Malt & Beer Sciences

LFoRCe
Leuven Food Science and Nutrition Research Centre

KULeuven- MBS at **M²S / site Leuven**

Primary gushing of over-carbonated beverages such as Belgian specialty beers

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Problem Statement

Gushing is a spontaneous and sudden overfoaming phenomenon occuring at the opening without any shaking. While secondary gushing is due to technical and technological problems (crystals of calcium oxalate, filter aids,…), primary gushing occurs when the weather conditions (high humidity and temperature) are favorable for fungal contamination.

It is assumed that primary gushing is provoked by fungal proteins called hydrophobins(Ref.1). These are small amphiphilic and surface-active proteins that are able to adsorb at the hydrophobic-hydrophilic interface such as the air-water interface. From malt to finished beer the concentration of hydrophobin is 10-fold-reduced(Ref.2).

Production of hydrophobin HFBI (*Trichoderma reesei*) at MBS-KUL

Culture(Ref.3,4)

Trichoderma reesei
MUCL 44908



Culture conditions

Working volume (300 mL), 25°C, 200 rpm, 4 days, addition of 6g glucose after 30h

Reactant	Concentration (g/L)
glucose	40.0
peptone	4.0
yeast extract	1.0
KH_2PO_4	4.0
$(NH_4)_2SO_4$	2.8
$MgSO_4.7H_2O$	0.6
$CaCl_2.2H_2O$	0.8
$CoCl_2.6H_2O$	4.0
$MnSO_4.H_2O$	3.2
$ZnSO_4.7H_2O$	6.9
$FeSO_4.7H_2O$	10.0

Extraction(Ref.3)



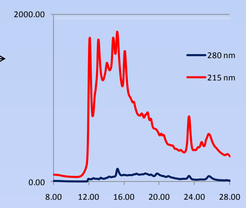
Buffer Mycelium extract

Extraction of HFBI (Class II)
Tris (170mM) HCl buffer pH9 containing 1% SDS

500 µL

Purification

Source 15RPC
Elution with linear gradient Acetonitrile (0-60% in 20minutes) containing 0.1% Trifluoroacetic acid at a flow rate 1mL/min



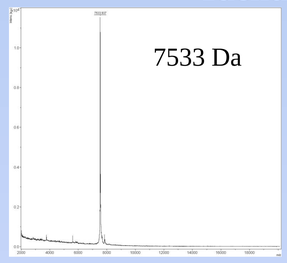
Detection by UV at the wavelengths of 215 and 280 nm.
Fractions between 50-60% ACN were collected and pooled

MALDI-TOF

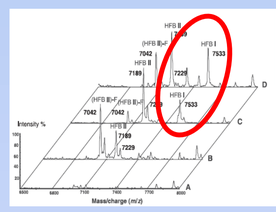
Matrix Assisted Laser Desorption Ionization – Time Of Flight

Edman degradation

Identification



Identification by comparison of the molecular weight with the results published by Neuhof and al. (Ref.5).



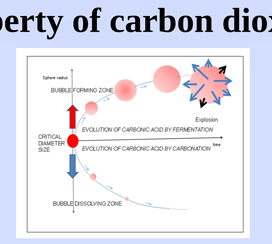
From Neuhof and al. (2007) (Ref.5)

HFBI sequence	S	N	G	N	G	N	V	C	P	P	G	L
Experimental sequence	/	/	/	N	G	N	V	X	P	P	G	L

In collaboration with : Laboratory of Food Microbiology-KUL Laboratory of Food Chemistry and Biochemistry-KUL ProMeta-KUL

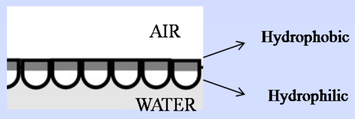
Theory of the primary gushing (Deckers *et al.*, 2010; MBS-KUL) (Ref.6) *

Property of carbon dioxide



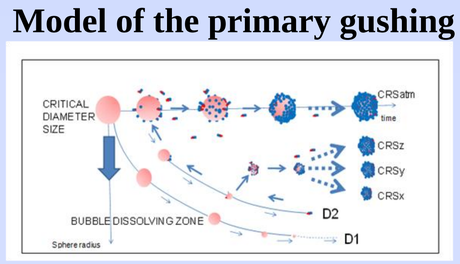
Carbon dioxide is a hydrophobic gas. For a gas, there is a critical diameter above which the gas bubbles will grow until they explode at the surface and under which they will shrink in size until their complete dissolution except in presence of a stabilizer.

Property of hydrophobin



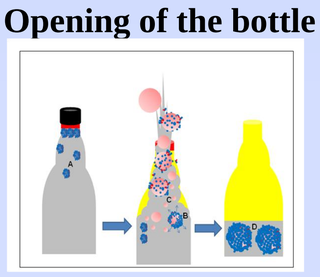
Hydrophobin are surface-active and can self-assemble at the hydrophobic-hydrophilic interface(Ref.7). While the self-assemby of Class I hydrophobin are insoluble (except in strong acid), the self-assembly of Class II hydrophobin are more easily soluble (60% ethanol, 2% SDS)(Ref.8).

Model of the primary gushing



The carbon dioxide nanobubbles represent a hydrophobic-hydrophilic interface at which hydrophobins can self-assemble to stabilize them and to avoid their complete dissolution. The size and the internal pressure of these stabilized nanobubbles depend on the internal conditions in the closed bottle.

Opening of the bottle

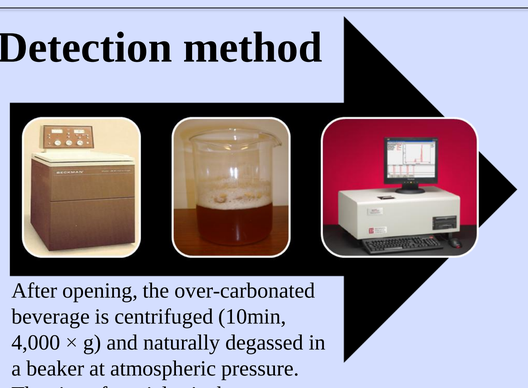


At the opening, the stabilized nanobubbles act as nanobombs. Due to the drop of pressure, the volume of nanobubbles increases (Gay Lussac's Law), the hydrophobin film is destroyed and nucleation sites are formed creating gushing. After natural degassing at atmospheric pressure, carbon dioxide molecules contaminated by hydrophobins arrange together to form bubbles with critical diameter

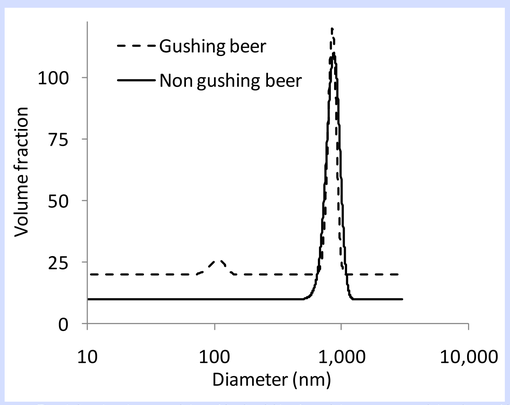
* Own original PhD finding

Detection method

As suggested by Nüter and Harms(Ref.9), particle size measurement can be used to do a comparison between a gushing and a non gushing beer. A method based on the Dynamic Light Scattering (DLS) technique is being developed to predict the gushing potential of over-carbonated beverages. DLS is a technique used to determined the size of particles (hydrodynamic diameter). It measures the Brownian motion (random movement due to collision with solvent molecules) and relates this to the size of particles. The larger (smaller) the particle, the slower (faster) the Brownian motion will be. The speed at which the particles are diffusing is determined by measuring the rate at which the intensity of the scattered light fluctuates when detected using a suitable optical arrangement. The small particles cause the intensity to fluctuate more rapidly than the large ones. A correlator is designed to measure the degree of similarity between two signals, or one signal with itself at varying time intervals. If the particles are large the signal will be changing slowly and the correlation will persist for a long time. If the particles are small and moving rapidly then correlation will reduce more quickly.



After opening, the over-carbonated beverage is centrifuged (10min, 4,000 × g) and naturally degassed in a beaker at atmospheric pressure. The size of particles is then determined by DLS



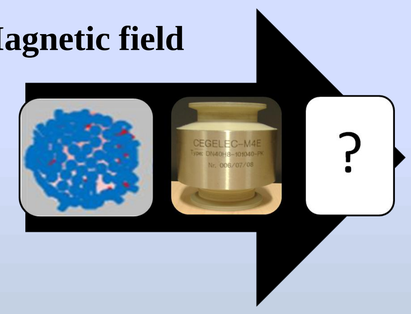
Particle size in a gushing (dashed line) and in a non gushing beer (dark line)

In collaboration with Centre for Surface Chemistry and Catalysis-KUL

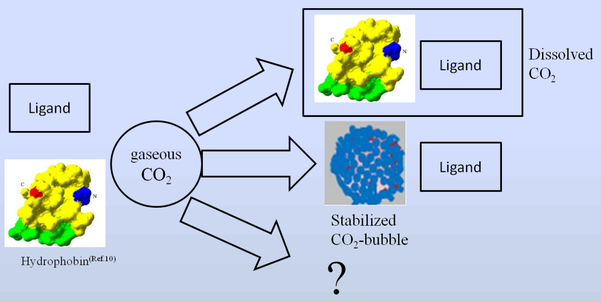
Preventive method
Curative method

Running

Magnetic field



Ligand



In collaboration with : M4E and Centre for Surface Chemistry and Catalysis-KUL Laboratory of Food Chemistry and Biochemistry-KUL

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