Different structures of Class I and II hydrophobins lead to different properties. At first this is due to variations in amino acid sequences. Class I have more amino acids (100–125 residues) than Class II (50–100 residues) (Hektor and Scholtmeijer, 2005), and more hydrophobicity (Linder et al., 2005). The hydrophobicity of Class Ib (Class I produced by Basidiomycetes) is higher than Class Ia (class I produced by Ascomycetes) and Class II (Linder et al., 2005).

The production levels of Class II hydrophobins are higher than for Class I and their purification is easier (Linder et al., 2005). The production of class I proteins has been obtained in amounts of about mg/L by heterologous methods, but for class II, g/l amounts were reached by homologous methods (Hektor and Scholtmeijer, 2005).

Class I proteins have more β-sheets than Class II which renders them more stable (Hektor and Scholtmeijer, 2005).

Class II hydrophobins have more tendency towards foaming than Class I and gushing (Linder, 2009), but Class I proteins adhere more to surfaces than Class II (Linder, 2009).

During self-assembly at air/water interfaces, Class I proteins show changes in the secondary structure, but no such changes in secondary structure of Class II proteins have been seen (Kisko, 2008).

The thickness of the interface films of Class I is higher than with Class II (10 nm and few nm, respectively) (Kisko, 2008). Class I does not contain an α-helix and does contain two large disordered regions. So the sequence between Cys (4 and 5) is shorter in class I hydrophobins (Kallio et al., 2007).

At air/water interfaces, both mono- and multilayers are made with Class I, but only monolayers with Class II (Kallio et al., 2007).

2.5. Properties of hydrophobins

Some of the important properties of hydrophobins which are used in some industrial cases are explained here:

- At low concentrations hydrophobins are able to make solution multimers (Linder, 2009), they would be dissolved in low concentrations (less than Critical Micellar Concentration: CMC) and above CMC they make dimers, tetramers. . . and multimer forms in a solution and also monomers (Kallio et al., 2007) (Fig. 4). Because of oligomerization of hydrophobins in solvents, they are readily soluble in water (Kallio et al., 2007).

Many disulfide bridges make the hydrophobins very stable proteins, which tolerate pH and temperature changes after secretion in the soil by fungi (Kisko, 2008). Fig. 4 shows that two monomers can produce two kinds of dimers: amphiphilic dimers and hydrophilic dimers. Amphiphilic dimers are formed when two monomers are bound together without shielding their hydrophobic parts. These dimers can link together and produce fibers (hydrophilic fibers) and also move to interfaces and produce monolayers. Hydrophilic dimers are produced when two monomers bind together with a shielding of their hydrophobic parts (Kallio et al., 2007). Both amphiphilic and hydrophilic dimers can produce tetramers in solution. The tetrameric assemblies tolerate heating and changes in pH (Christian et al., 2009a).

However addition of ethanol (65%) break the tetramers into monomers (without unfolding) and salts induce formation of larger aggregates (Kisko, 2008). Changes toward tetramer formation in solution do not affect the surface activity of the hydrophobins (Kisko, 2008). In fact monomers, dimers and tetramers are all surface active (Szilvay et al., 2007b).

- Hydrophobins produce surface films at the water-air interface (Fig. 4). These films are crystalline and viscoelastic. They are important for aerial growth of fungi (Kallio et al., 2007; Linder, 2009). Surface films on the air/water interface are monolayers for Class II but mono-multilayers for Class I (Kallio et al., 2007) and films formed by hydrophobins of Class I group are much more stable than those of Class II (Garbe et al., 2009).

During film formation at the air/water interfaces, the secondary structure of the hydrophobins does not change but through attachment to solid surfaces some degree of conformational changes occur (Kisko, 2008).

The self-assembly of molecules in interface films is mediated by non-covalent bonds (Kisko, 2008).

The difference between Class I and Class II films is that the Class I films are insoluble and turn into rodlets when the film is dried on a solid surface (the diameter of rodlets is within the range of 5–12 nm) (Garbe et al., 2009), and an α-helical structure is involved in the formation of the rodlet form (Garbe et al., 2009). The class II films are soluble reversibly, even when dried on a solid surface (Linder, 2009).

When the films are dried on a solid surface and studied by electron microscopy or atomic force microscopy (AFM), it is found that class I hydrophobins change to rodlets but class II change to rods, needles and fibrils (Fig. 5a and b) (Linder et al., 2005).

- Hydrophobins cause beer gushing and this is due to their foaming ability (Linder et al., 2005). Foaming ability and stability of hydrophobins have been seen in both classes but foaming tendency is stronger for Class II than class I (Linder, 2009). Beer gushing is caused by only Class II hydrophobins (Linder et al., 2005).
- Hydrophobins coat surfaces and so lower the surface tension. The surface tension will be decreased more in air/water interfaces than in liquid/liquid interfaces (Lumsdon et al., 2005).
- Hydrophobins can change the hydrophobic surfaces to hydrophilic (Teflon) (Fig. 6A), change hydrophilic surfaces (glass or paper) to hydrophobic surfaces (Fig. 6B) (Lumsdon et al., 2005).
- In low-molecular weight surfactants (500–1000 g/mol) adsorption at the oil/water or air/water interfaces happens very soon and spontaneously, but since hydrophobins are high-molecular weight molecules (7–15 kDa), their adsorption takes more time. But it is interesting that the amount of hydrophobins sufficient to reach a specific low surface tension is much lower than the

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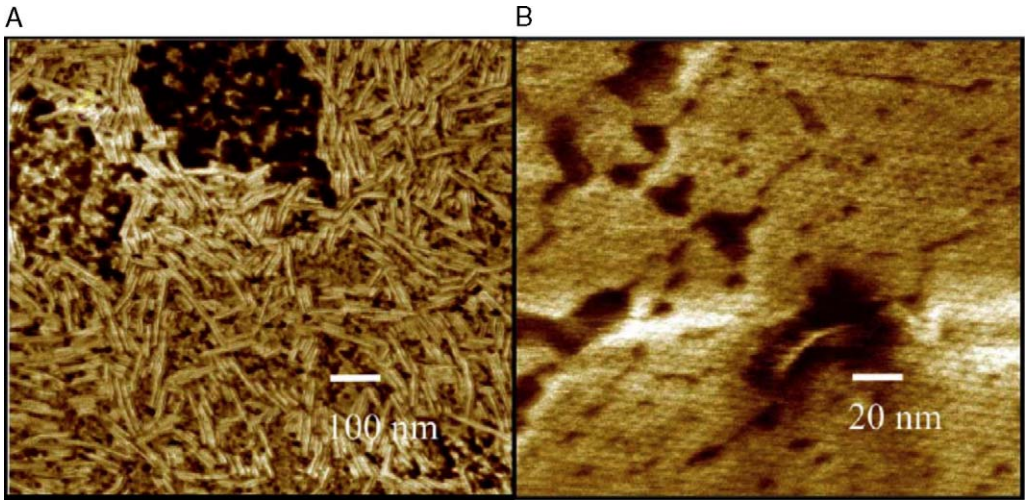

Fig. 5. (A) An atomic force microscopy (AFM) image of a *S. commune* SC3 sample showing rodlets. The sample was prepared by drying down a solution of protein on a sheet of mica. (B) An AFM image of an ordered film of *T. reesei* HFBI on the air/water interface. The film was deposited on a mica support using the Langmuir–Blodgett technique (Linder et al., 2005).

amount of other smaller molecular weight surfactants (Lumsdon et al., 2005).

- Hydrophobins can act as surfactants and emulsifiers (Linder et al., 2005).
- Immobilization of proteins and enzymes, e.g. some hydrophobins and EGI (an endoglucanase) co-immobilize on hydrophobic surfaces (Teflon). Denaturation of enzymes does not occur and EGI activity is retained upon binding. Binding is as monolayer (Linder et al., 2005).
- Application in protein–protein interaction columns. Thus hydrophobins bind to the hydrophobic glyoxyl-agarose surfaces, then lipase bind to hydrophobins by their hydrophilic part, allowing the hydrophobic part of lipase, which has the enzymatic activity, to remain free and also stable to heat treatment (Linder et al., 2005).

One of the most important properties of Class II hydrophobins are related to gushing of beverages, especially beer. For a good understanding of the gushing phenomenon, the foaming process must be treated first.

3. Foaming

3.1. Foam from formation to detachment

Foam is an emulsion of gas (i.e. dispersed phase) in liquid (i.e. continuous phase) that contains a soluble surfactant (Lewis and Bamforth, 2007). Foaming is a process including: bubble formation, bubble growth and bubble detachment (Fig. 7). There are two kinds of food foams; those created and consumed within a short space of time (i.e. long-term stability is not important) such as in beer and milk-shake and those where the rheology of the continuous phase is increased to stabilize the foam over a long space of time such as ice cream and mousse (Cox et al., 2009). The factors influencing the foam stability and the destabilization will be discussed in Section 3.2.

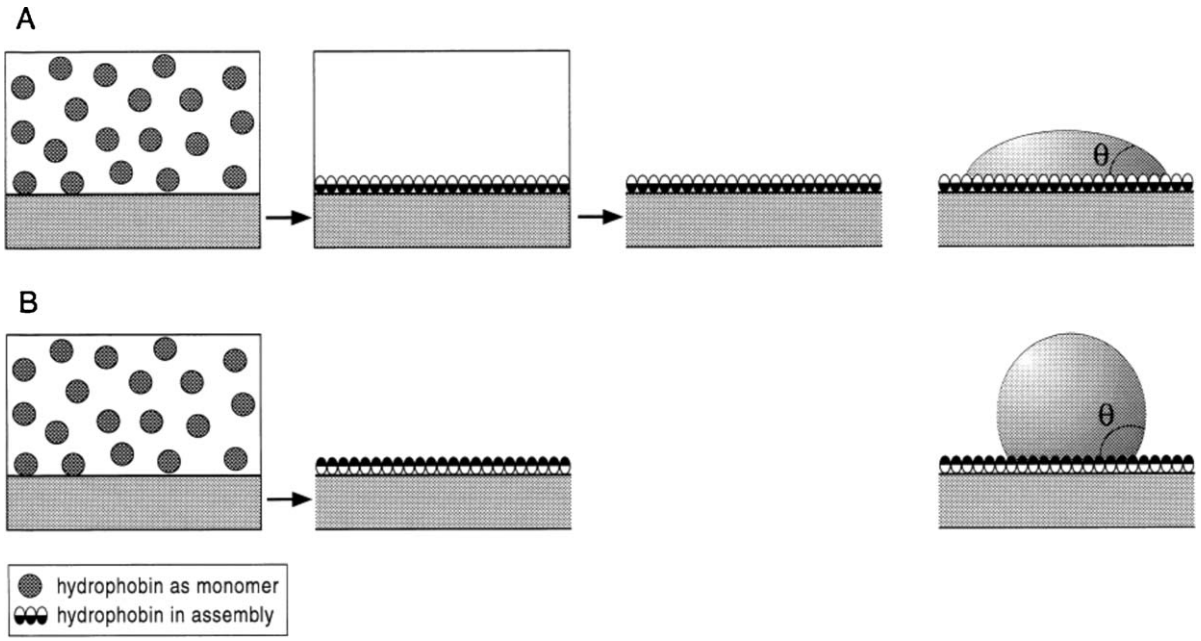

Fig. 6. Change of the nature of a surface by self-assembly of hydrophobins. (A) Change of the hydrophobicity into hydrophilicity and (B) change of the hydrophilicity into hydrophobicity (Wösten and de Vocht, 2000).

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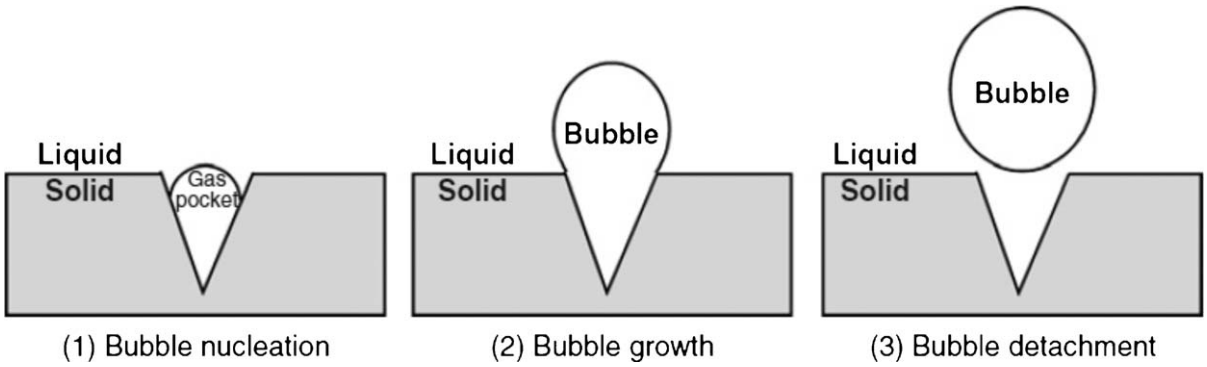

Fig. 7. Foam process (Evans and Bamforth, 2009).

3.1.1. Bubble formation

Even in supersaturated beverages, the formation of bubbles needs a nucleation process which may take place by the presence of particles, fiber or scratches in the glass surfaces (Prins and van Marle, 1999).

There are four types of bubble nucleation in a solution (Deckers et al., 2010a, 2011; Jones et al., 1999).

Type I (Classical homogeneous nucleation): this type is nucleation in a homogeneous solution. Because of the absence of gas cavities prior to the supersaturation, the required level of supersaturation is very high, in excess of 100 ATM or more (Fig. 8) (Jones et al., 1999).

Type II (Classical heterogeneous): In this type, foreign material or cavities, in the bulk or on the surface of the container must be present. The system is suddenly made supersaturated, for example by a sudden pressure reduction, resulting in a classical nucleation event. A bubble may then form in a pit in the surface of the container, on a molecularly smooth surface, or on a particle in the bulk. The bubble then grows, and detaches, leaving behind a portion of its gas. The production of the first bubble is referred to as type II nucleation (Fig. 8) (Jones et al., 1999).

Type III (Semi-classical nucleation): This nucleation includes homogeneous and heterogeneous nucleation at pre-existing gas cavities at the surface of the container, at the surface of suspended particles, and as metastable micro-bubbles in the solution bulk. As determined by the classical theory, even after supersaturation, the radius of curvature of each meniscus is less than the critical radius. Hence, for each cavity there exists a finite nucleation energy barrier which must be overcome. When supersaturated, local fluctuations in supersaturated environment occur, these are responsible for bringing to life the nucleation sites. Type III nucleation is achievable at low levels of supersaturation (Fig. 8) (Jones et al., 1999). The difference between this type and type II is that, before supersaturation, there is no bubble in type II but in this type, even before supersaturation, the bubbles are formed.

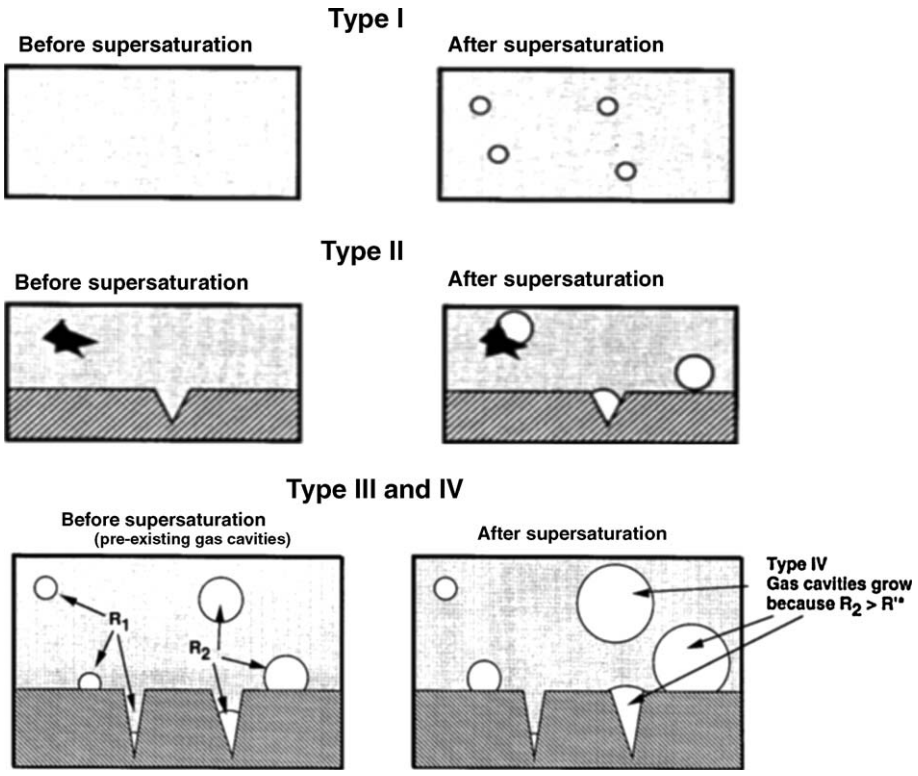

Fig. 8. Schematic nucleation types: Type I Classical homogeneous nucleation producing gas bubbles in the bulk at high levels of supersaturation of 100 ATM or more. Type II Classical heterogeneous nucleation, catalyzed by the presence of another material in the liquid. Type III, Gas cavities of size R1 < R'* may or may not grow, depending on local supersaturation fluctuations, Type IV non classical nucleation (Jones et al., 1999).

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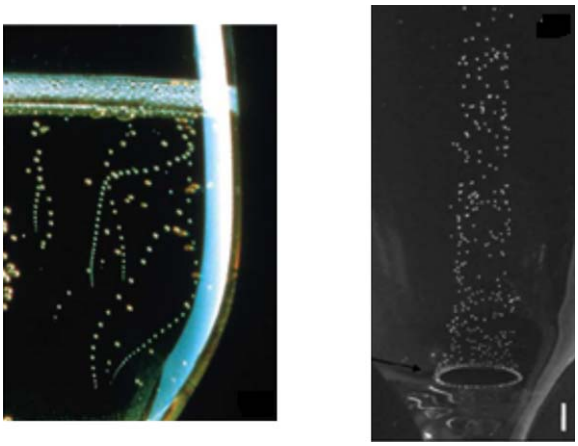

Fig. 9. "Natural" (left side) and artificial effervescence (Liger-Belair et al., 2008).

Type IV (Non-*classical* nucleation): This nucleation is considered non-classical because there is no nucleation energy barrier (activation energy) to overcome. The nucleation usually occurs at pre-existing gas cavities in the surface of the container or elsewhere in the liquid bulk, and may follow type II or type III nucleation events. Pre-existing gas cavities housing menisci with radii greater than the critical nucleation value provide a stable source for bubble nucleation. Over time, as the supersaturation decreases, the critical nucleation radius, increases to a value equal to the radius of a given cavity meniscus, and bubble production from that cavity ceases. This kind of nucleation is responsible for sustaining the cycle of bubble production in carbonated beverages, for example, long after the bottle is opened, or the liquid is poured into the glass (Fig. 8) (Jones et al., 1999).

As it is obvious from above, in type III and IV, pre-existing gas cavities is the reason for nucleation (Deckers et al., 2010a, 2011; Jones et al., 1999).

Bubble formation in beverages is heterogeneous nucleation from cavity walls and categorized as "type IV" nucleation. Cavity nucleation is typified by bubble formation from a gas–liquid interface entrapped in a wall cavity. On detaching, the bubble forms an elongated neck, which breaks to release the bubble and form a new gas–liquid interface for further bubble growth.

Bubble nucleation takes place within those areas of the substrate bounded by the abrasions, rather than within the abrasions themselves (Barker et al., 2002). When a supersaturated liquid is poured into a glass, bubbles are not formed in the whole liquid but appear at a specific spot. In this case, the type IV can be divided in "natural" and "artificial" bubble nucleation (Fig. 9). "Natural" effervescence is a bubbling process from a glass which has not experienced any specific surface treatment (Liger-Belair et al., 2008). The bubble nucleation sites were found to be located on preexisting gas cavities trapped inside cylindrical cellulose fibres (adhere to the glass by electrostatic force) and from gas pockets trapped inside tartrate crystals (precipitated on the glass wall resulting from the evaporation process after rinsing the glass with tap water) (Fig. 9, left side) (Liger-Belair et al., 2008; Polidori et al., 2009). Artificial effervescence is related to bubble nucleation from glasses with imperfections produced intentionally by the glassmaker (Fig. 9, right side) (Liger-Belair et al., 2008). The frequency of bubble formation from a given nucleation site is found to progressively decrease with time due to the fact that the concentration of CO_2-dissolved molecules progressively decrease as CO_2 continuously desorbs from the supersaturated liquid (Liger-Belair et al., 2008).

How is foam formed according to the above mentioned description?

Bubbles smaller than the critical size disappears altogether, unless the local energy fluctuations remain large enough, for long enough time. At high levels of supersaturation, the critical embryo size is correspondingly very small, and hence the presence of sufficient gas molecules coming together to achieve a successful nucleation is in high probability. By the presence of a solid substrate in the solution, the interfacial free energy of the nucleus lowers, by assuming that the interfacial energy between the solid and the liquid is lower than the interfacial energy between the liquid and the gas. In effect, the solid acts as a catalyst, lowering the size of the nucleation energy barrier. Although the radius of curvature of the critical size nucleus is unchanged, the number of molecules required to form the nucleus is reduced. Consequently, the nucleation becomes more probable. Heterogeneous nucleation, involving water in contact with smooth hydrophobic surfaces, still requires supersaturations of 100 ATM or more (Jones et al., 1999).

3.1.2. Bubble growth

Once the nucleation process has been completed, the bubble is free to grow and eventually to detach from the substrate. The growth rate is influenced by several parameters such as rate of molecular diffusion to the interface of the bubble, liquid inertia, viscosity and surface tension (Jones et al., 1999).

Bubble size is related to the degree and nature of the abrasions, and cannot be attributed to an enhanced rate of cavitation from the abrasions themselves (Barker et al., 2002).

As bubble size affects the sensory impact of the beverage, this becomes a very important quality factor. For example champagne is a superior beverage in comparison with sparkling wines, because it has smaller bubbles. The presence of small bubbles leads to enhanced mass transport of carbon dioxide when it is impinged upon the tongue, increasing the "tingling" sensation caused by the conversion of CO_2 to H_2CO_3 by the enzyme carbonic anhydrase. For decreasing the bubble size it is useful to add surfactant (Barker et al., 2002).

3.1.3. Bubble detachment

There are different forces affecting the bubble rise or rest in the gas cavity:

$$F_d + F_s = F_I + F_p + F_b$$

F_d is the relative velocity between the surrounding fluid and the bubble, caused by the bubble growth produced drag force; F_s: the surface tension; F_i: inertia force; F_p: pressure force; F_b: buoyancy force.

F_d and F_s are responsible for holding the bubble in the substrate, while, F_i, F_p and F_b are responsible for pulling the bubble out of the substrate. According to this equation, when overfoaming occurs, the right hand of this equation (sum of forces which pull the bubbles out of the liquid) is more than the left part. So for preventing the overfoaming, it is very useful to decrease the pulling forces for example by degrading the surfactants (Jones et al., 1999).

In this paper on beer overfoaming, the properties of beer constituents affecting foam will be considered. Different factors in general affect foam stability.

3.2. Foam stability

3.2.1. Factors which affect foam stability

The important factors which affect foam stability are:

(1) Surfactant (Fruhner et al., 1999): Molecular weights and size of complexes of surfactant affect foam stability: higher weight complexes cause lesser foam stability (Murray, 2007).

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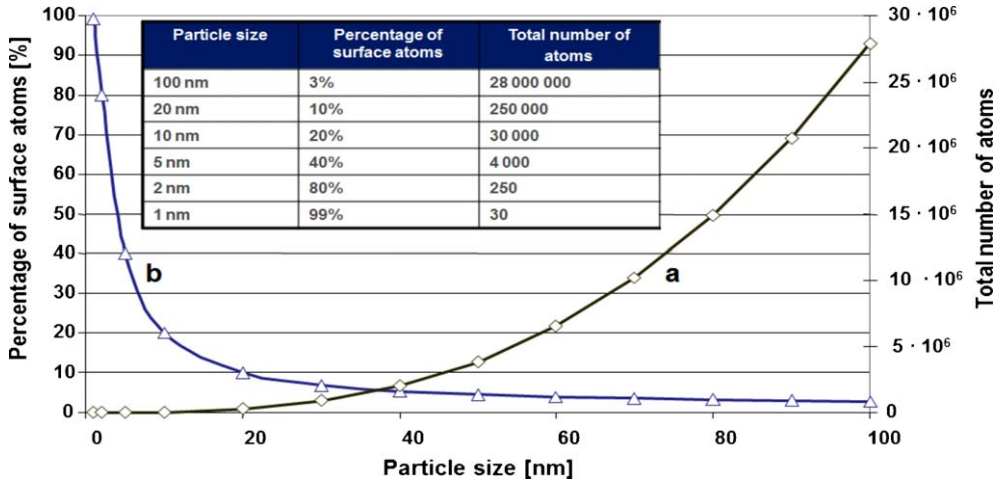

Fig. 10. Total number of atoms of a particle in dependence of particle size (a); and the percentage between surface atoms and total number of atoms of a particle in dependence of particle size (b) (Titze et al., 2010).

The surface potential of a particle is related to "Size" and "Electric charge".

Size means ratio of the surface to volume of a particle.

$$x = \frac{o}{V} = \frac{4\pi r^2}{4/3\pi r^3} = \frac{3}{r}$$

o is the surface and V is the volume.

When a particle is small, r will be small and the ratio will be high and vice versa (Titze et al., 2010).

This is shown in Fig. 10:

According to the above curve (Fig. 10b), as the particle size increases, the percentage of surface atoms decreases. This principle shows the importance of particle size in connection with particle surface. The particle surface is directly related to particle charge and surface potential, being the reason of interface phenomena. Among the materials with the required properties for a surfactant (surface atoms, size. etc).

Proteins are excellent and are good foaming agents because:

(a) they strongly adsorb to air/water interfaces
(b) they tend to give steric and electrostatic stabilization
(c) the adsorbed films tend to have structural coherence (Murray, 2007).

Any factor which increases the exposure of hydrophobic amino acids of protein to the solvent, will increase protein surface activity such as unfolding due to heat and addition of detergent (Murray, 2007). Hydrophobins are protein compounds, they are very good surfactants and can produce foams with high stability.

(2) Physicochemical properties of the foaming solution.
(3) The interaction of foam with the surrounding (Fruhner et al., 1999).

3.2.2. Destabilization of food foams

Foams will be destabilize according to 4 mechanisms; include Drainage, Ostwald ripening, Coalescence and Gas diffusion (Cox et al., 2009).

(1) Drainage: drainage happens when a wet foam turns to a dry foam due to drainage of liquid from the bubbles as a result of gravity of liquid. In dry foams, bubbles' film weakens and leads to bubble collapse (Ronteltap et al., 1991) (Fig. 11A).
(2) Ostwald ripening: Ostwald ripening tends *to Disproportionation,* meaning that the gas inside of a little bubble will diffuse to the larger ones because of pressure difference and Laplace pressure. Laplace pressure is measured according to this equation: $\Delta P = P_{inside} - P_{outside} = 2\gamma/r$ where: P_{inside} = pressure inside of the bubble, $P_{outside}$ = pressure outside of the bubble, γ = surface tension and r = radius of the bubble. According to this equation, the little bubbles have higher inside pressure than larger ones, resulting in diffusion (Fig. 11B). The factors affecting disproportionation are: gas content and bubble film thickness (Ronteltap et al., 1991; Bamforth, 2004a).

Disproportionation causes the radius of the bubbles to increase and the film around the bubble will be weaker which causes the bubbles to collapse.

For providing the elastic interfacial layer in foams, a thick insoluble interfacial layer (of the order of the emulsion droplet radius) is required to prevent disproportionation (Cox et al., 2008).

(3) *Coalescence*: This occurs when there are many bubbles with the same size and the same CO_2 pressure inside. They adhere together. The new larger ones are less stable (Ronteltap et al., 1991). Coalescence occurs as a result of a "hydrophobic particle mechanism" or of a "particle spreading mechanism" (Ronteltap et al., 1991) (Fig. 11C). Lipids in beer promote coalescence and destabilize foam stability (Ronteltap et al., 1991).
(4) *Gas diffusion*: when a bubble is in contact with the atmosphere, the pressure inside of the bubble is bigger than the atmospheric pressure and the gas diffuses out of a bubble. The bubble size decreases and consequently the pressure difference increases and there will be an acceleration of gas loss with time. Viscoelasticity of bubble surface would reduce the rate of gas diffusion (Dutta et al., 2004).

The higher the rate of Ostwald ripening, the lower the amount of gas loss is. Thus it can be concluded that the Ostwald Ripening retards the gas diffusion by transferring the gas to larger bubbles (Dutta et al., 2004).

According to the above mentioned foam destabilization factors, the rate of gas loss depends on these elements: Size of the bubbles, surface tension of the bubble (high surface tension makes the interface more stable and prevents that air passes across), permeability of the liquid film and distance from the free gas–liquid surface (Dutta et al., 2004); (as much as this distance is larger, the gas diffusion will be lesser).

3.2.3. Beer components that influence foam stability:

Several ingredients in beer affect foam stability.

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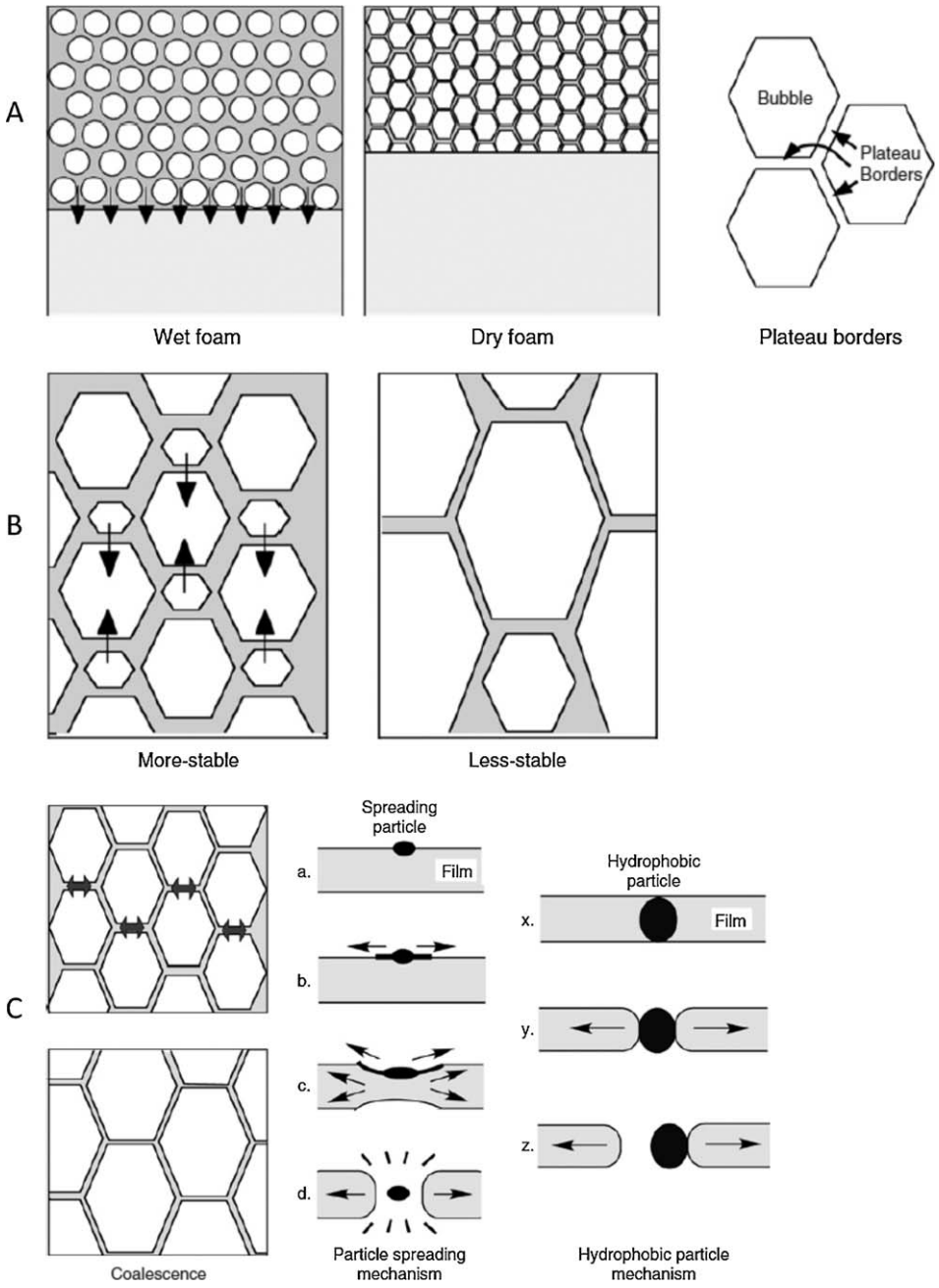

Fig. 11. Drainage (A), Ostwald ripening (B) and coalescence (C) (Evans and Bamforth, 2009).

1- *Proteins*:

(a) Lipid transfer protein (LTP1): ns-LTP1 is an abundant soluble protein of the aleurone layer from the grain endosperm. LTP1 is a basic protein in which amino acids such as lysine, histidine and arginine are responsible for the basic nature of ns-LTP1 (Hippeli and Elstner, 2002). Barleys with higher levels of LTP1 are found in more humid or wet environments. The LTP1 is a defense protein in barley, and in more humid growth conditions favoring the presence of more insects and pathogens, more LTP1 is produced (Evans and Bamforth, 2009). This component improves foam stability. Conformational changes cause LTP1 denaturation and can be deleterious for foam stability, especially when fatty acids are present (van Nierop et al., 2004). In comparison with its direct role as a foam promoting protein, the lipid binding capacity of LTP1 has a more important impact on foam stability (Bech et al., 1995).

(b) Hordeins: Hordeins are the major storage proteins of barley. They belong to the prolamine group of proteins insoluble in aqueous solutions and require proteolytic hydrolysis to become water-soluble. They improve foam stability but because of non-solubility in water, during brewing they should be submitted to proteolyltic enzymes to enter the final beer (Evans and Bamforth, 2009).

(c) Protein Z: is an albumin type protein that can improve foam stability. Although it has the highest surface viscosity and elasticity properties of all beer proteins, it has not been shown to be preferentially enriched in foams as observed for LTP1. Interactions between protein Z and other proteins

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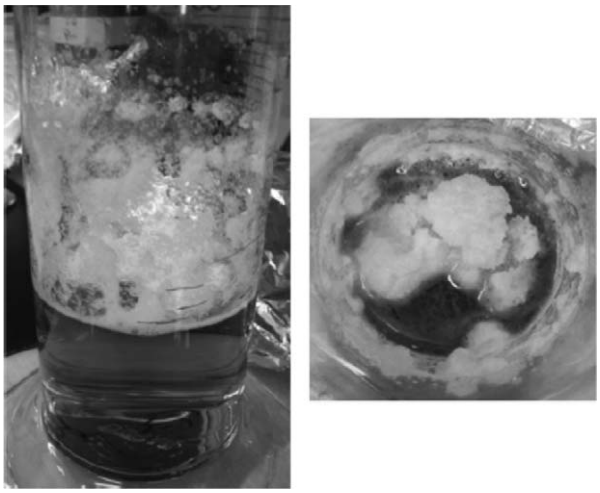

Fig. 12. Foam in "whipped egg-white icebergs" in tetra hop beers (Evans and Bamforth, 2009).

such as LTP1 are important for foam stability (Douma et al., 1997).

(d) Hydrophobins: the effects of hydrophobins are discussed below.

2- *Non-starch polysaccharides*: arbinoxylan and β-glucan or even oligosaccharides improve foam stability by increasing beer bulk viscosity, thus reducing the drainage of the liquid from foam, although beer viscosity is likely to be a minor factor (Stowell, 1985; Archibald et al., 1988; Lusk et al., 1995; Evans and Hejgaard, 1999; Evans and Sheehan, 2002; Lewis and Lewis, 2003).

3- *Hop acids*: Iso-α-acids of added hops cross-link with protein, and improve foam stability. But if they are used in large amounts (hydrogenated iso-α-acids) predominantly tetra hopped beers will degrade to produce a foam that is like "whipped egg-white icebergs" (Fig. 12) and foam stability is lost. As these acids can produce bad taste "vulcanized rubber" in the final beer, only low addition of hydrogenated iso-α-acid hop is a useful tool in optimizing foam quality. A high proportion of isohumulone to coisohumulone will result in more stable foams (Asano and Hashimoto, 1976).

4- *Cations*: Metal cations promote beer foam stability and gushing (Rudin, 1957; Rudin, 1958; Rudin and Hudson, 1958; Archibald et al., 1988). Multivalent cations improve foam stability via reversible cross-linking with hop acids and proteins (Simpson and Hughes, 1994).

5- *Lipids*: Sources of lipids in beer are mostly malt but also hops and yeasts producing lower molecular weight fatty acids such as C_6 or C_8 (Blum, 1969; Anness and Reed, 1985; Letters, 1992; Narziss et al., 1993). In the finished beer little amounts of lipids remain present. Among lipids, glycolipids are twice as damaging to foam stability as phospholipids or neutural lipids (Letters, 1992). Longer fatty acids (C_{16}, C_{18}) are more foam destabilizing than shorter fatty acids (C_6 to C_{10}). The degree of desaturation of fatty acids with double bonds ($C_{18:1}$, $C_{18:2}$) increases destabilization of foams (Wilde et al., 2003).

Long chain, saturated fatty acids are more hydrophobic, hence they have a more negative foam impact (Evans and Bamforth, 2009).

Among the most destabilizing fatty acids for foams, di and tri- hydroxyloctadecanoic acids are known. Their foam destabilizing effect will remain in beer because they are not utilized by yeasts during fermentation (Kobayashi et al., 2002).

By addition of lipids to beer, at first the foam destabilizes but after a rest for 24 h, its foam can be either fully or partially recovered. The reason for this reaction is the presence of lipid binding proteins in beer and the degree of recovery is related to the level of these proteins in beer, their state and the amount of lipids (Roberts et al., 1978; van Nierop et al., 2004).

There is no evidence that essential oils from hops have any impact on foam stability at the levels found even in the "hoppiest" products (Evans and Bamforth, 2009).

6- *Ethanol*: Different results on foam stability were obtained for ethanol. In 1996 it was found with a micro-conductivity test that high levels of ethanol reduced foam stability while low concentrations improved foam stability as evaluated by the Rudin test (Brierley et al., 1996). In 2003 a relatively weak positive association between ethanol and foam stability was observed as measured by the Constant method (Lewis and Lewis, 2003). Certainly, one readily observes that following the application of high concentrations of ethanol to foam, it collapses immediately (in the lacing index test), and so ethanol seems to act in an analogous fashion to lipids – disrupting interactions between proteins and iso-α-acids (Evans and Bamforth, 2009).

7- *Polyphenols*: polyphenols are of minor importance for foam stability (Evans and Bamforth, 2009).

8- *pH*: The pH of beer has an important impact on foam stability. The amphipathic nature of both proteins and hop acids leads to change of their surface characteristics and charge with pH. Certainly fermentation and the carbonation of beer with CO_2 will lower the pH. Lower pH values would be expected to result in greater dissociation of hop acids and protein charge to aid migration into foam and their interaction. Increasing concentrations of CO_2 will also of course push the equilibria of bubble nucleation towards formation, and also has a positive effect towards creaming and bubble recruitment and both will improve foam stability. In model systems studied in 2003 higher foam stabilitiy was found in the pH range of 3.8–4.6 (Evans and Bamforth, 2009).

9- *Amino acids*: Basic amino acids (arginine > lysine > histidine) interfere with the protein–iso-α-acid interaction to inhibit lacing (Furukubo et al., 1993; Honno et al., 1997).

10- *Malt manipulation*: Higher colored malt contains less foam active proteins available for extraction into beer (Ishibashi et al., 1997), such as LTP1 and Z7 but not Z4. Proteins like LTP1 and Protein Z are degraded with heating treatments, and their effect on foam stability is decreased (Ishibashi et al., 1996, 1997; Kakui et al., 1999; Evans and Hejgaard, 1999; van Nierop et al., 2004). The levels of β-glucan and arabinoxylan decrease during malt modification. Increasing their level is not a practical way of increasing foam stability (Evans and Bamforth, 2009).

11- *Removing of acrospires*: Acrospires include basic amino acids and trans-2-nonenal, so removing them will result in higher foam stability (Tada et al., 2004; Nishida et al., 2005).

12- *Adjunct selection*: Addition of wheat to barley causes more foam stability because of: (a) higher protein content of wheat than of barley (Bamforth, 1985), (b) the amount of arabinoxylan of wheat is also higher than in barley (Stowell, 1985). Thus the viscosity of finished beer will be higher which causes more foam stability (c) the size of bubbles will be decreased which results in higher foam stability (Kakui et al., 1999), (d) the puroindoline (lipid binding protein) level in wheat is high, and in beer there will be less lipids and foam stability will be higher (Douliez et al., 1999; Evans and Sheehan, 2002).

13- Brewing process

13.1 Mashing temperature: to produce stable foams in beer it is important to extract proteins as much as possible from malt to beer.

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13.1.1 If the mashing temperatures are low (<55 °C), the proteolysis remains active and causes loss of foam promoting proteins. More basic amino acids remain present in beer which cause foam destabilization as previously mentioned (Palmer, 2006; Evans et al., 2005; Jones, 2005).

13.1.2 If mashing is performed at high temperature (71 °C), protease activity is inhibited. Proteins are less degraded and keep their effect on foam stability. Hordein-derived polypeptides appear in beer and show their foam stabilizing effect .The enzyme lipoxygenase is deactivated and the production of fatty acid hydroperoxides which destabilize foams, stops (Ishabashi et al., 1997; Sheehan and Skerritt, 1997). If mashing is done at higher temperatures (>70 °C) the production of foam stabilizing glycoproteins occurs (Narziss et al., 1982a,b).

As a whole, mashing temperatures of 65 °C or higher have some benefits on foam stability but over 65 °C it results in reduced fermentation because of inactivation of some starch hydrolyzing enzymes including β-amylase (Evans et al., 2005).

13.2 Milling: Wet milling may improve foam stability, leading to increased levels of polypeptides in wort and beer. This was possibly due to inhibition of proteolytic enzymes (Kano and Kamimura, 1993).

13.3 Wort boiling (the best is at 103 °C) (Narziss et al., 1993): Wort boiling leads to foam promoting of beer by different reactions, such as increased hop acid extraction and isomeration, stopping of malt enzymatic reactions, concentration of wort, and increased Maillard reaction. The Maillard reaction improves foaming stability. The reason is increased glycosylation of protein Z and LTP1 which tends to more flexibility of molecules to move to the air/water interface (Roberts, 1975; Jackson and Wainright, 1978; Lusk et al., 1995; Hughes and Wilde, 1997; Curioni et al., 1995).

13.4 Pitching yeast into high gravity wort: this leads to severe stress on the yeast and reduces secretion of foam promoting proteases, thus reduing foam stability (Evans and Bamforth, 2009).

13.5 Yeasts: yeasts excrete proteinase A, which slowly degrades hydrophobic foam promoting proteins (LTP1), leading to foam destabilization (Shimizu et al., 1995; Wang et al., 2005).

13.6 Centrifugation: this process is used for yeast separation and due to the increased temperature, gravitational and shear forces, it reduces foam stability (Ormrod et al., 1991; Haukeli et al., 1993; Kondo et al., 1998).

13.7 Pasteurization: causes denaturation of enzymes like proteinase A which is detrimental for foam stability (Evans and Bamforth, 2009) and thus it may favor foam stabilization in beer.

14- *Enzymes*:

(a) Proteinase A: by degrading LTP1 and other foam stabilizing proteins, causes reduction of foam stability.

(b) Lipoxygenase: this enzyme produces fatty acid hydroperoxides which destabilize foams (Evans and Bamforth, 2009).

15- *Detergents*: Reduce foam stability by increasing foam coalescence (Evans and Bamforth, 2009).

3.2.4. Increasing of beer foam stability

1) Use N2 instead of CO_2 because:

(a) N2 has a lower partial pressure than CO_2. Thus it forms smaller bubbles (Carroll, 1979; Fisher et al., 1999).

(b) N2 has a lower aqueous solubility and thus reduced gas diffusion and disproportionation. The bubbles will be more stable (Carroll, 1979; Mitani et al., 2002; Bamforth, 2004a).

(c) N2 produces a more "creamy" foam texture while CO_2 makes the texture "prickle" (Carroll, 1979).

(By decreasing the CO_2 content in beer, replacing it with N_2, the beer will taste more watery and flat).

2) Apply nucleated glassware such as "headkeeper style" (Parish, 1997) or Widgets. It is a good solution but expensive (Evans and Bamforth, 2009).

3) Addition of PGA (Propylene Glycol Alginate): it has both desirable and undesirable consequences. Desirable impacts include: (a) PGA protects foam against impact of deleterious lipids (Jackson et al., 1980; O'Reilly and Taylor, 1996) and (b) PGA compensates the loss of protein due to papain treatment (Lusk et al., 2003). Undesirable impacts are: (a) it is a foreign chemical ingredient, (b) PGA is expensive and (c) PGA produces undesirable storage haze (Evans and Sheehan, 2002).

4. Gushing

4.1. Introduction

Gushing is a phenomenon observed with many carbonated beverages such as beer, in which without any agitation it vigorously overfoams on opening its container (Sarlin et al., 2007).

Gushing is the result of two mechanisms: nucleation and growth of bubbles (Sahu et al., 2006).

There are two types of nucleation to be considered: (1) homogeneous nucleation and (2) heterogeneous nucleation.

Homogeneous nucleation occurs only for supersaturated liquids because high levels of CO_2 are required and it is unlikely to occur in beer. Heterogeneous nucleation is related to pre-existing microbubbles in beer. The microbubbles with radius smaller than critical Diameter Size (CDS) will disappear and the larger will grow. By growth of these microbubbles, they explode after releasing of pressure by opening the bottle and gushing occurs.

4.2. Gushing types

There are two types of gushing:

a) Primary gushing induced by hydrophobins: Primary gushing occurs when using barley contaminated by filamentous fungi (*Fusarium* sp., *Trichoderma* sp., *Nigrospora* sp., *Aspergillus* sp., *Penicillium* sp., *Stemphylium* sp.) and is due to the presence of excreted hydrophobins. Hydrophobins could be found three weeks after seeding and their formation continued throughout the growing period of barley (Sarlin et al., 2007). Hydrophobins were also produced during malting, especially during the steeping and germination steps. Over tenfold higher amounts of hydrophobins were found in malt, compared to those in the corresponding barley If the relative concentration of hydrophobin in malt is 100%, only approximately 10% of the original hydrophobin content is present in the finished beer. The concentration needed to induce gushing depends on the fungus but a small concentration of 1 mg/L or even less is enough (Garbe et al., 2009; Sarlin et al., 2005). There is a consensus on a common mechanism for gushing in carbonated beverages (Fischer, 2001; Deckers et al., 2010a, 2011; Sahu et al., 2006; Draeger, 1996). Surface active molecules such as hydrophobins stabilize CO_2-bubbles by agglomeration at the gas–liquid interface of the CO_2-bubble. In a closed container, bubbles stabilized

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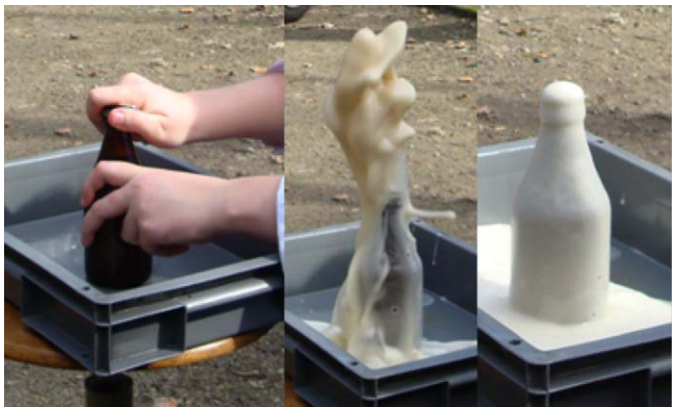

Fig. 13. Picture of gushing induced artificially after addition in a beer of a mycelium extract of *T. reesei* containing HFBI hydrophobin.

by hydrophobin are present. At the opening, the release of pressure is responsible for the growing of bubbles and their explosion (Deckers et al., 2010a, 2011). This explosion would bring the required energy for the simultaneous nucleation of many other bubbles, bubble growth, explosion and the gushing would appear instantaneously (Deckers et al., 2010a, 2011). Primary gushing is shown in Fig. 13.

b) Secondary gushing which seems to be the least problematic form of gushing (Garbe et al., 2009) is induced by haze, metal ions, calcium oxalate crystals, cleaning agents of bottles (tensides), excess of gas in bottled beer (Sarlin et al., 2005), isomerized hop extracts, the crown cork, filter aids and the inner surface of the bottle (Garbe et al., 2009).

Among the metal ions, Fe^{3+} can be responsible for gushing .The presence of Ni^{2+} and Co $^{2+}$ seems to be less important (Kieninger, 1976; Weideneder, 1992; Zepf, 1998).

The major source of calcium and oxalic acid is malt, brewery water and to a small extent hops. Ca^{2+} leads to gushing in concentration of about 20–30 mg/kg beer (Garbe et al., 2009).

At a concentration of 15 mg/kg, calcium oxalate in beer will precipitate as crystals (Jacob, 1998; Madigan et al., 1994; Schur et al., 1980; Schildbach and Müller, 1980; Zepf and Geiger, 1999, 2000). These crystals form nucleation sites and induce the release of CO_2 resulting in gushing (Burger and Becker, 1949; Brenner, 1957).

Isomerized hop extracts such as dehydrated humulinic acid are strong gushing promoters while hydrogenated iso-alpha acids (i.e. tetrahydro and hexahydro iso-alpha acids) are weak promotors. Polyunsaturated fatty acids present in hop oil show inhibitory effects on gushing (Carrington et al., 1972; Laws and McGuinness, 1972; Outtrup, 1980).

Crown corks cause gushing by the release of remaining detergents in beer, or by releasing iron ions from the scratch parts to the beer (Garbe et al., 2009).

Alkaline bottle washing deteriorates the glass surface and changes its properties and may induce gushing (Garbe et al., 2009).

4.3. Causes of beer gushing

The most important factors which can be responsible for inducing gushing are:

(a) special proteins: *hydrophobins* (especially when derived from barley grown in wetter weather conditions (Garbe et al., 2009)) and *plant typical non-specific lipid transfer proteins* (ns-LTPs). The protein ns-LTP1 causes gushing when it is glycosylated and degraded by proteolysis during the brewing process (Deckers et al., 2010a, 2011; Christian et al., 2010, 2009a).

(b) beer storage temperature (Deckers et al., 2010a, 2011): the lower the storage temperature of beer and the higher the temperature of beer after bottle opening, the greater the amount of gushing. Garbe et al. (2009) explained that gushing will occur only for beers that reach room temperatures or that are agitated.

(c) dry hopping (Deckers et al., 2010a, 2011).

(d) metal ions and calcium oxalate for secondary gushing (Christian et al., 2010) and Kieselguhr (Diatomaceous earth) (Christian et al., 2009a).

(e) multivalent cations cross-link to hop acids and proteins and promote foam stability and in the extreme, gushing (Evans and Bamforth, 2009).

(f) surface tension: according to the equation, $W = 4/3\pi\sigma r^2$ (W = work required to form a bubble, σ = surface tension and r = radius of the bubble), reducing the surface tension leads to more microbubbles (Garbe et al., 2009) and probably gushing will be increased.

(g) proteases: they can change the protein composition of the grain. The increase of the Kolbach index can change the colloidal structure in the finished beer. This influences the foam stability, the solubility of gases and ultimately the formation of micro bubbles, which are closely related to gushing (Garbe et al., 2009).

4.4. Producers of primary gushing in beer

The fungi *Fusarium* sp., *Nigrospora* sp. and *Trichoderma* sp. are the most active producers of hydrophobins that are found in beer and cause gushing (Sarlin et al., 2005). Species and strain specifity of *Fusarium* sp. found on barley depend upon both geographic location and climate. In the USA, Canada, China and southern and eastern Europe, *F. graminearum* predominates, while *F. culmorum* is more important in northern Europe (Garbe et al., 2009). *Fusarium* sp. causes disease which impacts the head or inflorescence of the grain. The pathogen exists as a saprophyte on crop residues and ascospores or conidia are wind-blown or rain splashed to the developing head (Garbe et al., 2009).

Fungi producing hydrophobins also may produce mycotoxins and *Fusarium* species are associated with the production of tricothecene mycotoxins and zearalenone. Deoxynivalenol (DON) is commonly produced by *F. graminearum* (Salas et al., 1999) and *F. culmorum* may produce DON or *nivalenol* (NIV) (Desjardins, 2006). DON present on the malt will largely be extracted into the beer. In many cases during steeping the barley mycotoxins may be significantly reduced or eliminated. However, in some cases the amount of mycotoxins may increase during malting, presumably through further growth of *Fusarium* sp., or perhaps by the liberation of bound mycotoxins (Garbe et al., 2009; Schwarz et al., 1995; Schwarz, 2003).

Levels of hydrophobin needed for beer gushing depend on the producer fungi, but some of the reports showed that 0.003 ppm hydrophobin in beer and 250 ppm in malt from *T. reesei* is sufficient for gushing (Sarlin et al., 2005) and for *F. poae*, 0.15 mg/l was needed to start gushing (Garbe et al., 2009).

4.5. Relationship between the presence of mycotoxins (e.g. DON) and hydrophobins in beer

No relationship between the presence of mycotoxins and the presence of hydrophobins in beer has been found (Sarlin et al., 2005). Consequently it is not possible to predict beer gushing from the presence of mycotoxins and also the presence of hydrophobins is not completely predictive for the presence of mycotoxins (Sarlin et al., 2007). According to Sarlin et al. (2005) gushing beers may be a signal for consumers concerning the presence of some mycotoxins.

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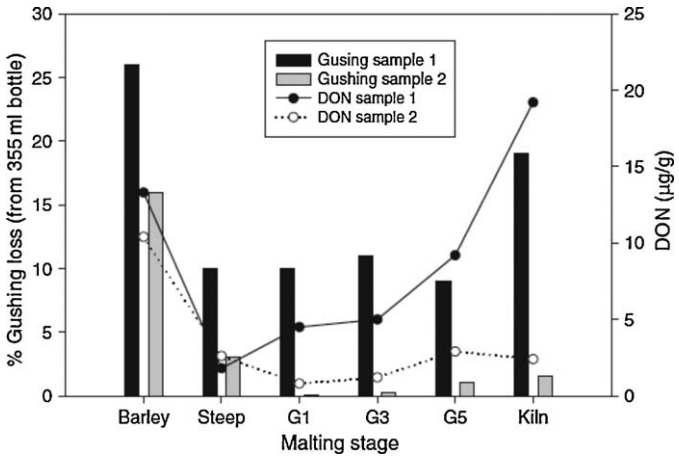

Fig. 14. Gushing potential changes during malting for two barley samples infected by *Fusarium* sp. G: days of germination (Garbe et al., 2009).

4.6. Changes of hydrophobin levels during malting

Hydrophobin amounts change during the treatment of barley:

(a) The gushing potential is not eliminated during barley rinsing. This is due to the production of hydrophobins beneath the husk (Garbe et al., 2009).
(b) There is a significant decrease in gushing potential during steeping. This is due to the partial solubility of hydrophobins in water (Vaag et al., 1993; Vaag and Pederson, 1992).
(c) Either mycotoxins or hydrophobins can be produced during germination.

The changes during malting are shown in Fig. 14.

4.7. Visible gas bubbles just before overfoaming of beer

In non gushing beer there is an amphiphilic layer around bubbles, made of ns-LTP1, which prevent the bubbles from disappearing after solution of CO_2 in bottled beer. In gushing beer this layer is made of mixture of hydrophobins with nsLTP1.This contamination leads to weak layer and tends to ascending, bursting and giving rise to condensation of more gas on the hydrophobic side of the layer which is now freely exposed to water. This phenomenon explains the generation of thousands of visible gas bubbles in a bottle shortly before overfoaming of beer (Stübner et al., 2010). The energy required for the CO_2 liberation and the beer gushing is brought by undisssolved CO_2 gas molecules which were contaminated by hydrophobins during artificial carbonation of beer most probably (Fig. 15).

4.8. Reduction of gushing

There are some possibilities to reduce gushing.

(1) Addition of proteolytic enzymes degrading hydrophobin structures (Garbe et al., 2009; Sarlin et al., 2005).
(2) Prolonged storage of barley tended to reduce the ability of fungi to produce hydrophobins in malting (Sarlin et al., 2007).
(3) Addition of hops: beer with higher hop addition leads to lower gushing propensity (Christian et al., 2010; Hanke et al., 2009). Hop oil concentration of 1 ppm in beer is a very good gushing inhibitor, but is not applicable because consumers would not appreciate a beer with such a high hop oil concentration (Hanke et al., 2009).

The use of hop extracts (containing hop oils and alcohols such as linalool: $C_{10}H_{18}O$ and humulones: $C_{21}H_{30}O_5$) instead of dry hops tend to lower gushing (Deckers et al., 2010a, 2011).

Addition of liquid oil droplets with small amount of insoluble hydrophobic material is the most efficient de-foaming agent (Murray, 2007).

The constituents of hop oil with gushing inhibitory effects are:

I. Humulones (reduce gushing less than hop oil)
II. Terpene (β-caryophyllene)
III. Hulupones
IV. Iso-α-acids
V. Linalool (reduce gushing more than hop oil) (Hanke et al., 2009).

The mechanism of gushing reduction by hop oils is due to the hydrophobic character of hop oil constituents and their accumulation on hydrophobic/hydrophilic interfaces. This would lead to gaps between the molecules of gushing promoting surfactants which then cannot form stable nuclei (Hanke et al., 2009).

The constituents of hop oil, found to have gushing promoting effects are:

I. Polyphenols
II. Saturated hop lipids (weak inducing property)
III. Isohumulones (powerful gushing promoter)
IV. Dehydrated humulinic acid (DHA): strong gushing promoter
V. α- and iso-α-acid derived oxidation products (Hanke et al., 2009).

(4) Pasteurization of beer at 60 °C: This increases the internal pressure in beer and the structure of the hydrophobin coatings around nano bubbles will be destabilized (Deckers et al., 2010a, 2011). Re-pasteurization increased pressure leads to solubilization of the micro-bubbles and gushing will be decreased (Garbe et al., 2009).
(5) Removing of hydrophobic material content (hydrophobin) by contacting with surfaces like nylon (Deckers et al., 2010a, 2011).
(6) Polar lipids excreted by *Fusarium* sp. such as phospholipids (Christian et al., 2009a).
(7) Membrane filtration of beer samples (0.1 μm pore size) suppressed the gushing affinity. The gushing affinity will be halved by applying pore sizes of 0.45 and 0.65 μm (Christian et al., 2009a).
(8) Lipids interfere with the foam stabilizing interactions of proteins, cations and hop acids and destabilize foam stability and promote coalescence (Evans and Bamforth, 2009).
(9) Electron beam irradiation: Electron beam irradiation at doses of 6–8 kGy reduces *Fusarium* sp. infection in malting barley with little impact upon germination (Kottapalli et al., 2003).
(10) Hot water treatment: either at 45 or 50 °C for 15 min results in reductions in *Fusarium* sp. infection from 32% to 1–2%, with only a slight reduction in germination (Kottapalli et al., 2003).
(11) Use of formaldehyde in the steep: 1000 mg/kg barley in the first steep suppresses the growth of *Fusarium* sp. Its use in commercial malting practice is not permitted (Gjersten, 1967; Haikara, 1980).
(12) Treatment of barley with hydrogen peroxide decreased *Fusarium* sp. infection by 50–98% within 5 min of exposure and had no effect on germination (Kottapalli et al., 2005).
(13) Use of gaseous ozone: after 5 min of ozonation at 0.16 and 0.10 mg ozone/g barley/min causes 96% inactivation for fungal spores. Inactivation of fungi continues in silos, as long as ozone gas was retained in the storage atmosphere, and the efficiency of ozone gas increases with water activity and temperature of the barley (Allen et al., 2003; Kottapalli et al., 2005).

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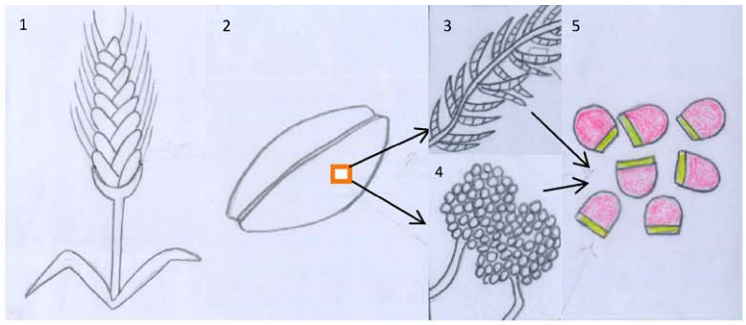
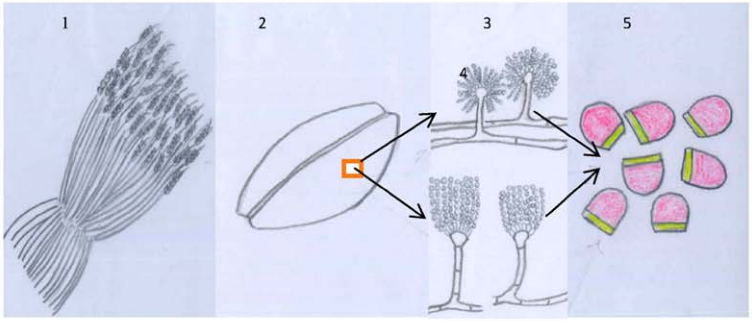
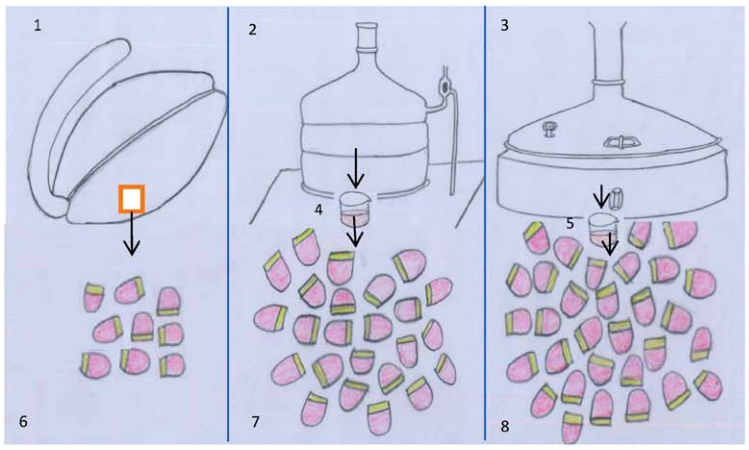


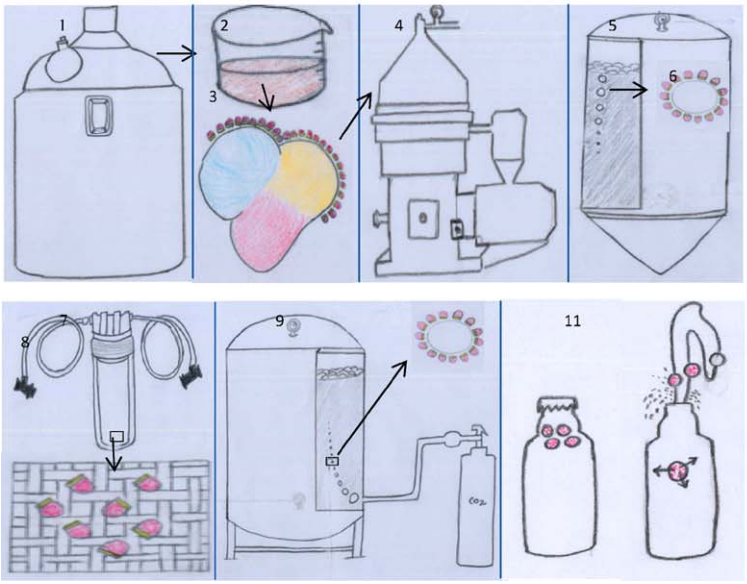
1. Barley
2. Barley grain
3. *Fusarium sp.*
4. *Trichoderma sp.*
5. Hydrophobin
   Hydrophobic patch
   Hydrophilic patch



1. Harvested barleys
2. Barley grain
3. *Aspergillus sp.*
4. *Penicillium sp.*
5. Hydrophobin
   Hydrophobic patch
   Hydrophilic patch



1.Malting
2. Mash tun
3. Lautering
4, 5. Wort
6, 7, 8. Increase of hydrophobin during these three processes



1. Boiling
2. Wort
3. Flocculation of proteins, lipids and carbohydrates (Hydrophobins around hydrophobic patches of flocculated particle)
4. Centrifuge (fluctuated particles removed here)
5. Fermentation (1.5 bar)
6. Hydrophobins around bubbles
7. Filtration
8. Adsorbed hydrophobins on the surface of filter
9. Carbonation (4.5 bar)
10. Hydrophobins around bubbles
11. Gushing and Exploding of contaminated bubbles with Hydrophobins (= Hydrophobins (red) arround bubbles)

Fig. 15. Schematic overview of the traject of an hydrophobin from field till gushing beer.

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(14) Use of lactic acid bacteria in the steeping step and also on the field shows significant reduction of *Fusarium* sp. as well as other fungi and bacteria (Garbe et al., 2009).
(15) Addition of *Geotrichum candidum* to the steep completely eliminates *Fusarium* sp. and DON. The reason is the fast competitive growth of *Geotrichum* sp. capturing more of the available nutrient resources (Boivin and Malanda, 1997).
(16) Addition of adsorbents such as charcoal, Fuller's Earth, Tansul, kaolin, activated alumina (Garbe et al., 2009).
(17) Addition of unsaturated fatty acids (Hanke et al., 2009).

As a conclusion mention must be made that not all methods are applicable in real scale and should only show what is theoretically possible.

4.9. Prediction tests for primary gushing

4.9.1. Modified Carlsberg test

In this method, an aqueous extract of ground malt is added to bottled sparkling water. The bottles are shaken for three days then opened and the amount of beer lost through gushing is determined by weighing (Haikara et al., 2005). From an interlaboratory test, it was shown that the precision of the method was not acceptable (Haikara et al., 2005) and a very good repeatability and reproducibility of test results can only be achieved in one and the same laboratory (Rath, 2008) due mainly to the variation of test conditions (Rath, 2008).

4.9.2. ELISA test

A competitive ELISA (Enzyme Linked ImmunoSorbent Assay) was developed by Sarlin et al. (2005) and patented by Haikara et al. (2006) for detection of hydrophobins in barley and malt. Antibodies against the hydrophobin of *Fusarium* sp. were used. The test could not distinguish between hydrophobin concentrations higher than 100 μg/mL (Sarlin et al., 2005).

4.9.3. Combination of particle size analysis and charge titration test

This method is based on the detection of particles with a size of 1–2 nm, the detected stray light intensities of these sized particles being significantly higher for gushing samples than for non-gushing samples. To differentiate gushing samples from non-gushing samples, the particle charge titration method was used. The results showed that higher titrated volumes for charge-neutralization were necessary for gushing samples (Christian et al., 2010).

4.9.4. Tracers test

This method is based on the presence and the detection of a particular molecule present in a gushing material. For example, DON (deoxynivalenol) a toxin produced by *Fusarium* sp. could be a tracer but there is no correlation between the gushing and the concentration of DON in malt (Sarlin et al., 2005). Alkaline foam protein A (AfpA), a member of a new protein class, fungispumin, can be isolated form pure liquid cultures of *Fusarium culmorum* and a homologous protein is synthesised by *F. graminearum*. The protein is produced in contaminated malt and enhances gushing of beer. The gene coding for AfpA is restricted to *Fusarium* sp. and presumably involved in the induction of beer gushing. AfpA may be useful as a marker for gushing in the future (Zapf et al., 2006).

4.9.5. MALDI-TOF test

MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time of Flight) can be used to detect hydrophobins (Neuhof et al., 2007). However, it is necessary to purify hydrophobins before to using mass spectrometry. The ns-LTP proteins are probably the most interfering molecules because they have a molecular weight in the same range as hydrophobins and have also four disulfide bridges.

5. Conclusion

Hydrophobins have important properties such as producing surface films at interfaces, forming multimers in solution of a solvent, changing hydrophobic surfaces to hydrophilic and vice versa, decreasing surface tension and act as emulsifier and foaming agents. However in the beverage industry they are responsible for gushing of over-carbonated bottled liquids, meaning strong over-foaming on opening the bottle.

To understand the gushing phenomenon a good knowledge of foam formation is needed. Foaming is a phenomenon which is related to the presence of gas capturing structures with high surface activity attracting proteins during its formation. In foaming, there are different types of nucleation which is type IV in beer. Foams are destabilized according to drainage, disproportionation, coalescence and gas diffusion. Different factors in beer affect foam stability. Proteins like LTP1, protein Z and hordeins favour foam stability. Non starch polysaccharides, hop acids and metal cations also improve foam stability while lipids, type of pitching yeasts for fermentation, centrifugation of beer and the presence of enzymes such as proteinase A, lipoxygenase and remaining detergents all decrease foam stability. Ethanol in high concentrations causes foam destabilization and in low concentrations it improves foam stability. Low pH improves foam stability of beer.

Gushing is also related to the binding of proteins to gas containing structures. Nanostructures (nanobubbles) are surrounded by hydrophobin molecules and become stabilized and then act as many nucleation sites. After opening the bottle, by releasing the pressure, the stabilized bubbles by a hydrophobin layer grow, explode and cause primary gushing. The effects of foam stabilizing or destabilizing factors on gushing foam containing hydrophobins certainly need further investigations. Thus HFBII forms exceptionally stable foams. Other foreign chemicals such as metal ions, crystals of calcium oxalate and remaining cleaning agents in bottles all cause secondary gushing. Gushing is a problem in industry, and could be solved or reduced by pasteurization of beer, addition of hop extracts, filtration of beers, long time storage of barleys and also addition of lactic acid bacteria to the barleys. A relatively simple and reliable method to predict gushing remains one of the priority research subjects however.

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