Evaluation of ascorbic acid microcapsules obtained by microfluidic devices

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INTRODUCTION AND OBJECTIVE

Ascorbic acid (AA) is a very effective antioxidant with vitamin function. However, it is very unstable and influenced by factors such as high temperature, light, pH, oxygen concentration and water activity, which limit its application (Fennema; Damodaran, Parkin, 2010). For this reason, the use of AA could be guaranteed with microencapsulation by microfluidic devices, which allow controlled production of microcapsules (Zhaol et al. 2011). No studies on AA encapsulation using this technique have been found in the literature. Therefore, a new alternative to improve the stability during storage and the masking of the sour taste of ascorbic acid is obtained.

Based on the above considerations, the aim of this work was the encapsulation of AA with production of solid lipid microcapsules (SLMs) by microfluidic devices, evaluate their structure by optical microscopy, the encapsulation efficiency and the stability of the encapsulated AA compared to free.

MATERIALS AND METHODS

The experiments were carried out using a glass microfluidic device (Figure 1). Five formulations were analyzed, differing in the composition of the inner aqueous phase (Table 1). The oil phase consisted of palm fat and the continuous phase consisted of polyvinyl alcohol solution (PVA) of concentration of 10% (w/w). The fluids were pumped through the microfluidic device using a syringe pump (Harvard PHD 2000 series). The flow rates used for formulations 1, 2 and 3 were 3000, 2500 and 14000 µL/h to the inner, oily and external aqueous phase, respectively. For the formulations 4 and 5, the flows were 1000, 3000 and 12000 µL/h. The heating temperature of the oil phase was kept at 66°C.

Morphological characterization was done by optical microscopy. The encapsulation efficiency and the stability of microencapsulated AA in comparison to the free form were monitored according to the spectrophotometric method described by Farajzadeh & Nagizadeh (2003). The encapsulation efficiency was determined after quantification of total AA present in the microcapsule and on the supernatant solution. The stability analyzes were done in duplicate at 0, 7, 15, 21 and 30 days after encapsulation, with the material stored in glass containers, protected from light, in the presence of O_2 at temperatures of 4°C and 20°C. The analyses were done at Harvard University, School of Engineering and Applied Sciences and Department of Physics, in Cambridge, Massachusetts, United States. Data were statistically analyzed by ANOVA and Tukey test at 5% significance level, with the use of statistical program SAS (Statistic Analisy System).

Table 1: Different compositions of the inner aqueous phase (w/w).

Formulations	
1	AA solution 20%
2	AA solution 20% + 1% Na_2CO_3
3	AA solution 20% + 2% Na_2CO_3
4	AA solution 3% + 0,25% de chitosan
5	AA solution 3% + 0,25% de chitosan + 1% Na_2CO_3

*The formulations 2, 3 and 5 were collected in a solution of 1%, 2% and 1% of $CaCl_2$, respectively.

RESULTS AND DISCUSSION

The microcapsules were collected at temperature below the melting point of palm fat, solidifying it and obtaining the SLMs. The production of the microcapsules within the microfluidic device was analyzed by optical microscopy, confirming the round format and presence of layers. Moreover, homogeneity in the size was observed in each formulation, expected characteristic for this type of encapsulation method (Fig. 1 and 2).

There were no differences among the formulations concerning to the morphology, proving that different compositions of the inner aqueous phase did not influence on morphology of the SLMs.

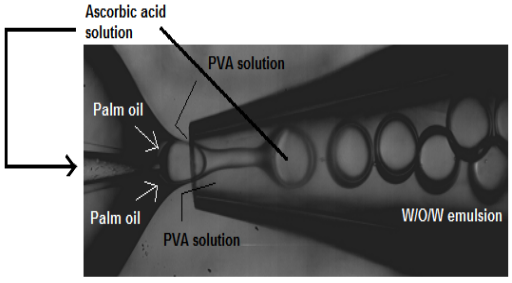

Figure 1. Diagram showing the formation of the microcapsules inside the device.

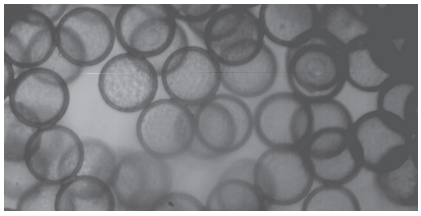

Figure 2. Optical microscopy (100x) for formulation 1.

Regarding to the encapsulation efficiency and stability of the encapsulated material and free AA, microcapsules with pores in the lipid phase were obtained, being responsible by lower encapsulation efficiency and stability of the formulation 1, possibly because AA diffusion through these pores, being exposed and therefore more susceptible to oxidation. There is a significant difference among the formulations, indicating that different compositions of the inner aqueous phase influenced on the amount of active material effectively encapsulated. (Table 2).

Na_2CO_3 was incorporated in the internal aqueous phase and $CaCl_2$ incorporated in the collection solution of the microcapsules due to the fact that these salts react and form the precipitate of calcium carbonate. Thus, the precipitate clogs the pores of the lipid phase, obtaining more stable structures. Another way to improve the encapsulation efficiency and the stability of SLMs is the use of chitosan in the internal aqueous phase, being possible the formation of a complex bigger than the pure AA. The diffusion of AA in this case would be difficult, keeping it in the internal aqueous phase and resulting in a great stability during storage.

Even with a significant reduction in the concentration of AA in all formulations studied at both temperatures, they were significantly lower than the control, which contained free AA in solution. After 30 days, the control remained around 4.6 and 3.4% of AA concentration at temperatures of 4 and 20°C, respectively. The formulations remained from 55 to 98% and from 46 to 97% at temperature of 4 to 20°C, respectively.

Table 2: Encapsulation efficiency of the formulations.

Formulations	Encapsulation efficiency (%)
1	73.40±2.84 [d]
2	91.74 ±2.83 [c]
3	92.13 ±1.50 [b,c]
4	96.64±0.47 [a]
5	95.14±0.88 [a,b]

* There were no significant differences among the samples with the same letters in the same column ($p < 0.05$).

CONCLUSIONS

The production of solid lipid microcapsules by microfluidic device aiming the encapsulation of ascorbic acid was feasible, since it showed high encapsulation efficiency and excellent performance regarding to the protection of AA during storage, even at room temperature. The formulation 4 should be considered the most suitable for AA encapsulation due to the results and the simplicity of the formula. Moreover, the use of microfluidic device for encapsulation of food ingredients is suggested because these compounds, in general, are sensitive to many factors that are dispensable when this technique is used.

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ACKNOWLEDGMENTS

The authors thank FAPESP for the scholarship conceded (Process 2012/04451-4).