Aroma release properties from yeast cell encapsulates in watery applications

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Introduction

The requirements to use natural flavours in foods rather than artificial ones are on the rise nowadays. In the same time there is a growing technological need in the food industry to get cook-stable flavours.
A recently introduced flavour encapsulation technology based on yeast cells appears to have some potential for addressing these needs (Pannell, 1987; Bishop et al, 1998). In this work the benefits and limitations of yeast cell encapsulates have been evaluated in water using limonene and ginger aroma essential oils. In particular the effect of heating temperatures applied to the system on the aroma retention properties of yeast encapsulates has been investigated analytically.

Material and methods

Instant baker's yeast (Saccharomyces cerevisiae) was purchased from DSM Bakery Ingredients (Dordrecht, NL). These yeast cells are partially deactivated by a heat treatment at 80°C during production. This yeast product was assessed as being microbiologically stable in an aqueous medium. Pure grade aroma internal standards (limonene and other aroma molecules naturally present in ginger flavour oil: citral, geraniol, alpha-terpineol and nerolidol) were obtained from Sigma-Aldrich. Ginger essential oil was purchased from Kalsec (Mildenhall, UK).

The procedure applied in this work for encapsulating aroma compounds in yeast cells was adapted from Bishop et al. (1998) and Pannell (1987). Yeast cells were pre-hydrated in demineralised water. The aroma essential oil was then added to the cell suspension under moderate vortex. The aroma/water/yeast ratio applied was 1/4/1. The encapsulation process was allowed to take place under gentle stirring during 4 hours at 40°C (in a bain-marie). The flavoured yeast cell medium was then washed by centrifugation at 2300 rpm during 10 minutes. After discarding the supernatant, fresh demineralised water was added before the next centrifugation cycle. 6 washing/centrifugation cycles were applied in total. The resulting paste-like product was freeze-dried for at least 48 hours.

In order to quantify the extent of essential oil encapsulated in yeast cells an aroma extraction method was applied to allow for an accurate characterisation by gas chromatography. The extraction procedure was based on the work published by Bishop et al. (1998). Ethanol was used as the extraction solvent (with a large volume fraction) to solubilise the lipid phase (including the yeast cell plasma membrane) and release the aroma essential oil.

The analytical characterisation of aroma retention and headspace release in temperature was carried out by Atmospheric Pressure Chemical Ionisation - Mass Spectroscopy (APCI-MS). The working principle of APCI-MS is first the production of protonated molecular ions $[M+H]^+$ that are then analysed via mass spectrometry. The analysis was done on a Finnigan Navigator mass spectrometer fitted with an APCI interface and operated via the MassLynx software.
The sample temperature was controlled by immersion of its glass container into a water bath installed underneath the APCI probe. The connection between the APCI probe and either the sample headspace or the laboratory air was achieved with a 3-way inox valve Swagelock. A second valve (simple on/off) was also connected to the sample headspace to allow ambient air to be sucked in when the Swagelock valve was directed towards the APCI probe for volatile release intensity measurements (pseudo-dynamic sample headspace). A preheating time of 5 minutes was allowed in all experiments at constant temperature so that the sample temperature could be close to the target temperature when starting the headspace aroma release measurements. In addition, the temperature

of the Swagelock valve was set 10 to 15°C above that of the sample with a heating line embedded into a covering insulating material in order to limit vapour condensation effects inside the tubing.
At temperatures above 55/60°C, the formation of water droplets in the gas tubes could not be fully prevented. This led to an elevated ratio of noise over signal intensity for measurements performed at temperatures higher than 60°C. However reliable data information could be exploited for sample temperatures up to 85°C.

Results and Discussion

The mechanism that has been proposed for the penetration of aroma essential oil inside yeast cells is passive diffusion across the cell wall and the cytoplasmic membrane (Bishop et al., 1998). A two-way process has been proposed by these authors to describe the encapsulation of aroma in yeast cells: the displacement of internal cell matter simultenaously with the uptake of oil emulsified by internal lipids. The result is then a partitioning of the oil into the yeast cells which become aroma capsules.
The plasma membrane appears to be the major permeability barrier to the diffusion of aroma essential oil into the yeast cells. The requirements for the aroma molecules to pass through the hydrated phospholipid bilayer are to be lipophilic enough as well as short enough in chain length. These 2 requirements were evaluated in more details with ginger essential oil. Furthermore the fluidity of the plasma membrane can be manipulated by changing the temperature, to alter the encapsulation rates of aroma compounds as well as the encapsulation kinetics (Bishop et al., 1998).

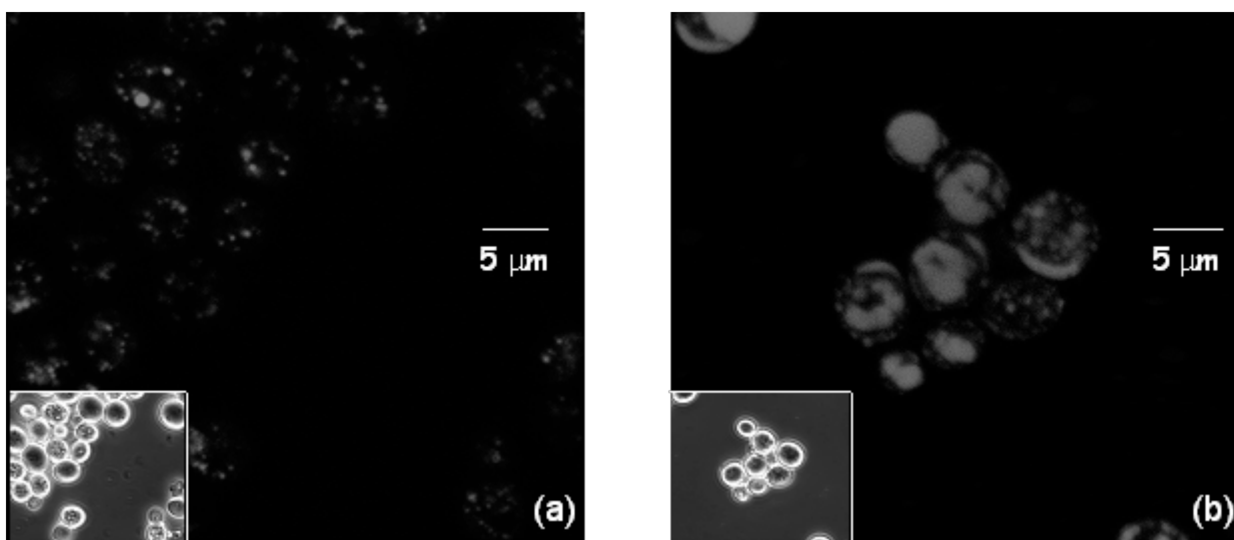

Figure 1: Fluorescence confocal laser scanning (Nile Blue staining at 0.1% w/v) / phase contrast (bottom-left corner) micrographs of yeast cells with (a) no added aroma (original baker's yeast) and (b) after encapsulation of limonene essential oil during 4 hours incubation at 40°C (loading ca. 32% w/w).

Figure 1a and 1b show the optical images of respectively the original yeast cells, with no added flavour, compared to yeast cells with encapsulated limonene essential oil after 4 hours of incubation at 40°C. The white spots appearing on the confocal light scanning micrograph in Figure 1a correspond to the 3 to 5% inherent lipid oil naturally present in yeast cells (stained with Nile Blue, a fat-soluble dye that fluoresces at an excitation wavelength of 488 nm). In Figure 1b it can be observed in the phase contrast image that the cell internal structure is no longer visible as before the aroma encapsulation process, suggesting indeed that the internal matter has been expelled from the cells (forming a floating layer observed on top of the cell suspension during the incubation with aroma essential oil). In addition the fluorescent spots present inside the yeast cells in the confocal microscopy image of Figure 1b are much bigger than prior to the aroma encapsulation. This is most likely due to the limonene essential oil cell intake during the incubation phase; after extraction of the yeast-encapsulated limonene aroma and quantification via gas chromatography with the help of

an internal standard, the flavour loading was measured to be of 32±3% w/w with respect to yeast cell weight. Encapsulation of citral and geraniol pure flavour oils in the same process conditions led to the same level of aroma loading in yeast: 27±2% and 23±2%, respectively.

In order to demonstrate that superior flavour retention could be achieved with yeast encapsulation, aroma release intensity with yeast encapsulates suspended in pure water was compared to that of equivalent aqueous systems with no encapsulates ('free' aroma, same concentration). The graph of Figure 2 shows the benefit of encapsulating a citrus flavour in yeast cells in terms of flavour retention when the samples are heated above 30°C. The released intensity of limonene (50 ppm) was measured after heating samples of 20 mL for 16 minutes at a constant temperature. These intensity values were obtained for different heating temperatures (ie. each data point corresponds to a different measurement with a new sample). The data are plotted as a function of the applied heating temperature. The behaviour of the encapsulated system was compared to the one of the non-encapsulated flavour. At ambient temperature the limonene release intensity measured in the 2 cases is more or less comparable; the loss of non-encapsulated aroma after 16 minutes at 20°C is indeed not expected to be significant. At higher temperatures the benefit of yeast encapsulates appears clearly from 30°C already. Furthermore this benefit increases further, up to a heating temperature of 50/60°C and then remains constant up to a heating temperature of 80°C. These data demonstrate with no ambiguity that yeast encapsulation offers excellent flavour retention in temperature (at least up to 80°C).

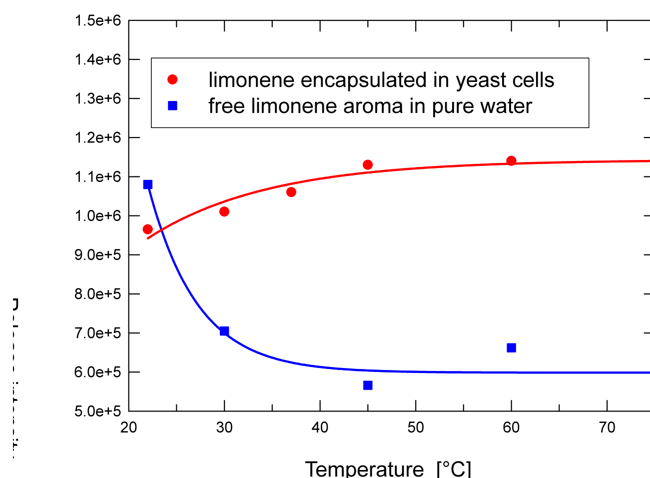

Figure 2: Limonene release intensity measured by APCI-MS after 16 minutes heating, at a constant temperature, of limonene essential oil encapsulated in yeast cells dispersed in water (circles) or free limonene aroma dispersed in pure water (squares). Aroma concentration was 50ppm in all samples.

The flavour retention properties of yeast encapsulates were further investigated with ginger sesquiterpenes. Here the focus was on the comparison of the time release profiles between the encapsulated and the free flavours (at the same sample aroma concentration of 100 ppm). The experiment was conducted at a measured sample temperature of 73°C. It can be seen in Figure 3 that significant noise level is appearing in the signal measured for the non-encapsulated flavour; though the true data can be easily extrapolated (see dotted line). This result shows a remarkable flavour retention effect since the spurt-like initial peak is 21 times smaller in surface area with yeast capsules than without. In other words the free flavour aqueous system has been significantly depleted in sesquiterpene compounds whereas the encapsulated system keeps a heavily loaded reservoir of aroma until at least 17 minutes heating at 73°C (plus pre-heating). Further data, that will be shown in the poster, demonstrate that the steady sesquiterpene aroma release intensity can be sustained in fact during several hours at the same level of applied temperature.
In Figure 3 it can be seen that the level of aroma release intensity remaining after 17 minutes of heating is comparable in the 2 cases. However, the heating temperature in this experiment was only

73°C. In many real conditions of cooking, the food product temperature can be much higher than that. Therefore it is expected on the one hand that in the free flavour case the depletion in aroma compounds would occur much quicker (for same aroma volatility). And on the other hand it is hypothesised that in the encapsulated case the level of steady-state aroma release intensity versus time will be comparable at higher applied temperatures (before dropping rapidly when the yeast capsules reservoir of aroma is starting to be depleted). This hypothesis will be demonstrated in the poster by aroma release intensity data obtained by APCI-MS measurements of ginger compounds when slowly increasing the temperature of the encapsulates suspension from 10 to 85°C.
Similar aroma release experiments when slowly increasing the temperature showed a very unique heat stability property of yeast-encapsulated flavours, together with an apparent critical temperature of 60°C above which the rate of aroma diffusion through the hydrated cellular phospholipid bilayer appears to remain constant. A mechanistical hypothesis to account for this peculiar aroma heat stability, believed to be related to the properties of the phospholipid bilayer, will be discussed in the poster. Furthermore sensory evaluation of yeast-encapsulated flavours in product submitted to high temperature cooking confirmed this exceptional aroma retention. Equally, the limitations of this technology in terms of the extent of its area of applications in aqueous systems containing triglyceride fats and/or emulsifiers will be discussed in the poster as well.

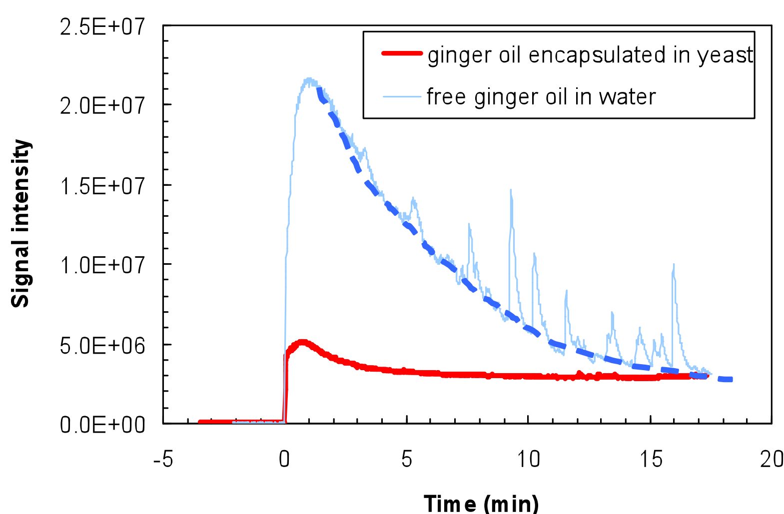

Figure 3: Sesquiterpenes aroma release intensity profiles, measured by APCI-MS, of yeast-encapsulated ginger essential oil (thick line) and free ginger oil (thin line), both dispersed in pure water and heated at a constant temperature of 73°C. Aroma concentration was 100ppm in the 2 samples.

Conclusions

The benefits of encapsulating flavours in yeast cells on their retention during cooking have been demonstrated in model aqueous applications. Yeast cell encapsulates appear to have the rather unique property to offer excellent aroma heat stability in purely aqueous media. It has been demonstrated indeed that the rate of aroma depletion with time from the yeast capsule reservoir is very limited. Furthermore, it appears that the rate of aroma diffusion through the yeast cell bilayer membrane remains constant above a critical temperature of about 60°C. A mechanistical hypothesis related to a phase transition of the phospholipid bilayer is proposed to explain this peculiar aroma release property.

References

Bishop J.R.P., Nelson G. and Lamb J. (1998), 'Microencapsulation in yeast cells', Journal of Microencapsulation, vol.15, no.6, 761-773.
Pannell N.A. (1987), 'Microbial encapsulation', AD2 Ltd, European Patent no. 0242135B1.