Microencapsulation of curcumin in cells of *Saccharomyces cerevisiae*

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**ABSTRACT**

Curcumin was successfully encapsulated in yeast cells of *Saccharomyces cerevisiae*. The verification of encapsulation was achieved by Fluorescence and Scanning Electron Microscopy (SEM). The confirmation as well as the distribution of curcumin molecules in yeast cells and their interactions with yeast cell components was also studied with Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FT-IR). In all microcapsules prepared, curcumin was integrated in the plasma membrane bilayer participating in hydrophobic interactions, but also interacted with constituents of the cell wall network. The amount of finally encapsulated curcumin was expressed by the %Encapsulation Yield (%EY) and %Encapsulation Efficiency (%EE) and was related to the preparation conditions including temperature, plasmolysis of yeast cells, presence of ethanol and mass ratio curcumin:cells. Encapsulation was favored 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. Prior plasmolysis of yeast cells was rather redundant. Proper combination of the abovementioned parameters can result in microcapsules that contain up to 35% w/w curcumin. Encapsulation of curcumin in yeast cells can enhance the aqueous solubility of curcumin by maximum 91.7% at 25°C, whereas the *in vitro* dissolution profile of curcumin in simulated gastric fluid (SGF) showed a slow and prolonged release of curcumin and higher stability against alkaline degradation in simulated pancreatic fluid (SPF).

*Keywords: Encapsulation Yield; Differential Scanning Calorimetry; Infrared Spectroscopy; Solubility; Plasmolysis*

**INTRODUCTION**

Curcumin is a yellow-orange polyphenol derived from the herb *Curcuma Longa* (turmeric), widely studied for its antioxidant and anticancer properties. In the food industry curcumin is used as a colorant, but its applications are limited by its poor water solubility, instability at light and alkaline pH, and susceptibility to oxidation and heat, some of which are overcome by microencapsulation. Microencapsulation is defined as a process in which a micro-component is surrounded by another material (wall material/coating/carrier). Encapsulation can impart some degree of stabilization to the active compound since the wall material acts as a physical barrier. The scope of this research was to evaluate the feasibility of cells of the baker’s yeast *Saccharomyces cerevisiae* as encapsulation carrier for curcumin. The most appropriate conditions for effective encapsulation were determined. Moreover, the effect of encapsulation on the solubility of curcumin was evaluated in addition to the *in vitro* dissolution profile of encapsulated curcumin in SGF and SPF.

**MATERIALS & METHODS**

Encapsulation of curcumin in yeast cells was studied in relation to the following parameters: temperature (25, 35, 45 and 55°C), plasmolysis (in NaCl) of yeast cells, encapsulation solution (water or 50% v/v ethanol) and mass ratio curcumin:cells (from 10.0 to 0.2) [1]. Quantification of curcumin was performed by HPLC [1]. The efficiency of encapsulation was expressed by %EY and %EE. %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 [1]. The verification of the encapsulation was mainly achieved by fluorescence microscopy and SEM. The confirmation, as well as the interactions of curcumin molecules with yeast cell components was also studied by DSC and FT-IR [1]. The dissolution profile of encapsulated curcumin was studied *in vitro* in SGF and SPF [2,3]. Furthermore, solubility studies of curcumin were performed from yeast microcapsules [4] and in the presence of increasing amounts of yeast cells [5].
RESULTS & DISCUSSION

Figure 1. %EY (a) and %EE (b) of curcumin yeast microcapsules prepared at 25, 35, 45 and 55°C with mass ratio curcumin:cells ranging from 0.2 to 10.0, using plasmolysed (i and ii) as well as non-plasmolysed (iii and iv) yeast cells. Encapsulation was carried out in water (i and iii) or in 50% v/v ethanol (ii and iv).

Figure 1 depicts the %EY (fig. 1a) and %EE (fig. 1b) of curcumin microcapsules as affected by: (i) mass ratio curcumin:cells, (ii) temperature, (iii) preparation solution (water or 50% v/v ethanol) and (iv) physical state of cells (plasmolysed or not). The mass ratio curcumin:cells was critical; when curcumin was in excess (mass ratio>3.3) the %EY did no longer increase, explainable by the limited loading capacity of each cell. The trend of the %EE was reverse to %EY and was particularly favored when cells were in excess. From a practical point of view, when the aim is the production of fully-loaded with curcumin yeast microcapsules (high %EY), curcumin should be used in excess (mass ratio>2.0). On the contrary, when the aim is to exploit
as much of the initially added curcumin as possible (high %EE), the mass ratio should be lower than 0.5. %EY and %EE were, also, both favored when the preparation of microcapsules was carried out at the highest temperatures (45 and 55°C). As regards the preparation solution, when water instead of ethanol was used, the %EE and %EY values were at least 2-fold higher [1].

In Figure 2 brightfield/fluorescence (fig. 2I) as well as SEM micrographs (fig. 2II) of empty yeast cells and curcumin microcapsules are shown. Empty yeast cells could not emit fluorescence (fig. 2Ib), contrary to yeast microcapsules (fig. 2Id) which emitted fluorescence due to curcumin, indicating that curcumin interacted with yeast cell components. All the prepared microcapsules emitted fluorescence, suggesting that independently of the preparation conditions, curcumin was encapsulated in yeast cells [1]. SEM revealed the spherical shape of the empty cells (fig. 2Ia) with a diameter, slightly higher than 4 μm. SEM image of microcapsules (fig. 2Ic) showed that yeast cells have been shrank and their cell wall remained thick, while curcumin molecules interacted with yeast cells.

DSC and FT-IR analyses provided extra information regarding the possible distribution of the curcumin molecules in the cell wall and membrane (fig. 3). The DSC thermogram of the freeze-dried non-plasmolysed yeast cells had a broad endothermic peak at 158°C (fig. 3a,i), attributed to the phase transition temperature (Tm) of yeasts’ phospholipid bilayer from gel to liquid-crystalline state [6, 7]. Curcumin’s DSC scan (fig. 3a,ii) demonstrated an endothermic peak at 183°C, corresponding to its melting point and a second one with onset at 204°C attributed to its gradual decomposition. The physical mixture of curcumin/cells (fig. 3a,iii) clearly exhibited the two characteristic endothermic peaks of its components, showing that no significant interaction occurred between them. On the contrary, in the DSC thermogram of microcapsules prepared in 50% v/v ethanol (fig. 3a,iv) the melting point of curcumin disappeared and a new endothermic peak at 123°C appeared, attributed to Tm, significantly lower than 158°C, suggesting that the yeast membrane became more fluid due to curcumin’s integration. Therefore, curcumin’s aromatic groups were involved in hydrophobic interactions with the acyl-chains of membrane phospholipids, lowering the van der Waals interactions between the lipid acyl-chains. The DSC thermogram of the microcapsules that was prepared in water instead of 50% v/v ethanol and had high %EY (~27%) is depicted in Fig. 3.v. In this case the melting point of curcumin did not disappear while Tm appeared at 110°C, significantly lower than the empty ones (158°C), indicating that part of the initially added curcumin had been integrated in the cell membrane. The peak “signature” of curcumin at 183°C, was attributed to curcumin molecules that strongly interacted (e.g. cross-linked) with constituents of the surface of yeast cell (i.e. cell wall mannoproteins or glucans) mainly through hydrogen-bonding. DSC analysis, also, revealed that the microcapsules remained stable up to 262°C and up to 230°C under oxidative conditions. Taking into consideration that curcumin started to decompose at 204°C, it seems that yeast cell microencapsulation can enhance its heat oxidative stability [1].

The IR spectrum of the tested samples are shown in Fig 3.b. In the spectrum of the empty cells (fig. 3b,i) the 3000-2800 cm⁻¹ and 1740-1500 cm⁻¹ regions provide significant information regarding yeast lipids and proteins of the cell wall, respectively. The band region from 1500 to 1390 cm⁻¹, also, refer to yeast proteins mainly involving the membrane and the wall, whereas the IR region from 1166 to 780 cm⁻¹ includes
absorptions of mannans and glucans [8]. The IR spectrum of the physical mixture of curcumin and yeast cells (fig. 3b,iii) was a combination of the IR spectra of yeast cells (fig. 3b,i) and curcumin (fig. 3b,ii) and the characteristic absorption peaks of curcumin appeared as identical as those of pure curcumin. On the contrary, the spectra of the microcapsules prepared in 50% v/v ethanol (fig. 3b,iv) was similar to the spectrum of the empty yeast cells (fig. 3b,i) while the IR absorption bands of curcumin nearly disappeared. Thus, the curcumin molecules are rather located inside the yeast cells, therefore their IR ‘signature’ was ‘hidden’. In particular, the disappearance of the 3501 cm\(^{-1}\) band of curcumin in the spectrum of the microcapsules indicated interactions of its phenolic -OH with cell components most likely through hydrogen bonding, while the disappearance of the 1600 and 966 cm\(^{-1}\) peaks indicated that its aromatic rings interacted with cell components. Differences in the absorption bands of 1659 and 1553 cm\(^{-1}\) between empty yeast cells (fig. 3b,i) and microcapsules indicated curcumin-protein interactions, while changes in the 1108-1000 cm\(^{-1}\) region, such as the disappearance of 1027 cm\(^{-1}\) band of β-1,4 yeast glucans, suggested curcumin-glucan interactions.

The IR spectrum of the yeast microcapsules prepared in water suspension, is shown in Fig. 3b,v and included many characteristic absorption peaks of curcumin, but their absorption intensity and frequency had changed. The spectrum of these microcapsules was obviously different from the physical mixture (fig. 3b,iii). For instance, the 3501 and 1600 cm\(^{-1}\) absorption peaks of curcumin disappeared, implying the presence of curcumin-cell hydrogen bonding and hydrophobic interactions, correspondingly [1].

The in vitro dissolution profile of curcumin in SGF and SPF is depicted in Figure 4. It is evident that in SGF (fig. 4a) the release of curcumin from yeast microcapsules was gradual and lasted for several hours, probably due to the barrier properties of the cell membrane and the outer protein layer which limit the disintegration of the microcapsules. After 3 h incubation in SGF, the concentration of non-encapsulated curcumin in SGF was 22.31 μg/mL, whereas after encapsulation an increase of 8.5% (microcapsules with %EY~3.0) and 27.2% (microcapsules with %EY~12.0) in concentration was obtained. Further exposure to SGF, increased the dissolution of curcumin from yeast microcapsules and after 72 h the concentration in SGF of non-encapsulated curcumin, was 29.6 μg/mL, lower by approximately 75% compared to yeast encapsulated. Thus, after encapsulation, curcumin’s concentration in SGF increased, possibly due to curcumin’s interactions with yeast cell components (mannoproteins, glucans or membrane phospholipids) [5, 7] or with constituents of the SGF, to the absence of aggregation or agglomeration and to its increased dispersion. In order to mimic the periodical emptying of gastric fluid into the intestinal tract, fractions from the SGF were exposed to SPF (pH 8.0) (Fig. 4b). The concentration of curcumin, during the first 5 min increased most likely due to the high pH, where curcumin has a better water solubility. However, further exposure to SPF decreased the concentration of non-encapsulated curcumin, probably due to its alkaline labile nature. Taking as a reference point the curcumin’s concentration 5 min after sample addition in the SPF, the decrease after
30 min exposure to SGF followed by 240 min exposure in SPF (fig. 4b1) was 68.74% for non-encapsulated curcumin, contrary to yeast encapsulated (approximately 22% decrease). Similar trend was observed after samples’ exposure in the SGF for 60 min (fig. 4b2) and 180 min (fig. 4b3). Therefore, yeast microcapsules were able to release their content in simulated gastrointestinal fluids, to prolong the dissolution of curcumin and lower its degradation rate in SPF.

**Figure 4.** Dissolution profile of curcumin from yeast microcapsules (with %EY 3 and 15) in: (a) SGF and (b) SPF following 30 min (b1), 60 min (b2) and 180 min (b3) of prior exposure to SGF.

Since yeast encapsulation of curcumin tended to enhance its dissolution in SGF, a double experiment was also conducted in order to estimate whether yeast microencapsulated curcumin can exhibit higher aqueous solubility. In the first experiment (fig. 5) increasing amounts of yeast cells were added in curcumin-water suspensions. The aqueous solubility of non-encapsulated curcumin was 121, 210, 242 and 310 μg/100 ml at 25, 35, 45 and 55°C, respectively. The presence of non-plasmolysed yeast cells (fig. 5a) enhanced the solubility of curcumin. In particular, when cells were present in lower quantity than curcumin (<100 mg), the solubility of curcumin was almost logarithmically increased. When 100 mg of yeast cells were present (mass ratio curcumin:cells 1) the solubility of curcumin increased by 90.1%, 42.4%, 50.8% and 32.2% at 25, 35, 45 and 55°C, respectively. At all temperatures tested, excess amount of yeast cells (curcumin:cells mass ratio<1) did not further enhance the curcumin's aqueous solubility. Similar trend was obtained when plasmolysed cells were used, however, in comparison with non-plasmolysed cells, their presence enhanced curcumin's solubility to a lower extend. Plasmolysis of cells possibly affected the organization and composition of yeast cell wall and membrane [1] and, thus, slightly restricted yeast cell's ability to enhance curcumin's solubility. In the second experiment, the water solubility of curcumin from microcapsules, prepared with either non-plasmolysed (fig. 6a) or plasmolysed yeast cells (fig. 6b), was measured. An appropriate amount of each microcapsule was used so that equal amount of curcumin was present. All microcapsules enhanced curcumin’s water solubility, suggesting that independently of the preparation conditions of microcapsules which affected the %EY, microencapsulation, particularly in non-plasmolysed cells, increased curcumin's solubility.
Figure 5. Effect of increasing amounts of (a) non-plasmolysed and (b) plasmolysed yeast cells, on the aqueous solubility of curcumin at 25, 35, 45 and 55°C.

Figure 6. Aqueous solubility of various curcumin microcapsules prepared with (a) non-plasmolysed yeast cells and (b) plasmolysed yeast cells at 25, 35, 45 and 55°C.

CONCLUSION

*S. cerevisiae* yeast cells, fundamental in the fermentation industry, comprise a natural, safe (food grade), cheap and abundant food material. By this work it was shown that the yeast cells can effectively be used as encapsulation carrier for curcumin. Microencapsulation of curcumin in yeast cells can increase the aqueous solubility of curcumin and modify, but not restrict, its dissolution in simulated gastrointestinal fluids. The phospholipid membrane of the cells can behave as liposomes and can be used for the encapsulation of not only hydrophobic but also of hydrophilic bioactive substances.

REFERENCES


