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Molecule structural factors influencing the loading of flavoring compounds in a natural-preformed capsule: Yeast cells

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ABSTRACT

Yeast cells are efficient microcapsules for the encapsulation of flavoring compounds. However, as they are preformed capsules, they have to be loaded with the active. Encapsulation efficiency is to a certain level correlated with LogP. In this study, the effect of structural factors on the encapsulation of amphiphilic flavors was investigated. Homological series of carboxylic acids, ethyl esters, lactones, alcohols and ketones were encapsulated into the yeast *Yarrowia lipolytica*. Although, in a single homological series, the length of the molecule and thus the LogP were correlated with encapsulation efficiency (EY%), big differences were observable between series. For instance, carboxylic acids and lactones exhibited EY% around 45%–55%, respectively, for compounds bigger than C8 and C6, respectively, whereas ethyl esters reached only about 15–20% for C10 compounds. For a group of various C6-compounds, EY% varied from 4% for hexanal to 45% for hexanoic acid although the LogP of the two compounds was almost similar at 1.9. In total our results point out the importance of the level of polarity and localization of the polar part of the compound in addition to the global hydrophobicity of the molecule. They will be of importance to optimize the encapsulation of mixtures of compounds.

1. Introduction

Aroma compounds are organic molecules of low molecular weight (<400 Da). They are rather hydrophobic and have various physicochemical characteristics. Their hydrophobicity may be estimated through their logP which corresponds to the logarithm of the partition coefficient between water and *n*-octanol. Aroma compounds with logP value below 1.5 are considered polar and are relatively soluble in water. In food, aroma compounds are present in very small quantities; their concentrations vary from a few milligrams per tones (ppb) to a few milligrams per kilogram (ppm). However they play an important role in food sensorial properties which may affect the satisfaction of consumers and thus the choice of these latter for foods. Therefore the stability of these compounds in a product is very important as it relates directly to the appreciation of the product. Nevertheless it is very difficult to control their degradation. Most of these substances are very volatile and highly hydrophobic which makes challenge for food processor to incorporate and keep them in food matrix until consumption. Their modification or loss caused by fabrication and conservation treatment may induce the loss of consumer's perception. There are many ways to enhance their retention in food such as the addition of sugars [1] or of a fat phase [2,3]. However, due to the consumption tendency of reduction of sugar and fat contents to provide better healthier foods, this method gradually declines in food processing today. A powerful solution that can be cited here is microencapsulation.

Microencapsulation can limit this degradation or loss during manufacture or storage of a product. It is a technique using a wall material to form a protection layer around the sensitive material which makes the active core of the particle [4]. The most popular food application of microencapsulation can be found in the flavors domain [5]. The use of encapsulated flavors instead of free compounds has attracted technologists since the surrounding wall may protect them from oxygen damage, heat, moisture or other hostile environmental conditions and thus enhance their stability, especially the stability in water continuous systems. The capsules can also act as a system that helps to improve the dispersion of flavors molecules in food matrices. Alleviation of the flavor volatility is one of the objectives that can be attained through microencapsulation as well. This approach is carried out through many different methods such as spray-drying, emulsification, building of liposomes, etc with various wall materials including maltodextrins, phospholipids, gums, etc. [6].

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Yeast has subsisted in human culinary life for long time ago, especially in beer, wine and bread production. It is a friendly material for consumer. Since it provides a natural and pre-formed capsule, it has been interesting for many researchers using it as a novel means for encapsulation process. Over the last few years, various studies focused on highlighting yeast advantages when acting as a capsule. Yeast cell wall is proved to have antioxidant potential [7] that can protect encapsulated compounds from oxidation and photodegradation [8] as well as heat treatment [9,10]. Apart from these interesting properties, the yeast cell is a high volume low cost material for encapsulation [11]. Using yeast cells as microcapsules for flavor encapsulation has been increasingly developed. Up to now, there are many patents exploiting this technique for use in cosmetics, drug delivery, and especially in food flavors.

Most encapsulation processes consist in building a capsule around the active, however, in some cases using hollow core prepared by the layer-by-layer technique [12] or already existing cell/tissues structures [13], to encapsulate means to load the active molecule into the already existing structure. This is the case when using yeast cells as microcapsules. The encapsulating material must pass through the cell wall and the cell membrane prior to be retained inside the cell. This process is proved to occur via a passive diffusion way in which molecules go down the concentration gradient [14,15] and no cellular or chemical energy is required. Flavor encapsulation by yeast is therefore governed by their physicochemical properties such as their molecular size and hydrophilic properties as well as by the physiological state of the yeast cell. Some studies showed that the more lipophilic and small molecules are, the better encapsulation will occur [14,16]. Another work raised the role of the cell wall in controlling molecular fluxes through the cell [17]. It can be inferred that molecular encapsulation using yeast cells depends on the selectivity process managed by the cell envelope. It also means that not all molecule types can pass through the cell envelope. Some years ago, by experiments on 98 flavor molecules stretching on a wide range of logP value (−1.09–6.39), Dardelle et al. [18] demonstrated that the molecular logP value highly affects the encapsulation achievement. Particularly, high encapsulation efficiency was achieved when high logP molecules were used. However, some of their results did not fit with this principle. Some substances had the same logP value but displayed different encapsulation efficiency result. Or in another case, molecules with different logP value from 2 to 6 reached the same result. The authors just raised a hypothesis that other properties such as molecular size, sharp, affinity with the wall material could explain this situation. This phenomenon is still rather unexplained. Beside the logP value, the importance of the molecular structure on the encapsulation achievement needs to be investigated. This study therefore aims to point out the impact of molecular structure and cell physiological and physical state on the encapsulation yield in yeast-based microencapsulation processes.

2. Materials and methods

2.1. Microrganism and cultivation conditions

The strain Yarrowia lipolytica W29 (ATCC20460; CLIB89) was used in this study. Stock cultures were prepared with subsequent storage at −80 °C. Cells from thawed stock cultures were activated in yeast extract peptone dextrose agar (YPDA) medium (all concentrations are given in g 100 mL^{-1}) (1 yeast extract, 2 peptone, 2 glucose, 2 agar) for 48 h at 27 °C and used to inoculate YPD (1 yeast extract, 2 peptone, 2 glucose, 2 agar) or YPO (1 yeast extract, 2 peptone, 0.5 methyl oleate, 0.002 Tween 80) liquid media cultivated at 27 °C under a140 rpm agitation (Gerhardt Thermoshake Incubating Orbital Shaker, Germany) in a baffled Erlenmeyer flask. Cells in the mid-logarithm growth phase (19 h for YPD and 24 h for YPO) were harvested and washed three times with sterile physiological water (NaCl 0.9) (pH_2O). The optical density at 600 nm was monitored with a ThermospectronicBiomate 3 UV–vis Spectrophotometer (USA).

2.2. Flavor compounds

The compounds used for encapsulation in this study have been chosen in homologous series of carboxylic acids, ethyl ester, lactones, alcohols and ketones. They are given in Table 1 and are all from Sigma Aldrich, Saint-Quentin Fallavier, France except hexanol (Prolabo VWR) and hexanoic acid (Merck Chimie), both located in Fontenay-sous-Bois, France.

2.3. Yeast treatment before encapsulation

2.3.1. Heat treatment

200 mL of yeast culture at OD_{600} = 2 were heated at 95 °C for 1 h in a water bath. Cells were then collected and washed twice in physiological water for further encapsulation process.

2.3.2. β-mercaptoethanol (β-ME) treatment

Cells harvested after cultivation (3 g wet weight) were suspended in 165 mL of ammonium carbonate (1.89 g L^{-1}) and 1% β-ME (v/v) solution, and incubated at 37 °C for 30 min according to Casanova and Chaffin [17]. After incubation, cells were washed twice with 0.6 M KCl solution for further encapsulation process.

2.3.3. Enzyme treatment

Cells harvested after cultivation were suspended in Tris-HCl/EDTA (TE) buffer solution (pH 7.5), 200 mL of cellular suspension at OD_{600} = 2 were treated with 500 μL of protease (Protease inhibitor cocktail P8215, Sigma) in a 500 mL Erlenmeyer flask tightened with screw cap. The incubation took place at 35 °C for 60 min with gentle agitation (65 rpm). Cells were then harvested and washed twice in TE buffer solution for further encapsulation process.

2.4. Flavor yeast based microcapsules preparation

Cells were harvested in the mid-logarithmic phase and were washed 3 times in pH_2O. Wet yeast cells with or without supplementary treatment (heat treatment or enzyme treatment) were mixed directly with desired flavor following the ratio: 1 g wet yeast cell, 4 g distilled water, 1 g desired flavor in a small hermetic tube. The incubation was carried out for 4 h at 50 °C and 180 rpm. Loaded yeasts were harvested by centrifugation. The microcapsules were then washed twice with distilled water (dH_2O), the resulting slurry was freezed at −78 °C for at least 1 h for complete freezing, the frozen microcapsules were lyophilized for 20 h using a freeze dryer (Thermo Scientific HetoPowerDry PL6000 Freeze Dryer) with a condenser temperature of −55 °C and a pressure of 0.025 hPa. The resulted capsules were stored at room temperature until further experiments.

2.5. Determination of the encapsulation yield

2.5.1. Extraction procedure

The extraction process was performed as described by Dardelle et al. (2007) with some modifications [18]. Briefly, 100 mg of flavor yeast microcapsules were mixed with 1.5 mL dH_2O and 5 μL of the internal standard (1-pentanol, 99%, Sigma) in a 50 mL-corning tube. After 15 min resting at room temperature, 10 mL hexane were added in the tube and the mixture was forcefully agitated (200 rpm, Gerhardt Thermoshake Incubating Orbital Shaker, Germany) for