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Microencapsulation of curcumin in cells of *Saccharomyces cerevisiae*

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ABSTRACT

Curcumin was successfully encapsulated in yeast cells of *Saccharomyces cerevisiae* as confirmed by fluorescence microscopy, Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FT-IR). Emphasis was given on the encapsulation parameters including temperature, plasmolysis of yeast cells, presence of ethanol and mass ratio curcumin:cells that affected the amount of curcumin finally encapsulated, as expressed by the %Encapsulation Yield (%EY) and %Encapsulation Efficiency (%EE). Encapsulation was favoured at temperatures above 35 °C and preparation of microcapsules in water instead of 50% v/v ethanol increased the %EY and %EE values by at least 2-fold. Although plasmolysis of yeast cells modified membrane's fluidity and cell wall's composition, the microcapsules prepared with plasmolysed cells did not differ in their curcumin content when compared to those prepared with non-plasmolysed cells. Proper combination of the abovementioned parameters resulted in microcapsules that contained up to 35.8 ± 0.86% w/w curcumin. In all microcapsules prepared curcumin was integrated in the plasma membrane bilayer but also interacted with constituents of the cell wall network.

1. Introduction

Microencapsulation is a technique that has widely been used in the drug industry and during the last decades finds many applications in the food industry. It is defined as a process in which tiny particles or droplets of a micro-component (antioxidant, vitamin, natural colourant, enzyme, etc.) are surrounded by another material, which is called wall material/coating/carrier or embedded in a homogeneous or heterogeneous matrix, resulting to the formation of small capsules. Encapsulation imparts some degree of stabilisation to the active compound since the wall material acts as a physical barrier for oxygen or other molecules, preventing deleterious reactions. Besides, the encapsulated compound can be released in a controlled way in complex biological systems or in product applications (Madene, Jacquot, Scher, & Desobry, 2006). Since the stability and release properties of microcapsules are highly dependent on wall material's composition, various kinds of carriers have been used, including sugars, cyclodextrins, maltodextrins, modified starches, gums, proteins and nanoparticles, micelles or liposomes (Gibbs, Kermasha, Alli, & Mulligan, 1999). However, cost and legal constraints in the food industry impose the need for new or novel coatings (Gouin, 2004).

Saccharomyces cerevisiae yeast cells, which are fundamental in the fermentation industry, can be regarded as food-grade, low-cost and abundant food ingredients. Besides, their phospholipid membranes can behave as liposomes and have been used for the encapsulation of both hydrophobic and hydrophilic molecules, like resveratrol (Shi et al., 2008), essential oils (Bishop, Nelson, & Lamb, 1998), as well as for the water-soluble chlorogenic acid (Shi et al., 2007) and enzymes (Chow & Palecet, 2004). Normand, Dardelle, Bouquerand, Nicolas, and Johnston (2005) used the hydrophobic volative compound limonene, after encapsulation in the yeast cells, as a model and studied the release mechanism as affected by temperature and water uptake. An exceptional behaviour of this delivery system was demonstrated where temperature above 260 °C and water activity above 0.7 were necessary for release to occur. Moreover, Dardelle et al. (2007) used yeast cells for the encapsulation of 98 different substances, covering a large range of log*P* (where *P* refers to the partition coefficient) and found that %EE could exceed 50% when log*P* was higher than 2. Furthermore, it was shown that the dry loaded cells could not release their content in oil or in air, contrary to the wet loaded cells. It is noteworthy that the concept of encapsulation into yeast cells has also been the topic of several patents (Inoue, Ishiguro, Ishiwaki, & Yamada, 1991; Round & Nelson, 2006). Compared to liposomes, the yeast cell has the extra advantage of the external thick cell wall, which mainly consists of a β-1,3-glucan network, a mannoprotein layer and a small amount of chitin. The wall provides mechanical strength to the cell and allows to molecules with molecular weight up to 760 to diffuse freely. On the contrary, the cell membrane is the major permeability barrier for a permeating molecule (De Nobel, Klis, Munnik, Priem, & Van Den Ende, 1990).

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Curcumin (diferuloylmethane) is a polyphenol derived from the perennial herb *Curcuma longa* (turmeric), commonly used in India. In powder form it has a yellow–orange colour whilst in solution a yellow hue at pH 2.5–7.0 and red at pH > 7.0 (Goel, Kunnumakkara, & Aggarwal, 2008). In human nutrition, curcumin is greatly used since it is the main constituent of the herb curry and in the food industry is added in commercial jellies as a stabilizer or as a natural colourant in cheeses, pickles, mustard, cereals, soups, ice cream and yogurts, replacing artificial colourants.

Curcumin is, also, a very interesting pharmacological compound since it possesses anticarcinogenic properties and suppresses proliferation of a wide variety of tumour cells. It has protective action against skin diseases (scleroderma, psoriasis), autoimmune diseases (rheumatoid arthritis, psoriasis, inflammatory bowel disease) as well as against Alzheimer's disease and HIV-replication (Pari, Tewas, & Eckel, 2008). Besides, curcumin reduces blood cholesterol and prevents LDL oxidation (Pari et al., 2008). Due to the above-mentioned health-promoting effects, curcumin is commercially available in the form of tablets, soft-gels, capsules or trans-dermal film.

Major barriers for curcumin's use in the food processing industry are its poor water solubility and susceptibility to alkaline conditions, light, oxidation and heat (Sharma, Gescher, & Steward, 2005) which also limit its clinical efficacy. Encapsulation has been used to overcome most of the drawbacks of curcumin using a variety of encapsulation coatings: gelatin (Aziz, Pen, & Tan, 2007), cyclodextrins (Tomren, Masson, Loftsson, & Tonnesen, 2002), cationic micelles (Leung, Colangelo, & Kee, 2008), liposomes (Li, Braitech, & Kurzrock, 2005), and modified starch (Yu & Huang, 2010).

The aim of this study was to evaluate the feasibility of cells of the baker's yeast *S. cerevisiae* as an encapsulation carrier for curcumin. The main parameters involved in the encapsulation mechanism were assessed and the most appropriate conditions for effective encapsulation were determined. Moreover, the microcapsules formed were studied by fluorescence microscopy, whilst the confirmation as well as the distribution of curcumin molecules in yeast cells and their interactions with yeast cell components was studied with DSC and FT-IR.

2. Materials and methods

2.1. Materials

Curcumin was obtained from Sigma (St. Louis, MO, USA). Ethanol and sodium chloride used for yeast cell plasmolysis were of analytical grade and obtained from Merck (Darmstadt, Germany). Deionised water was used for the preparation of the complexes. All the chemicals used for HPLC analysis were HPLC grade and purchased from Merck. The yeast cells used in all the experiments were commercially available compressed *S. cerevisiae* yeast cells (L' hirondelle® blue pack, Lesaffre group, France), which according to the industrial process used they had been harvested in the stationary growth phase.

2.2. Yeast cell plasmolysis and freeze-drying

Prior to encapsulation, 500 g of the compressed yeast cells were suspended in phosphate buffer (pH 6.8) and then harvested by centrifugation (6000 rpm, 10 min). The washing procedure was repeated five times. Part of the washed cells was directly freeze-dried (referred as non-plasmolysed cells) whilst the rest was plasmolysed in sodium chloride (NaCl) solutions (plasmolysed cells). Yeast cell plasmolysis was carried out as follows: suspensions of 10% w/w of yeast cells were added to four conical flasks containing 10%, 20%, 30% and 40% w/w NaCl solutions. The flasks were agitated at 180 rpm for 48 h at 55 °C. The plasmolysed cells were harvested by centrifugation (6000 rpm, 10 min), washed five times with deionised water to remove cytoplasmic material and NaCl and finally freeze-dried. Plasmolysed and non-plasmolysed yeast cells, both freeze-dried, were evaluated for their ability to encapsulate curcumin.

During all the above-mentioned treatments, the number of yeast cells, both viable and non-viable, per mg of freeze-dried powder was microscopically assessed by using a Neubauer counting chamber (ProSciTech, Thuringowa, Australia) and methylene-blue stain (0.01% w/w in phosphate buffer, pH 6.8). Specifically, 100 mg of each freeze-dried powder of cells was suspended in 100 ml of phosphate buffer (pH 6.8) and 1 ml of this suspension was added to 1 ml of the methylene-blue stain solution. A coverslip was then placed over the counting's chamber surface and the stained suspension was introduced into the V-shaped wells with a pipet. The charged counting chamber was placed on the microscope stage and the counting grid was brought into focus. Cells with active enzymes (living cells) remained colourless, whereas the dead cells were blue-stained. The percentage of dead cells was related to the total number of cells.

2.3. Parameters that affect encapsulation of curcumin in yeast cells

2.3.1. Plasmolysed, non-plasmolysed and alive yeast cells

Plasmolysed (100 mg) and non-plasmolysed (100 mg) cells, both freeze-dried, or fresh alive (non-freeze-dried) yeast cells (300 mg) were suspended in conical flasks containing 50 ml of deionised water. The number of the cells, as measured in the Neubauer chamber, in each cell suspension prepared, was approximately 4.7×10^9 cells. Then, in each flask curcumin (100 mg) was added, either dispersed into 50 ml of water or dissolved into 50 ml ethanol (EtOH) thus, the resulted preparation solution consisted of either water or 50% v/v ethanol. The flasks were constantly agitated at 180 rpm for 48 h, at 55 °C and then each curcumin-cell suspension was collected by centrifugation (6000 rpm, 10 min). In order to remove non-encapsulated (unbounded) curcumin, cell washing was carried out, either with ethanol or with deionised water, depending on the loading protocol used. In particular, each curcumin-cell suspension that was prepared in 50% v/v ethanol was added to deionised water, thoroughly shaken, centrifuged (6000 rpm, 10 min) and the biomass was collected. The washing process was repeated three times. Each curcumin-cell suspension that was prepared in water was washed in a similar way with absolute ethanol. Finally, all washed cell preparations were freeze-fried for 24 h and kept at −38 °C for further analyses. From each freeze-dried curcumin microcapsule made, curcumin was extracted as described below (Section 2.4) and 'Encapsulation Yield' (%EY) as well as 'Encapsulation Efficiency' (%EE) was determined. %EY is defined as mg of encapsulated curcumin per 100 mg of microcapsule and %EE as the ratio of the quantity of encapsulated curcumin over its total quantity initially added:

$$\mathrm{EE}(\%) = \frac{C_E}{C_T} \times 100, \quad \mathrm{EY}(\%) = \frac{C_E}{C_M} \times 100$$

where C_E refers to the mass of encapsulated curcumin, C_T to total initially added curcumin mass, and C_M to the mass of the resulted microcapsule.

2.3.2. Mass ratio of curcumin:cells, temperature, ethanol

Curcumin (100 mg) was dispersed into flasks containing 50 ml water or was dissolved in 50 ml ethanol (Fig. 1). Different amounts (10, 30, 50, 70, 100, 200, 300 and 500 mg) of freeze-dried, non-plasmolysed or plasmolysed (in 10% w/w NaCl solution) yeast cells were also suspended in flasks containing 50 ml of water. Then, the