



Doubly Modified Carlsberg Test combined with Dynamic Light Scattering allows prediction of the primary gushing potential of harvested barley and malt

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ABSTRACT

Despite intensive research on the gushing of carbonated beverages during the last decades, there is no universal method to predict its occurrence and consequently how to avoid the economic losses it induces. Primary gushing can be visualized as the strong overfoaming and/or strong liquid expulsion of liquid when a bottle of carbonated beverage is opened. This process results from the interaction between gaseous CO₂ and class II hydrophobins. Both chemicals are present in the pressurized liquid as CO₂ nanobubbles coated by hydrophobins which explode when at bottle opening the pressure is released, which results in a vigorous expelling of CO₂. Hydrophobins are produced by filamentous fungi in the field or during storage and processing. To avoid gushing of beers, their early detection in the barley-to-beer chain is of capital importance. To ascertain with more certainty the presence of hydrophobins on barley and malt and their gushing inducing property, the gushing test mostly often used in practice, the doubly Modified Carlsberg Test, was used but it was followed by a new test based on the detection of CO₂-hydrophobin nanoparticles by Dynamic Light Scattering (DLS). This allowed to certify that the potential of provoking gushing by samples of barley and malt is due to the potential of provoking a primary gushing and the presence of fungal products: hydrophobins. The results showed also that only 5% of gushing provoking grains in the grist is sufficient to induce gushing and the detection of the nanoparticles, typical for primary gushing.

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Introduction

In 1909, Kastner, a German brewing scientist, was the first to report strong foam and beer overflows after opening “normal” beer bottles without shaking (Kastner, 1909). This phenomenon known as gushing was later observed with other CO₂ saturated beverages such as champagne and sparkling wine (Zoecklein, 1999), cider (Wilson et al., 1999), soda and even mineral water (Fischer, 2001) although it seems to be most relevant in beer (Pellaud, 2002) where it represents severe economic losses and bad brand image. In the 1960s, gushing is divided in two types based on the causes. While

secondary gushing is due to technical and technological problems related to the brewing process, primary gushing occurring periodically, is related to the quality of raw material and becomes of more concern for the malster (Pellaud, 2002). In the 1960–1970s, some causes of primary gushing were proposed and a fungal contamination of raw material by molds such as *Fusarium* sp. was suggested. Molecules involved remained unknown (for a review see Pellaud, 2002). Wessels et al. (1991) discovered an amphiphilic protein produced by filamentous fungi and called it hydrophobins on the base of its amino-acids sequence. In 1996, the hypothesis that organic materials such as proteins produced by molds could form solid pellicles around CO₂ gas bubbles is advanced but it was not explained how and why (Gardner, 1973; Casey, 1996). At the end of 1990s hydrophobins were considered as the major contaminants responsible for primary gushing (Haikara et al., 1999) but the mechanism

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still remained unexplained. Despite this knowledge, parts were still missing in the gushing puzzle: how does primary gushing occur and how to prevent it? (Winkelmann and Hinzmann, 2009). These questions remained unanswered due to the fact that people put all emphasis on hydrophobins and not on the true responsible molecule, which is CO₂ (Deckers et al., 2011) which can be deduced from the observation that gushing can be reduced by reducing the bottle opening temperature. To understand this phenomenon, it was necessary to study the whole process with consideration of the physico-chemical properties of both hydrophobins and CO₂. It was important to understand the notion of the critical diameter of a gas bubble, which is ruled by the Young-Laplace and the Henry's law (Nelson, 2009; Deckers et al., 2010). Recently, Deckers et al. (in press) showed by molecular dynamics simulation that CO₂ molecules go to interact with the hydrophobic patch of the HFBII hydrophobin and described in successive steps the formation of nanobubbles stabilized by crystalline layer of Class II hydrophobin. These nanobubbles stabilized by hydrophobins and having an internal pressure corresponding to approximately 4 bars will act as nanobombs when the bottle will be opened (i.e. release of the pressure resulting in the detend of the gas) (Deckers et al., 2010, in press).

The economic disastrous effects caused by gushing become more and more apparent and explain why research was intensified in different countries such as Germany and Finland mainly on the development of a method to detect and predict primary gushing. Different methods based on a correlative factor (i.e. do not measure gushing but a factor correlated to gushing) were developed such as standard plate counts for barley or malt infection, ELISA test, PCR-based methods, presence of mycotoxin test, etc. (for more details see Garbe et al., 2009; Shokribousjein et al., 2011). Three "different" more direct tests to detect gushing were also developed. The first one called the Carlsberg test consists in adding a water extract of malt to a bottle of commercial beer and measuring the overflow. In the Modified Carlsberg Test (MCT), the non-standardized beer matrix used in the Carlsberg test is replaced by sparkling water (7 g CO₂/L). However, an inter-laboratory test underlined the lack of reproducibility of this test, most probably due to many parameters different from laboratory to laboratory (Haikara et al., 2005; Rath, 2008). In the doubly Modified Carlsberg Test (M²CT) fine malt grist was used for the preparation of Congress worts and these worts were added to sparkling water (7 g CO₂/L) (for more details about the gushing tests, see Garbe et al., 2007, 2009). From these three gushing tests, Garbe et al. (2007) showed that the best way for a reliable prediction of gushing from six malts was to prepare fine grist and congress wort (i.e. using the M²CT in place of MCT). In Germany (for a complete review, see Christian et al., 2011) Christian developed new ideas to quantify gushing more precisely because the overfoaming amount for the gushing test can fluctuate. He proposed to determine the minimal volume of a wort needed to induce gushing but also to determine the amount of a CO₂ hop extract to inhibit gushing (Christian et al., 2009a, 2010b). He combined also particle size analysis and charge titration test (Christian et al., 2010a). In 2011, a new method based on Dynamic Light Scattering (DLS) was developed to detect primary gushing in final products by the detection of particles with a diameter of approximately 100 nm in the gushing beverages (Deckers et al., 2011). Dynamic Light Scattering is a method to determine the size distribution profile of small particles undergoing Brownian motion in a solution. A laser provides a light source to illuminate a sample contained in a cell. The small particles cause the intensity to fluctuate more quickly than the large ones as they are moving faster. The scattering intensity signal from the detector is passed to a digital processing board called a correlator which compares the scattering intensity at successive time intervals to derive the rate at which the intensity is varying. The information of the correlator is then analyzed by

Table 1
Composition of the grind.

Non-gushing barley – non-gushing malt							
NG barley (%)	0	10	20	30	40	50	n.d. ^a
NG malt (%)	100	90	80	70	60	50	n.d.
Gushing barley – non-gushing malt							
G barley (%)	0	10	20	30	40	50	n.d.
NG malt (%)	100	90	80	70	60	50	n.d.
Non-gushing malt – Gushing malt							
NG malt (%)	100	90	80	70	60	50	0
G malt (%)	0	10	20	30	40	50	100
Non-gushing barley – Gushing malt							
NG barley (%)	0	10	20	30	40	50	n.d.
G malt (%)	100	90	80	70	60	50	n.d.

^a Not determined.

the machine software and the size information is obtained (Hunter, 2005). The objective of this study was the combination of the M²CT and the DLS methods to analyze malt samples but also harvested barley regarding the primary gushing potential and to show how the DLS method gives more certainty about the causes of gushing.

Materials and methods

Characterization of gushing potential of barley and malt by doubly Modified Carlsberg Test (M²CT)

A gushing (variety Azurel) and a non-gushing (variety Sebastian) barley samples were kindly provided by the malt house Dingemans (Stabroek, Belgium). Gushing and non-gushing malt samples (variety Prestige) were kindly provided by Cargill (Herent, Belgium). In order to extract hydrophobins from grains, standard laboratory Congress wort was produced according to Analytica-EBC (2004) method 4.5.1. Laboratory-scale mashing experiments were carried out in an automated mashing bath (LB8 Electronic Mashing Bath, Funke Gerber GmbH, Berlin, Germany). The grains were ground in a Bühler-Miag mill (Bühler-Miag, Minneapolis, MN) set for fine grist coarseness (0.2 mm gap between the grinding discs). Fine grind (50.0 g; the composition (in %) containing gushing and non-gushing grists is given in Table 1) was mixed with 200 mL of water at 46 °C. A temperature of 45 °C was maintained in the mash for 30 min. The temperature was then raised at 1 °C per min to 70 °C before 100 mL water (70 °C) were added. The temperature was maintained at 70 °C for 1 h before cooling down to room temperature in 10–15 min (4 °C per min). The mash was continuously stirred at 100 rpm. After adjusting the beaker content to 450 g, the contents were stirred thoroughly and emptied immediately and completely into a filter (filter Macherey-Nagel MN 614 ¼ 320 mm diameter, Filter Service S.A., Eupen, Belgium). The first 100 mL of the filtrate were returned to the funnel. The filtration was stopped when the cake appeared dry and the wort was obtained. Each combination (Table 1) was used at least two times to produce a wort. For each wort obtained, 20 mL of sparkling water (1 L, 7 g CO₂/L, 2 °C) were replaced by 20 mL of wort. The bottles were crowned, weighed, and shaken at 150 rpm (Bühler GmbH SM30, Berlin, Germany) in a horizontal position for 3 days at room temperature (25 °C; Bühler GmbH TH30 incubator hoods, Berlin, Germany). After shaking, the bottles were left standing for 10 min and then manually turned upside down and right side up three times, with 10 s of standing between each turn. After the last turn and 30 s of standing, the bottles were opened. Once overfoaming ended, the bottles were weighed to determine the amount of overfoaming that occurred (Garbe et al., 2007; Rath, 2008; Deckers et al., 2011). Two bottles were prepared at least two times for each wort produced.

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