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Dynamic light scattering and the confirmation of Nanobomb theory in primary gushing

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INTRODUCTON

Hydrophobins and primary gushing

Class II Hydrophobins are fungal amphiphilic surface active proteins (Fig. 1). They are produced during their vegetative growth covering spores and hyphae to make them hydrophobic and more resilient to the weather conditions (Linder, 2005), as a particular feature, hydrophobins can interact very strongly with CO_2 molecules through their hydrophobic patch causing a well known phenomenon called primary gushing.

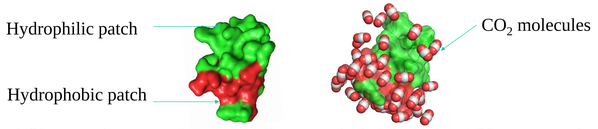


Fig. 1. Right: graphic representation of a class II hydrophobin, Left: interaction of CO_2 molecules with the hydrophobic patch of a hydrophobin

Primary gushing is a physical phenomenon caused by the interaction of hydrophobins with gaseous CO_2 producing a strong spontaneous overfoaming out of the container without any shaking (Fig. 2).

Fig. 2. Primary gushing produced by class II hydrophobins

Class II hydrophobins encapsulate CO_2 molecules into nanobubbles structures (Deckers et al, 2012) stabilizing and solubilizing them**;** these nanobubbles will remain stable until bottle opening. The sudden pressure drop will break the nanobubbles, releasing all their energy causing gushing. The diameter of the particles was calculated to be 100 nm, at atmospheric pressure and when the pressure increases inside the bottle, they shrink to 63 nm. Currently, detection of 100 nm nanoparticles is possible using dynamic light scattering. However, finding these particles inside of a bottle under pressure has not been confirmed. In this work**,** a specially designed DLS equipment was used to detect 63 nm particles inside a pressurized bottle.

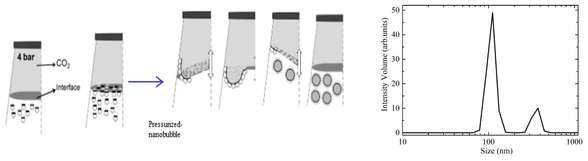
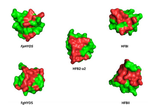


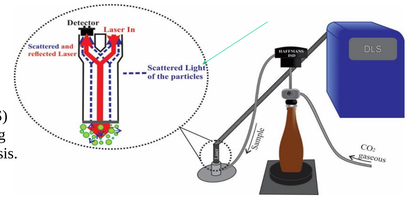
Fig. 3. Right: Nanobomb formation theory, Left: Detection of 100 nm nanobubbles at atmospheric pressure after opening the bottle of a gushing positive sample

MATERIALS AND METHODS



Five different class II hydrophobin solutions were used to test the formation of nanobubbles under pressure HFBI (*Trichoderma reesei*), HFBII (*T. reesei*), HFB2-a2 (*T.harzianum*), FpHYD5 (*Fusarium poae*) and FgHYD5 (*F. graminearum*).

Solutions of pure hydrophobin were inoculated into beer bottles filled with sparkling water, each bottle was then shaken for 3 days at 25°C and 75 rpm. Afterwards, each bottle was removed and allowed to rest for a minimum of 10 minutes before DLS analysis.



A specially designed dynamic light scattering (DLS) equipped with a sampling device capable of holding the sample under pressure at all times during analysis.

RESULTS AND DISCUSSION

Detection of nanobombs at different pressures

Using the special pressurized chamber for the DLS detector. Detection of nanoparticles at 4 bar was possible, as shown in Fig 4. There was a decrease in the nanobubble diameter along with the pressure increase. This is clear evidence that nanobombs are present inside the bottle and they are responsible for primary gushing events.

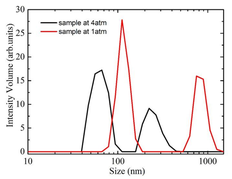


Fig. 4. Detection of the shrinking process of hydrophobin nanobubbles with increasing pressure

Formation and detection of nanobombs with different hydrophobins

Nanobubble formation with different class II hydrophobins was also tested, showing a similar pattern across all the proteins**.** As shown in Fig. 5, nanoparticles around 63 and 71 nm were found. Although the gushing potential can be different depending of the hydrophobin involved; these differences are related to how the hydrophobins self-assembl**e**, which causes the amount of CO_2 fixed to vary (Riveros, 2015). However, the mechanism of action remains the same for all hydrophobins tested (nanobubbles formation).

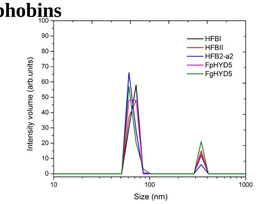


Fig. 5. Presence of nanoparticles using five different hydrophobins

RESULTS AND DISCUSSION

Effect of hydrophobin concentration in the formation and detection of nanobombs

The ability to detect different concentrations of purified hydrophobins was tested, as shown in Fig. 6. The detection of nanoparticles at 63-65 nm was possible using concentrations ranging from 0.5-1.5 mg/mL. However, concentrations below 0.5mg / mL were not able to detected due to the sensitivity of the machine (too diluted to be detected).

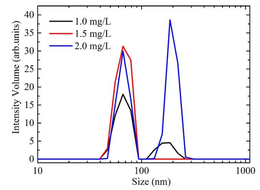


Fig. 6. Effect of different hydrophobin concentrations in nanobombs formation

Influence of headspace in the formation of nanobombs

Since the formation of nanobubbles takes place at the gas/liquid interface, the ratio of Hydrophobin to interface diameter was investigated using three different levels of filling: high (330 mL), medium (270 mL) and low (230 mL) (Fig. 7). Once again, nanoparticles around 68-72 nm were founded at all three levels. It is noteworthy that independent of the headspace volume, nanobubble assembly took place. It is clear that class II hydrophobins and CO_2 molecules interacting at an interface are necessary for nanobubble formation.

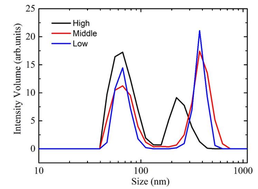


Fig. 7. influence of the headspace level in nanobombs formation

CONCLUSIONS

Dynamic light scattering was successfully used to detect nanoparticles of class II Hydrophobins/CO_2 under pressure (4 bar) in beer bottles filled with sparkling water. The presence of 63 nm nanobubbles were found under different concentrations and filling levels. Nanobubbles were also detected when different hydrophobins were used.

It has been theorized that a nanobomb of 100 nm formed at atmospheric pressure will shrink to around 63 nm as the internal pressure of the bottle increases and stabilizes. The pressure will remaining stable until the bottle is opened**,** where after a primary gushing will occur. The detection of 63 nm nanoparticles in samples contaminated with pure hydrophobins confirms the nanobomb theory.

The use of dynamic light scattering with pressure sensitive detectors is a powerful tool to predict primary gushing events. Some of the advantages of this technique are: no additional equipment required, minimal sample preparation, and rapid analysis time. This makes DLS a suitable solution for routine analysis in the laboratory and carbonated beverage industries.

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