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Review

The Gushing Experience—A Quick Overview

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Abstract: Beer lovers all over the world like to get their drink with a certain volume of stabile foam, which mainly depends on the beer style. However, sometimes this foam comes in form of a sudden, eruptive, and uncontrolled over-foaming (gushing) of beer. Gushing occurs after the bottle has been opened, without previously being treated inappropriately (exposure to high temperatures, shaking, or any other kind of agitation). According to recent scientific and professional literature, gushing may be induced by many factors, but fungal proteins are directly connected to this phenomenon. Gushing caused by fungal proteins—hydrophobins—is called primary gushing, and depends solely on raw material quality. Other reasons for extensive foaming after the bottle has been opened can be of chemical or technological nature in the course of the brewing process. This is called secondary gushing, which can be influenced and reduced by applying good manufacturing practice protocols.

Keywords: gushing; beer; hydrophobins

1. Introduction

Beer foam—some consumers enjoy it, some do not. However, it is the most alluring component of beer, that makes every consumer's mouth water when looking at a full, cold, foamy beer mug. Beer head makes beer a friendly drink. Depending on the type of beer, it can be bigger, smaller, longer-lasting, creamy, etc. However, some foams are not that desirable. Foam created during a gushing experience is not stable and goes away very fast. In short, an immediate release of carbon dioxide (CO_2) is observed on opening the bottle [1]. This is a sudden and extremely undesirable phenomenon when it comes to the brewing industry's reputation. It affects the beer quality and consequently causes financial problems for breweries. Providing constant beer quality is one of the biggest challenges for the brewing industry. Therefore, product quality is defined by product stability. Factors affecting beer stability are divided in three major areas: sensory stability (e.g., appearance, taste, or smell), microbiological stability, and physico-chemical stability. Gushing reportedly affects both sensory and physico-chemical stability of beer [2]. Besides the financial problems, the customer's desire to completely change the brand after a gushing experience contributes to the overall negativity towards this phenomenon. Note that gushing is not limited to only beer, but is a well-known occurrence in the carbonated beverages industry (fruit sodas, sparkling wines, champagnes, lemonades, and mineral water) [3–6].

Gjertsen et al. [7,8] recognized two types of gushing: primary and secondary. Primary gushing is induced by fungal activity, especially of *Fusarium* species. Other species of the genera *Aspergillus*, *Rhizopus*, *Penicillium*, and *Nigrospora* [9] are also linked with the appearance of gushing. Secondary gushing—or non-malt-related gushing [10]— is caused by manufacturing factors which one can easily influence by applying good manufacturing practices (GMPs). Coarse bottle surface, oversaturation with CO_2, increased oxalate concentrations, components from hops (hop oils), etc. contribute to gushing [6,10,11]. However, *Fusarium* infected grains are still considered as a serious problem

in malting and brewing industries merely because they are directly associated with the gushing phenomenon [4,12–15]. Recent investigations have proven that hydrophobins—small surface-active proteins synthesized and excreted by fungi—are responsible for gushing in beer [15,16].

Alongside hydrophobins, the well-known gushing inducers fungispumins [17,18], as well as elevated levels of pathogenesis-related proteins (PRs), might be responsible for gushing [18].

Some studies attempted to link the gushing effect with mycotoxin content in malt. Schwarz et al. [19] reported that approximately 90% of all malts containing deoxynivalenol (DON) are prone to gush, but Sarlin et al. [3] disputed this statement, reporting that hydrophobins levels and DON in malts do not correlate, indicating no connection between the synthesis of those two fungal metabolites. In addition, no correlation between DON content and the gushing potential of malt has been observed.

Apart from causing massive economic losses to malting and brewing industries, beer gushing also makes consumers reconsider the brand they are buying, and drives them away from the product, giving the brewery a bad name [20–22].

2. Types and Causes of Beer Gushing

2.1. A Short Overview of Hydrophobins and Gushing Mechanism

Hydrophobins are small proteins produced by filamentous fungi (*Fusarium* sp., *Nigrospora* sp., and *Trichoderma* sp.) and dimorphic yeasts [23]. According to Wessels et al. [24], who first named hydrophobins in 1991 and continued research on hydrophobins, these proteins are highly surface-active and self-assemble at hydrophilic–hydrophobic interfaces in order to form amphipathic membranes. This gives them the ability to lower water surface tension and take part in numerous functions in fungal growth and development. They are thus are able to form a protective coating on fungal structures. This allows the aerial growth of hyphae, protects aerial conidia against desiccation and wetting, and helps the aerial dispersal of spores [25]. Considering their properties, Wessels [26] divided hydrophobins into Class I and Class II. Class I hydrophobins are insoluble in aqueous solutions but are soluble in strong acids (trifluoroacetic acid, TFA; formic acid). Class II hydrophobins are soluble in organic solvents (60% ethanol; 2% hot sodium dodecyl sulfate, SDS) [24,27–31]. Szilvay et al. [32] reported that hydrophobins can form monomers, dimers, and tetramers in hydrophilic solutions. One of their properties is to self-assemble at hydrophilic–hydrophobic interfaces [33]. Zapf et al. [17] link primary gushing with class II hydrophobins. The research Deckers et al. conducted in 2012 [34] shed additional light to the understanding of primary gushing.

According to Shokribousjein et al. [33,35], self-assembly in carbonated beverages occurs around hydrophobic CO_2 gaseous molecules and causes primary gushing of beer. One of the role of fungal hydrophobins is to help fungus to adhere on different surfaces. This occurs by changing the hydrophobicity of surfaces and provides fungus a better grip on the grain.

According to Sahu et al. (2006) [36], gushing is a result of nucleation and bubble growth. A more detailed description of the gushing mechanism is provided in several papers [33,34,37], but they all refer to the explanation Deckers et al. [34,38] in their research. In short, carbonated beverages in closed containers sustain an equilibrium between gaseous and dissolved CO_2 molecules. Hydrophobins adhere to CO_2 bubbles and stabilize them by forming nanobubbles. Nanobubbles agglomerate at the gas-liquid interface and, on opening the container, when the pressure in the container suddenly drops, the particles instantly grow and subsequently explode, causing an extreme foam propensity.

2.2. Primary Gushing

As mentioned before, beer gushing can be induced by many factors. The most infamous and most damaging cause of gushing belongs to primary gushing, and is a result of fungal contamination of raw material. Cereals are often infected in the field by different filamentous fungal species, and many of them are known fungal hydrophobins producers (*Fusarium*, *Aspergillus*, *Rhizopus*, *Penicillium*,

and *Nigrospora*) [3,9]. *Fusarium* species are often and in detail considered in the brewing industry as the most potent and most threatening regarding not only hydrophobins production, but also mycotoxins and overall malt quality. *Fusarium graminearum* and *Fusarium culmorum* are marked as ubiquitous on malted and un-malted cereals used for brewing, and are for that reason closely monitored for gushing production. Rath [39] dealt with the addition of *Fusarium* infected grains, so called "relevant red grains" in reference material. The results showed that the addition of five red grains stimulates gushing. Kleemola et al. [40] and Sarlin et al. [32] demonstrated that the addition of purified fungal hydrophobins to beer causes gushing, and in 2007 Sarlin et al. [14] found that even a small concentration of 3 μg/L of hydrophobins in beer, and 250 μg/g in malt cause gushing. According to Zapf et al. [16], fungi used in their studies did not have relevance as gushing inducers in central Europe (*Trichoderma reesei, Fusarium poae,* and *Nigrospora* sp.). However, different research by Stenglein [41] and Yli-Mattila et al. [42,43] refer to *F. poae* as a common plant pathogen in Northern Europe, Asia, and North America.

The influence of fungicides on the gushing potential of malt is also a subject of many studies. Havlova et al. [9] reported that the application of some fungicides can induce, rather than reduce, the gushing potential of malt. In some cases, the fungicide treatment stimulated the fungus to produce hydrophobins in order to sustain on the grain surface. One of the methods to reduce the risk of gushing is an early detection of gushing potential of raw materials for beer, such as wheat or barley malt. *Fusarium*-infected grains are a serious problem for the malting and brewing industry, not only because *Fusarium* species produce mycotoxins, but also because they produce hydrophobins—a cause of gushing [3,6,13,14]. Thereunto, *Fusarium* infection affects other malt quality parameters as well: lower grain weight and germinative capacity, yield loss, endosperm protein and starch degradation, etc. [13,44,45].

Even though hydrophobins are proteins, they are thermostable; because of that, they "survive" the brewing process. In fact, hydrophobins are among the most stable proteins that resist temperatures of boiling water [46]. During mashing, hydrophobins are extracted into wort, and about 10% of them will—post-boiling, fermentation, and maturation phases—end up in the final beer. Most hydrophobins will be removed with spent grains, hot trub, or spent yeast [14]. Habschied et al. [47] conducted research in which gushing was related with wheat genotype, fungicide application, and *F. culmorum* infection. Results showed that the infection of wheat with *F. culmorum* significantly affects and increases the gushing potential of wheat malt, and that the application of fungicide did not contribute to gushing suppression.

However, there is still not enough data on how the brewing process influences hydrophobins. More detailed research is needed to evaluate the effects of different process steps on the gushing potential of wheat and barley malt.

2.3. Secondary Gushing

As mentioned before, agents of secondary gushing are many, and very often they can be avoided by applying GMPs. Some of them will be stated below:

2.3.1. Proteins

Proteins originating from barley are usually not inducers of gushing. However, if during the brewing process plant typical non-specific lipid transfer proteins (ns-LTPs) get degraded, they can contribute to the gushing potential of beer [3,6,38,48]. Heightened Kolbach index values in malt usually mean the increased protease activity. This affects the colloidal stability of finished beer, gas solubility, and microbubbles formation, again contributing to gushing [49].

2.3.2. Metal Ions and Oxalic Acid

In the course of the brewing process, many adjuncts are used that can contain metal ions. The presence of metal ions (Fe^{3+}) from kiselguhr also triggers secondary gushing [50,51]. Oxalic

acid and its crystals are the usual constituents of beer, in relatively high amounts (about 40 mg/L in wort and 10–25 mg/L in beer) and mainly stem from malt. Oxalic acid crystals form aggregates with hydrophobins and create bubbles that cause gushing [52]. A common cause of secondary gushing is the formation of calcium oxalate precipitations in bottled beer. Insoluble calcium oxalate precipitates in many crystalline and amorphous forms. These particles act as seed crystals, entrap gas bubbles and induce beer gushing [53–55].

2.3.3. Hops Constituents

Adding different common compounds such as hops can also trigger over-foaming. Namely, it is considered that hop oils contain different compounds that induce gushing: polyphenols [56], saturated hop lipids (weak inducer), isohumulones (strong inducer), dehydrated humulinic acid (DHA) (strong inducer), α- and iso-α-acid-derived oxidation products [11].

2.3.4. Storage Temperature

According to Deckers et al. [38], lower storage temperature in combination with higher temperature after bottle opening will result in the appropriate amount of gushing. Garbe et al. [49] suggested that gushing will occur in beers left at room temperature or which are agitated. This is very often in households, where beer was kept in a refrigerator and then opened at room temperature. A similar effect can be achieved by storage at room temperature before opening. CO_2 bubbles expand their volume upon opening of the bottle or can. With sudden temperature increase, the gaseous CO_2 is less soluble in the solution (beer or any other liquid) and erupts from the bottle. In addition, CO_2 pressure in the bottle (liquid phase) is high, and on opening, the pressure falls in order to equilibrate with partial CO_2 pressure in the air (gaseous phase), resulting in spontaneous over-foaming [57].

2.3.5. Bottle Conditioning

Impurities originating from bottles can also contribute to beer gushing upon bottle opening. Detergents remaining in crown corks or scratched parts of the bottle may be released into the beer and cause gushing [49]. Cracks of rough internal bottle surfaces can also contribute to beer gushing by trapping small gas bubbles.

3. Methods Concerning the Prevention of Beer Gushing

Today's research deals with the causes of gushing, and investigates methods that suppress the induction of gushing, and their application in different phases of beer production. Many of the suggested methods are not suitable for industrial scale, and so preventing or reducing primary gushing is still challenging in real systems. Several methods are described as effective in the industry; they will be described in the following sections.

3.1. Biological Methods

Gushing reduction can be influenced while barley is still in the field. According to Garbe et al. [49], the dispersion of lactic acid bacteria on barley in the field can be applied in order to reduce *Fusarium* infection and suppress gushing. Lactic acid bacteria (LAB) or yeast *Geotrichum candidum* can also be added during the steeping phase of the malting process. Boivin and Malanda [58] reported a significant drop of *Fusarium* infection after the application of the above-mentioned microorganisms. LAB bind mycotoxins and remove them from the medium [59]. Martin [60] reported and patented the use of *Pythium oligandrum* in different industries, including the food industry. Its antifungal effect was successfully applied during the malting process, where it suppressed the growth of other fungi. Its effect on secondary metabolites production was not described in this research [61]. In 2002, Laitila et al. [62] successfully applied two *Lactobacillus plantarum* strains into steeping water that acted against several *Fusarium* species: *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, and *F.*

oxysporum. Laitila et al. [63] also tried to suppress *Fusarium* growth and to prevent the production of hydrophobins during malting by using *Pichia anomala* C565. They also experimented with *P. anomala* in combination with *Lactobacillus plantarum* VTT E-78076 starter culture in order to enhance malt processability. The results were promising in terms of *Fusarium* growth suppression, and hydrophobins production was also lowered.

3.2. Physical Methods

3.2.1. Storage Time

Sarlin et al. [14] reported that longer storage time of barley can contribute to the reduction of gushing. This is because the *Fusarium* fungi loses the ability to produce hydrophobins.

The best way to prevent gushing is to ensure un-infected barley and malt in the beer production chain. This can be achieved by imposing strict in-house quality control of raw materials. Before malting, barley selection starts with cleaning and grading. This ensures the removal of small and most heavily *Fusarium*-infected kernels from the cereal lot.

3.2.2. Mixing of Different Batches

Mixing of different malt batches can also contribute to the reduction of gushing. However, the lack of storage space is a common problem in breweries, and it would affect the brewing process price. Furthermore, in years with lower yield or higher *Fusarium* contamination there is not enough "healthy" raw material [50].

3.2.3. Membrane Filtration and Pasteurization

According to Christian et al. [50], membrane filtration (0.1 μm pore size) of beer helps gushing reduction. Beer pasteurization at 60 °C also results in reduced gushing potential of beer [38,48].

3.2.4. Radiation

The utilization of electron beam irradiation (6–8 kGy) for the sterilization of barley significantly reduces the number of *Fusarium* fungi [64]. Considering that the radiation does not affect the germination during malting, this method can be applied for malting barley.

3.2.5. Magnetic Field Application

Most recent is the application of a magnetic field to disperse the hop extract [37]. According to the results of this research, the application of magnetic field for the addition of low concentrations of hop extract antifoam is an effective method to decrease gushing, before mixing with the wort. A higher concentration of hop extract antifoam induces gushing. As for the brewery scale, the application of magnetic field for the dispersion of hop extract antifoam showed very good foaming control with small amounts of extract, and no gushing was observed.

3.2.6. Ultrasonic Vibrations

According to Sahu et al. [36], ultrasonic vibrations act to appease to gushing. The change in the number of microbubbles (or embryos) and their size distribution actually calms down the disturbed liquid prone to gushing.

3.3. Addition of Different Compounds

3.3.1. Chemicals

Barley can be treated with hydrogen peroxide in order to reduce *Fusarium* infection. Kottapalli et al. [65] a 50–98% reduction if infection after a 5 min treatment. Hydrogen peroxide has no effect on germination. Hot water (45 or 50 °C) for 15 min results in reduction of *Fusarium*

infection from 32% to 1–2%, with only a slight reduction in germination [64]. The addition of different calcium-rich silicates can also reduce gushing [52]. The application of silicate salts does not affect the sensory properties of beer; indeed, it only makes it more appealing to the consumers. Foam stability and physical-chemical characteristics also do not undergo any changes [52]. Gaseous ozone can also significantly reduce *Fusarium* fungi when applied during steeping, without any negative consequences for malt quality [66,67].

3.3.2. Enzyme Addition

The addition of proteolytic enzymes in wort causes a degradation of hydrophobins and plant proteins originating from barley. This significantly reduces the gushing phenomenon [3,20,49].

3.3.3. Hop Compounds

Hop components such as free linalool (50 µg/L) and humulones (5 mg/L) count as gushing decreasing methods [11]. Hop extracts are commonly used in brewing in order to control the foam in boiling kettles and fermenters. Adding such an extract before carbonation is effective for gushing reduction [68]. The extract addition has a better effect when added to wort after cooling, and before fermentation. According to Christian et al. [51] and Hanke et al. [11], beer with higher hop addition tends to have a lower gushing potential. Hop oil at a concentration of 1 µg/L in beer is a good gushing inhibitor. Hop oils, as surface-active compounds, act suppressively on gushing by lowering the surface tension and viscosity [69]. Since hop oils are a common ingredient in the perfume industry, such a high hop oil concentration does not agree with the consumers' preferences, because it negatively affects the beer flavor [11].

3.3.4. Polar and Non-Polar Molecules

The addition of non-polar molecules to a hydrophobin-rich solution reportedly affects their binding with gaseous CO_2 and contributes to gushing reduction [37]. The inhibition of gushing in model substances was shown as a success, but their addition to beverages may not be the most appropriate. Besides, these substances interfere with the taste and aroma of the finished beer.

Laibl and Geiger [70] determined that some lipids excreted by *Fusarium* fungi can also influence and decrease the gushing potential of beer.

Postulkova et al. [67] published an excellent review on gushing, describing in detail the causes and novel preventive techniques of this phenomenon. However, by now no safe and reliable procedure is known to prevent gushing. All methods that are currently in use require additional or modified process steps, which makes malting and brewing more expensive, significantly affects the aroma profile of the beer product [52], or are not appropriate for industrial scale application.

4. Gushing Potential Evaluation Methods

There are a few gushing prediction methods developed for malt, wort, beer, and other beverages. Since gushing is a very specific phenomenon, it is hard to obtain reliable results. The substances causing gushing are rarely identified, but the potential of raw materials for gushing can be determined. Some of them are being modified and upgraded in order to ensure more accurate and precise data on gushing. Garbe et al. [49] and Shokribousjein et al. [33] described in their papers some of the gushing determination methods:

1. *Carlsberg test*—developed by Vaag et al. in 1993 [71], it is relatively simple to perform: 100 g of malt and 400 mL of water is subjected to mixing in a blender. Fifty milliliters of this extract is then added to a bottle of commercial beer, pasteurized, weighed, and closed. The bottle is then attached to a shaker and left for 3 days. After 3 days, the bottle is opened, and the quantity of beer escaped from it is measured by weighing. However, standard deviations can be quite high, and according to Garbe et al. [50], the type of used beer has a significant influence on the results.

2. *Modified Carlsberg test (MTC)*—in order to set a more uniform test material, Radau et al. [72] introduced an aqueous extract of coarse-ground malt to bottled carbonated water (7 g/L CO_2) instead of beer. The bottles are shaken for 3 days and then opened. The quantity of beer loss from the bottles is measured by weighing Radau et al. [72]: 0–5 g qualifies as no gushing; 5–50 g is described as possible gushing, and >50 g is indicative of gushing. This test was accepted by MEBAK®, but as it turns out, this test also has a high potential for false negative results.
3. *Doubly modified Carlsberg test (M^2TC)*—Garbe et al. [73] modified the original Carlsberg test by introducing the preparation of congress wort, which was then added to carbonated water as a test agent. This modification contributed to a much greater gushing expression.
4. *Enzyme-linked immunosorbent assay (ELISA)*—a method implemented by Sarlin et al. in 2005 [3] and patented by Haikara et al. in 2006 [74]. This method is used for the screening and detection of hydrophobins in barley and malt. The downside of this test is that it has a limit of 100 µg/mL, and beyond this number, the distinction between hydrophobins concentration is not detectable (Sarlin et al.) [3]. Before ELISA analysis, samples can be diluted.
5. *Combined method of particle size analysis and charge titration test*—this method was originally developed for beer colloidal stability analysis [75]. Based on the intensity of light going through dispersed protein particles in beer (particles size of 1–2 nm), it turned out that the detected stray light is significantly higher for samples prone to gushing than for non-gushing samples. In order to confirm the gushing samples, the particle charge titration method was used, and the results showed that samples prone to gushing needed higher titrated volumes in order to neutralize the charge [76].
6. *Tracers test*—this method is based on the detection of a particular molecule present in a gushing material, linked with gushing potential. For example, alkaline foam protein A (AfpA) is a fungispumin produced by *Fusarium* sp. that can be found in infected malt, and contributes to beer gushing. According to Zapf et al. [17], AfpA may be used as a gushing marker.
7. *Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) test*—according to Neuhof et al. [77], this method can be used to asses hydrophobins, with mandatory purification of hydrophobins prior to analysis. The non-specific lipid transfer proteins (ns-LTP) interfere with hydrophobins, having similar molecular weight and four disulfide bridges, and should be eliminated before analysis.

5. Conclusions

The research activity related to gushing is extremely intense, especially recently. However, there is still a lack of general methods for the effective suppression of this phenomenon. Considering that fungal proteins are identified as the main gushing inducers, it can be assumed that further research will lean towards finding effective strategies to reduce the raw material contamination with fungi—especially from the genus *Fusarium*. The insurance of microbiological safety is extremely important when admitting cereals, weather barley or wheat, to the malt factory. A better and deeper knowledge of beer gushing inducers and finding new methods for the suppression or complete avoidance of this phenomenon would enable loss reduction in malting and brewing factories caused by gushing.

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References

1. Pellaud, J. Gushing: State of the art. *Cerevisia* **2002**, *27*, 189–205.
2. Titze, J. Qualitätssiegel oder Medaille?—Güte und Sicherheit von Produkten richtig kommunizieren. *Getränkeindustrie* **2010**, *64*, 52–55.

3. Sarlin, T.; Nakari-Setälä, T.; Linder, M.; Penttilä, M.; Haikara, A. Fungal hydrophobins as predictors of the gushing activity of malt. *J. Inst. Brew.* **2005**, *111*, 105–111. [CrossRef]
4. Lutterschmid, G.; Stübner, M.; Vogel, R.F.; Niessen, L. Induction of gushing with recombinant class II hydrophobin FcHyd5p from *Fusarium culmorum* and the impact of hop compounds on its gushing potential. *J. Inst. Brew.* **2010**, *116*, 339–347. [CrossRef]
5. Stübner, M.; Lutterschmid, G.; Vogel, R.F.; Niessen, L. Heterologous expression of the hydrophobin FcHyd5p from *Fusarium culmorum* in *Pichia pastoris* and evaluation of its surface activity and contribution to gushing of carbonated beverages. *Int. J. Food Microbiol.* **2010**, *141*, 110–115. [CrossRef] [PubMed]
6. Christian, M.; Titze, J.; Ilberg, V.; Jacob, F. Novel perspectives in gushing analysis: A review. *J. Inst. Brew.* **2011**, *117*, 295–313. [CrossRef]
7. Gjertsen, P.; Trolle, B.; Andersen, K. Weath- microflora of possible importance to malting and ered barley as a contributory of gushing in beer. In Proceedings of the European Brewery Convention Congress, Brussels, Belgium, 1963; pp. 320–341.
8. Gjertsen, P. Gushing in beer; its nature, cause and prevention. *Brew. Digest.* **1967**, *42*, 80–84.
9. Havlova, P.; Lancova, K.; Váňová, M.; Havel, J.; Hajšlová, J. The effect of fungicidal treatment on selected quality parameters of barley and malt. *J. Agric. Food Chem.* **2006**, *54*, 1353–1360. [CrossRef] [PubMed]
10. Sarlin, T.; Kivioja, T.; Kalkkinen, N.; Linder, M.B.; Nakari-Setälä, T. Identification and characterization of gushing-active hydrophobins from *Fusarium graminearum* and related species. *J. Basic Microb.* **2012**, *52*, 184–194. [CrossRef] [PubMed]
11. Hanke, S.; Kern, M.; Herrmann, M.; Back, W.; Becker, T.; Krottenthaler, M. Suppression of gushing by hop constituents. *Brew. Sci.* **2009**, *62*, 181–186.
12. Haikara, A. Relationship between malt and beer. In Proceedings of the European Brewery Convention, Monograph VI, Helsinki, Finland, 1980; pp. 251–258.
13. Sarlin, T.; Laitila, A.; Pekkarinen, A.; Haikara, A. Effects of three *Fusarium* species on the quality of barley and malt. *J. Am. Soc. Brew. Chem.* **2005**, *63*, 43–49. [CrossRef]
14. Sarlin, T.; Vilpola, A.; Kotaviita, E.; Olkku, J.; Haikara, A. Fungal hydrophobins in the barley-to-beer chain. *J. Inst. Brew.* **2007**, *113*, 147–153. [CrossRef]
15. Lowe, P.D.; Arendt, E.K. The use and effects of lactic acid bacteria in malting and brewing with their relationship to antifungal activity, mycotoxins and gushing: A review. *J. Inst. Brew.* **2004**, *110*, 163–180. [CrossRef]
16. Zapf, M.W.; Theisen, S.; Vogel, R.F.; Niessen, L. Cloning of wheat LTP1500 and two *Fusarium culmorum* hydrophobins in *Saccharomyces cerevisiae* and assessment of their gushing inducing potential in experimental wort fermentation. *J. Inst. Brew.* **2006**, *112*, 237–245. [CrossRef]
17. Zapf, M.W.; Theisen, S.; Rohde, S.; Rabenstein, F.; Vogel, R.F.; Niessen, L. Characterization of AfpA, an alkaline foam protein from cultures of *Fusarium culmorum* and its identification in infected malt. *J. Appl. Microbiol.* **2007**, *103*, 36–52. [CrossRef] [PubMed]
18. Gorjanović, S. A Review: The role of barley seed pathogenesis-related proteins (PRs) in beer production. *J. Inst. Brew.* **2010**, *116*, 111–124. [CrossRef]
19. Schwarz, P.B.; Beattie, S.; Casper, H.H. Relationshio between Fusariun infestation of barley and the gushing potential of malt. *J. Inst. Brew.* **1996**, *102*, 93–96. [CrossRef]
20. Aastrup, S.; Legind-Hansen, P.; Nielsen, H.; Jørgensen, A. Enzymatischer Abbau der Gushing-Neigung im Bier. *Brauwelt* **1995**, *135*, 1385–1387.
21. Bellmer, H.-G. Forschungsprojekt "Gushing". *Brauwelt* **1995**, *135*, 1167–1170.
22. Kunert, M.; Sacher, B.; Back, W. Ergebnisse einer Umfrage in deutschen Brauereien zum Thema "Gushing". *Brauwelt* **2001**, *141*, 350–362.
23. Scholtmeijer, K.; Wessels, J.G.H.; Wösten, H.A.B. Fungal hydrophobins in medical and technical applications. *Appl. Microbiol. Biotechnol.* **2001**, *56*, 1–8. [CrossRef] [PubMed]
24. Wessels, J.G.; de Vries, O.M.; Asgeirsdottir, S.A.; Schuren, F.H. Hydrophobin genes involved in formation of aerial hyphae and fruit bodies in *Schizophyllum*. *Plant Cell* **1991**, *3*, 793–799. [CrossRef] [PubMed]
25. Wösten, H.A.B.; Wessels, J.G.H. Hydrophobins, from molecular structure to multiple functions in fungal development. *Mycoscience* **1997**, *38*, 363–374. [CrossRef]
26. Wessels, J.G.H. Developmental regulation of fungal cell-wall formation. *Annu. Rev. Phytopathol.* **1994**, *32*, 413–437. [CrossRef]

27. Hektor, H.J.; Scholtmeijer, K. Hydrophobins: Proteins with potential. *Curr. Opin. Biotechnol.* **2005**, *16*, 434–439. [CrossRef] [PubMed]
28. Lumsdon, S.O.; Green, J.; Stieglitz, B. Adsorption of hydrophobin proteins at hydrophobic and hydrophilic interfaces. *Colloids Surf. B Biointerf.* **2005**, *44*, 172–178. [CrossRef] [PubMed]
29. Linder, M.B.; Szilvay, G.R.; Nakari-Setälä, T.; Penttila, M.E. Hydrophobins: The protein-amphiphiles of filamentous fungi. *FEMS Microbiol. Rev.* **2005**, *29*, 877–896. [CrossRef] [PubMed]
30. Kisko, K. Characterization of Hydrophobin Proteins at Interfaces and in Solutions Using X-rays. Ph.D. Dissertation, University of Helsinki, Faculty of Science, Department of Physics, Helsinki, Fenland, 2008.
31. Linder, M.B. Hydrophobins: Proteins that self-assemble at interfaces. *Curr. Opin. Colloid Interface Sci.* **2009**, *14*, 356–363. [CrossRef]
32. Szilvay, G.R.; Nakari-Setälä, T.; Linder, M.B. Behavior of *Trichoderma reesei* hydrophobins in solution: Interactions, dynamics, and multimer formation. *Biochemistry* **2006**, *45*, 8590–8598. [CrossRef] [PubMed]
33. Shokribousjein, Z.; Deckers, S.M.; Gebruers, K.; Lorgouilloux, Y.; Baggerman, G.; Verachtert, H.; Delcour, J.A.; Etienne, P.; Rock, J.-M.; Michiels, C.; et al. Hydrophobins, beer foaming and gushing. *Cerevisia Belg. J. Brew. Biotechnol.* **2011**, *35*, 85–101. [CrossRef]
34. Deckers, S.M.; Venken, T.; Khalesi, M.; Gebruers, K.; Baggerman, G.; Lorgouilloux, Y.; Shokribousjein, Z.; Ilberg, V.; Schönberger, C.; Titze, J.; et al. Combined modeling and biophysical characterisation of CO_2 interaction with class II hydrophobins: New insight into the mechanism underpinning primary gushing. *J. Am. Soc. Brew. Chem.* **2012**, *70*, 249–256. [CrossRef]
35. Shokribousjein, Z.; Philippaerts, A.; Riveros, D.; Deckers, S.; Khalesi, M.; Michiels, C.; Delcour, J.A.; Gebruers, K.; Verachtert, H.; Derdelinckx, G.; et al. Effect of mashing process on performance of hop extract antifoam regards primary gushing. *Cerevisia Belg. J. Brew. Biotechnol.* **2013**, *38*, 71–76. [CrossRef]
36. Sahu, K.K.; Hazama, Y.; Ishihara, K.N. Gushing in canned beer: The effect of ultrasonic vibration. *J. Colloid Interface Sci.* **2006**, *302*, 356–362. [CrossRef] [PubMed]
37. Shokribousjein, Z.; Riveros Galan, D.; Michiels, C.; Gebruers, K.; Verachtert, H.; Martens, J.; Peeters, C.; Derdelinckx, G. Effect of a magnetic field on dispersion of a hop extract and the influence on gushing of beer. *J. Food Eng.* **2015**, *145*, 10–18. [CrossRef]
38. Deckers, S.M.; Gebruers, K.; Baggerman, G.; Lorgouilloux, Y.; Delcour, J.A.; Michiels, C.; Derdelinckx, G.; Martens, J.; Neven, H. CO_2-hydrophobin structures acting as nanobombs in beer. *Brew. Sci.* **2010**, *63*, 54–61.
39. Rath, F. Gushing in 2008—Trialling the "Modified Carlsberg test". *Brauwelt Int.* **2009**, *27*, 26–29.
40. Kleemola, T.; Nakari-Setälä, T.; Linder, M.; Penttilä, M.; Kotaviita, E.; Olkku, J.; Haikara, A. Characterisation and detection of the gushing factors produced by fungi. In Proceedings of the European Brewery Convention Congress, Budapest, Hungary, 12–17 May 2001; Fachverlag Hans Carl: Nürnberg, Germany, 2001; pp. 129–138.
41. Yli-Mattila, T.; Paavanen-Huhtala, S.; Jestoi, M.; Parikka, P.; Hietaniemi, V.; Gagkaeva, T.; Sarlin, T.; Haikara, A.; Laaksonen, S.; Rizzo, A. Real-time PCR detection and quantification of *Fusarium poae, F. graminearum*, *F. sporotrichioides* and *F. langsethiae* in cereal grains in Finland and Russia. *Arch. Phytopathol. Plant Prot.* **2008**, *41*, 243–260. [CrossRef]
42. Stenglein, A.A. *Fusarium poae*: A pathogen that needs more attention. *J. Plant Pathol.* **2009**, *91*, 25–36. [CrossRef]
43. Yli-Mattila, T. Ecology and evolution of toxigenic *Fusarium* species in cereals in Northern Europe and Asia. *J. Plant Pathol.* **2010**, *92*, 7–18. [CrossRef]
44. Siranidou, E.; Kang, Z.; Buchenauer, H. Studies on symptom development, phenolic compounds and morphological defence response in wheat cultivars differing in resistance to *Fusarium* head blight. *J. Phytopathol.* **2001**, *150*, 200–208. [CrossRef]
45. Španic, V.; Drezner, G.; Horvat, D. Changes of agronomic and quality traits in *Fusarium*-inoculated wheat genotypes. *Croat. J. Food Technol. Biotechnol. Nutr.* **2012**, *7*, 85–89.
46. Wösten, H.A.; de Vocht, M.L. Hydrophobins, the fungal coat unravelled. *Biochim. Biophys. Acta Rev. Biomembr.* **2000**, *1469*, 79–86. [CrossRef]
47. Habschied, K.; Krstanović, V.; Velić, N.; Šantek, B.; Novak, M.; Slačanac, V. Gushing potential of wheat malt infected with *Fusarium culmorum*. *J. Hyg. Eng. Des.* **2014**, *6*, 166–170.

48. Deckers, S.M.; Lorgouilloux, Y.; Gebruers, K.; Baggerman, G.; Michiels, C.; Neven, H.; Derdelinckx, G.; Delcour, J.A.; Martens, J. Dynamic light scattering (DLS) as a tool to detect CO_2-hydrophobin structures and study the primary gushing potential of beer. *J. Am. Soc. Brew. Chem.* **2011**, *69*, 144–149. [CrossRef]
49. Garbe, L.A.; Schwarz, P.; Ehmer, A. Beer Gushing. In *Handbook of Alcoholic Beverages Series, Beer: A Quality Perspective*; Bamforth, C.W., Russell, I., Stewart, G., Eds.; Elsevier Ltd.: San Diego, CA, USA, 2009; Chapter 6.
50. Christian, M.; Illberg, V.; Aydin, A.A.; Titze, J.; Jacob, F.; Parlar, H. New gushing mechanism proposed by applying particle size analysis and several surfactants. *Brew. Sci.* **2009**, *62*, 100–107.
51. Christian, M.; Titze, J.; Ilberg, V.; Jacob, F. New cognitions on gushing in the wort production process and in quantifying the gushing potential of malt. *Cerevisia* **2010**, *35*, 35–37. [CrossRef]
52. Müller, V.; Besier, A.; Pätz, R.; Fröhlich, J. Method for the Treatment of the Phenomenon Gushing in Beer and Malt Beverages. Available online: www.erbsloeh.com/downloads/en/130128_Method%20for%20the%20treatment%20of%20the%20phenomenon%20gushing%20in%20beer%20and%20malt%20beverages.pdf (accessed on 10 June 2017).
53. Jacob, F. Calcium oxalsäure: Technologische relevanz. *Brauwelt* **1998**, *138*, 1286–1287.
54. Zepf, M.; Geiger, E. Gushing problems with calcium oxalate I. *Brauwelt* **1999**, *139*, 2302–2304.
55. Zepf, M.; Geiger, E. Gushing problems with calcium oxalate II. *Brauwelt* **2000**, *140*, 222–223.
56. Aron, P.M.; Shellhammer, T.H. A discussion of polyphenols in beer physical and flavour stability. *J. Inst. Brew.* **2010**, *116*, 369–380. [CrossRef]
57. Šavel, J.; Košin, P.; Brož, A. Spontaneous liberation of carbon dioxide from beer and gushing. *Kvasny Prum.* **2013**, *59*, 33–40. [CrossRef]
58. Boivin, P.; Malanda, M. Improvement of malt quality and safety by adding starter culture during the malting process. *MBAA Tech. Q.* **1997**, *34*, 96–101.
59. El-Nezami, H.S.; Chrevatids, A.; Auriola, S.; Salminen, S.; Mykkanen, H. Removal of common *Fusarium* toxins in vitro by strains of Lactobacillus and Propionibacterium. *Food Addit. Contam.* **2002**, *19*, 680–686. [CrossRef] [PubMed]
60. Martin, F.N. Biocontrol of fungal soilborne pathogens by *Pythium oligandrum*. U.S. Patent WO 1,998,016,110 A1, 10 May 1999.
61. Rezanina, J. Application of Pythium Oligandrum as Biofungicide in Malting. Unpublished Diploma Thesis, V SCHT Praha, Praha, Czech, 2014. (In Czech)
62. Laitila, A.; Alakomi, H.-L.; Raaska, L.; Mattila-Sandholm, T.; Haikara, A. Anti-fungal activities of two *Lactobacillus plantarum* strains against *Fusarium* moulds in vitro and in malting of barley. *J. Appl. Microbiol.* **2002**, *93*, 566–576. [CrossRef] [PubMed]
63. Laitila, A.; Sarlin, T.; Kotaviita, E.; Huttunen, T.; Home, S.; Wilhelmson, A. Yeasts isolated from industrial maltings can suppress *Fusarium* growth and formation of gushing factors. *J. Ind. Microbiol. Biotechnol.* **2007**, *34*, 701–713. [CrossRef] [PubMed]
64. Kottapalli, B.; Wolf-Hall, C.E.; Schwarz, P.; Schwarz, J.; Gillespie, J. Evaluation of hot water and electron beam irradiation for reducing *Fusarium* infection in malting barley. *J. Food Prot.* **2003**, *66*, 1241–1246. [CrossRef] [PubMed]
65. Kottapalli, B.; Wolf-Hall, C.E.; Schwarz, P. Evaluation of gaseous ozone and hydrogen peroxide treatments for reducing *Fusarium* survival in malting barley. *J. Food Prot.* **2005**, *68*, 1236–1240. [CrossRef] [PubMed]
66. Dodd, J.G.; Vegi, A.; Vashisht, A.; Tobias, D.; Schwarz, P.; Wolf-Hall, C.E. Effect of ozone treatment on the safety and quality of malting barley. *J. Food Prot.* **2011**, *74*, 2134–2141. [CrossRef] [PubMed]
67. Postulkova, M.; Riveros-Galan, D.; Cordova-Agiular, K.; Zitkova, K.; Verachtert, H.; Derdelinckx, G.; Dostalek, P.; Ruzicka, C.M.; Branyik, T. Technological possibilities to prevent and suppress primary gushing of beer. *Trends Food Sci. Tech.* **2016**, *49*, 64–73. [CrossRef]
68. Shokribousjein, Z.; Philippaerts, A.; Riveros, D.; Titze, J.; Deckers, S.M.; Khalesi, M.; Delcour, J.A.; Gebruers, K.; Verachtert, H.; Ilberg, V.; et al. A curative method for primary gushing of beer and carbonated beverages: Characterization and application of antifoam based on hop oils. *J. Am. Soc. Brew. Chem.* **2014**, *72*, 12–21. [CrossRef]
69. Gardner, R.J. The mechanism of gushing: A review. *J. Inst. Brew.* **1973**, *79*, 275–283. [CrossRef]
70. Laibl, B.; Geiger, E. Primary gushing and polar lipids—An important addition to gushing research. In Proceedings of the European Brewery Convention Congress, Dublin, Germany, 17–22 May 2003; Fachverlag Hans Carl: Nürnberg, Germany, 2003; pp. 915–922.

71. Vaag, P.; Riis, P.; Knudsen, A.D.; Pedersen, S.; Meiling, E. A simple and rapid test for gushing tendency in brewing materials. *J. Inst. Brew.* **1993**, *99*, 209.
72. Radau, B.; Linemann, A.; Krüger, E. Modifizierter Carlsberg—Test (MCT). *Brauerei Forum* **1995**, *10*, 377–378.
73. Garbe, L.A.; Nagel, R.; Rauschmann, M.; Lamers, M.; Khmer, A.; Tresek, R. Correlation of DON, hydrophobins and gushing. In Proceedings of the European Brewery Convention Congress, Venice, Italy, 6–10 May 2007; pp. 6–10.
74. Haikara, A.; Kleemola, T.; Nakari-Setälä, T.; Pentillä, M. Method for Determining a Gushing Factor for a Beverage. U.S. Patent US 7,041,464 B2, 9 May 2006.
75. Poštulková, M.; Vitoušová, K.; Novák, P.; Fiala, F.; Růžička, R.; Brányik, T. History and New Trends of Research Into Over-foaming of Beer. *Kvasny Prum.* **2013**, *59*, 317–320. [CrossRef]
76. Christian, M.; Titze, J.; Ilberg, V.; Jacob, F. Combined particle analysis as a new tool to predict gushing shown with alcohol-free beverage products. *Brew. Sci.* **2010**, *63*, 72–79.
77. Neuhof, T.; Dieckmann, R.; Druzhinina, I.S.; Kubicek, C.P.; Nakari-Setälä, T.; Penttilä, M.; Döhren, H. Direct identification of hydrophobins and their processing in *Trichoderma* using intact-cell MALDI-TOF MS. *FEBS J.* **2007**, *274*, 841–852. [CrossRef] [PubMed]