# Microencapsulation of DL-α-tocopherol in bakers' yeast cells

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## INTRODUCTION AND OBJECTIVE

Vitamin E (α-tocopherol) is a lipid-soluble compound mainly located in cell membranes (Brigelius-Flohé 1999). It works as a natural free radical scavenger involved in termination of the lipid peroxidation chain reactions (Atkinson 2007). Recent study showed that α-tocopherol can help reduce risk of cardiovascular and cancer diseases (Quiñones 2013). Vitamin E is therefore widely used in pharmaceutical, food and cosmetic industries. However, as it is sensitive to oxygen and light, encapsulation is needed for protection of this vitamin during storage.

The *Saccharomyces cerevisiae* yeast cell wall made it a promising encapsulation wall material for bioactive compounds. Natural properties of yeast cells provide many benefits compared to other microencapsulation methods (Nelson 2002). In fact, yeast cells have been successfully applied in the encapsulation of essential oils (Bishop 1998), water-soluble antioxidant (Shi 2008) and drug (Sangwai 2011).

The objective of this study was to encapsulate α-tocopherol within yeast cells as core material. The aim was to achieve α-tocopherol improved storage stability in a powder form under different relative humidity conditions.

## MATERIAL AND METHODS

### *Materials*

DL-α-tocopherol (αTP) was obtained from Sigma (St. Louis, MO, USA). The yeast cells were commercially available pressed *S. cerevisiae* bakers' yeast (Lesaffre, France). Methanol, ethanol, sodium chloride and other chemicals were of analytical grade and purchased from POCH (Poland).

### *Yeast pretreatment*

Yeast cells suspension containing 10% of solids was placed in 500 ml glass bottle and 5% of sodium chloride was added. Plasmolysis was conducted for 24 h in 50 °C with gentle stirring. Cells were then centrifuged (3950 x g), washed three times in water and lyophilised. Fresh, untreated washed cells after lyophilisation were also used for αTP encapsulation (referred as non-plasmolysed).

### *Microencapsulation of α-tocopherol*

The encapsulation procedure was based on the method adopted from Bishop et al. (1998). Yeast cells (0,4 g), plasmolysed or not, were suspended in amber glass vial contained 15 ml of water. Then, αTP (0,2 g), dissolved in 15 ml of absolute ethanol, was added to each vial. The mixture was constantly agitated at 250 rpm at 25, 35, 55 and 65 °C for 24 h. The cells were then centrifuged (3950 x g), washed three times with water and lyophilised.

Yeast-encapsulated αTP (10 mg) was rehydrated with water and extracted three times with 2 ml of absolute ethanol. The total αTP was determined with HPLC (Agilent 1200) with C18 column used in isocratic conditions with 98% methanol as mobile phase. Quantification was performed at 280 nm by external standard calibration.

The encapsulation efficiency (EE) was calculated as a ratio of αTP amount in yeast cells to its initial mass in encapsulation mixture.

### *Effect of relative humidity (RH) and light on the stability of αTP*

Approximately 100 mg of dry microcapsules were stored in open vials in desiccators containing saturated salt solutions of 33% RH ($MgCl_2$), 53% RH ($NaNO_3$) and 90% RH ($KNO_3$). The desiccators were sealed and stored for 30 days in an incubator under HQI daylight lamp with ca. 2000 lm (Osram, Germany) at 25 ± 0,2 °C. The indicated samples (10 mg) were removed and analysed for αTP content with the above described method. The results were expressed as a retention percentage. For comparison, non-encapsulated αTP was also stored and determined under the same conditions.

## RESULTS AND DISCUSSION

Plasmolysis has been described as a process when intracellular water (with dissolved sugars, proteins, etc.) flows out of the cell, which may increase internal cell space and, therefore, promote encapsulation of a compound (Shi 2008). However, our results showed that encapsulation efficiency values were similar ($P > 0,05$) amongst plasmolysed and non-plasmolysed cells, at the same incubation temperature (Figure 1). It is in accordance with results of Paramera et al. (2011), who encapsulated water insoluble curcumin in yeast cells without prior plasmolysis.

Incubation temperature was the main parameter affecting encapsulation of αTP (Figure 1). Efficiency of the process was significantly higher when it was carried out at 55 and 65 °C rather than the lowest temperature (25 °C). Similar effect was observed for

encapsulation of essential oils in *S. cerevisiae* cells (Bishop 1998).

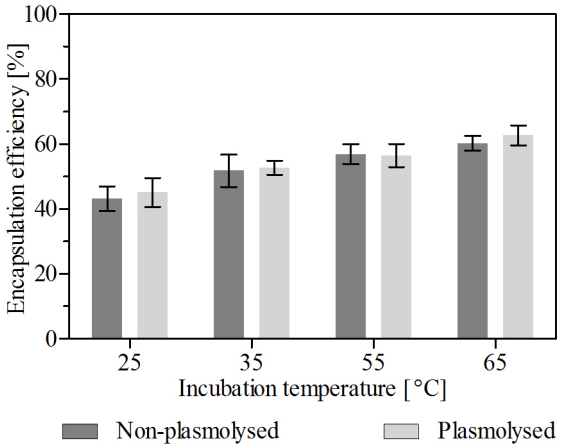


**Figure 1: Encapsulation efficiency of αTP in yeast cells, either non-plasmolysed and plasomylysed, in different incubation temperatures**

In a storage stability study, the samples were kept for 30 days at constant RH (from 33% to 90%), at 25 °C, under light. The stability was expressed as percentage retention of αTP at the end of storage (Table 1). The results showed that stability depended both of the RH value and form of vitamin. During storage, retention of both vitamin form decreased. In particular, retention of encapsulated αTP decreased from 99,1% and 89,9%, at moisture of 33% and 53%, to 84,2% at 90% RH ($P< 0,05$). This is in accordance with Normand et al. (2005), who reported that at high water content ($a_w> 0,7$), the cell wall swells, which promotes diffusion of a substance through the wall, where its more susceptible to oxidation. Non-encapsulated αTP exhibited similar stability at all tested RH values ($P> 0,05$).

**Table 1: Retention percentage (mean ± SD, *n = 3*) of yeast microcapsules and non-encapsulated αTP**

| | Storage conditions | | |
|---|---|---|---|
| | 33% RH | 53% RH | 90% RH |
| | Retention [%] | | |
| Microcapsules | $99,1^{a} \pm 0,5$ | $98,9^{a} \pm 0,9$ | $84,2^{c} \pm 0,9$ |
| Non-encaps. αTP | $59,4^{b} \pm 0,6$ | $58,6^{b} \pm 0,7$ | $57,3^{b} \pm 1,4$ |

Values in the column marked with the same letter are not significantly different ($P> 0,05$)

Encapsulation increased the stability of αTP at all tested RH values ($P< 0,05$). Cell wall effectively protected vitamin from oxidation over 30 days storage under light. Similar effect was observed in stability test for yeast-encapsulated curcumin at different moisture content (Paramera 2011).

## CONCLUSIONS

This work shown that yeast cells could be used as natural wall material for microencapsulation of α-tocopherol with encapsulation efficiency up to 60%. The encapsulation was simple and involved only mixing of yeast cells water suspension with vitamin and ethanol. Encapsulated αTP was highly stable at different humidity for 30 days. This study could be helpful to solve the problem of low stability of bioactive vitamin E in different formulations.

## ACKNOWLEDGMENTS

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