HPLC Evaluation of the Ascorbic Acid Yield in the Microencapsulation Process

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Ascorbic acid (AA) plays an essential role in the human body, and because it can not be synthesized, the daily intake has to be assured by our daily meals. Since is a water soluble vitamin, show low stability while dissolved. In addition, the effect of external factors such as temperature, heating process, pH or oxygen, will promote significant AA losses. In order to avoid these drawbacks, different encapsulation techniques for AA has been evaluated and the effectiveness of the encapsulation was evaluated. Different batches of gluten-free cookies were fortified with microencapsulated AA, and the % of losses was determined by HPLC technique. The protective effect of AA was more evident by spray-drying whereas the highest effectiveness was achieved by lyophilization.

Keywords: ascorbic acid, microencapsulation, HPLC

Microencapsulation (ME) has been defined as the technology of packaging solids, liquids and gaseous materials, by surronding or enveloping them within a secondary material, known as the encapsulant, matrix or shell to form a microcapsule [1, 2].

The role of ME is to protect "the core" creating a barrier that avoids chemical reactions and/or enables the controlled release of their contents at a specific moment, or over prolonged periods of time [3, 4].

The ME technology is widely used, here are some of the industry fields that are using microencapsulated products: food industry (vitamins, minerals, fibre, probiotics, antioxidants, volatile substances etc.), personal care (antiperspirants, fragrances etc.), pharmaceuticals (drugs, DNA etc.), building construction materials, household care(odor control agents, fragrances etc.), paper industry, agrochemicals, industrial sector, textile industry [1, 5].

Food ingredients ME into coating materials can be achieved by several techniques: spray-drying, spray-chilling, spray-cooling, lyophilization, extrusion, centrifugal extrusion, fluidized-bed coating, coacervation, centrifugal suspension separation, cocrystallization, inclusion complexation, liposome entrapment [6, 7].

Food industry applies ME as the technology that improves the nutrients retention time in food [4], controls and isolates the release of a specific substance [8], protects 'the core' from the deteriorating effect of oxygen or unpleaseant chemical reactions, masks the bitter taste or odours, improves the handling, by turning liquids into powders and slows down the evaporation of a volatile substance [1, 9, 10].

Vitamin C (ascorbic acid) is a water-soluble vitamin stable as a powder, but when it is dissolved in water its stablity drops significantly [11]. In addition, the effect of external factors such as temperature, heating process, prolonged storage, pH or oxygen, will promote significant AA losses [4, 12].

Ascorbic acid is an essential human nutrient and its biological functions are centred on its antioxidant properties in biological systems, in which it prevents common degenerative processes [13, 14]. It is known that vitamin C chelates heavy metal ions [15], reacts with singlet oxygen and other free radicals and suppresses peroxidation [16], reducing the risk of arteriosclerosis, cardiovascular diseases and some types of cancer [17, 18].

Due to their excellent health effects, the purpose of this study was to investigate the effect of different ME techniques on the protection/stabilization of ascorbic acid during processing and storage of different types of fortified gluten-free cookies.

Experimental part

Materials and methods

For this study ascorbic acid (AA) (PANREAC QUIMICA S.A.U., Barcelona, Spain) was microencapsulated using both spray-drying and lyophilization (also termed as "freeze-drying") techniques; the coating material was a mixture of maltodextrin (Maldex 190, CARINSA, Creaciones Aromaticas Industriales SA, Barcelona, Spain), modified starch (HI-CAP 100, CARINSA) and β-cyclodextrins (Zhengzhou Sigma Chemical Co., LTD., Henan, China),.

The wall materials (encapsulating agents) were chosen due to their good encapsulating properties [19, 20]. The coating materials were mixed with Milli-Q purified water using a T 25 digital ULTRA-TURRAX blending equipment; the mixture was covered with aluminium foil and stored in the dark for 24 h at room temperature.

For spray drying (SD) a Bûchi Mini Spray Dryer B-290 was used, working at a feed rate of 15ml/min, inlet air temperature of 180 °C and outlet air temperature of 90°C [21]; as shown in table 1 (g AA), three experiments with different concentrations of AA were carried out.

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No.	ME type	Samples	g AA	ml sol AA.20%	g obtained powder	mg/kg AA powder	g of ME AA	g powder used for GF cookies	mg/kg AA in GF cookies	g/400g AA in GF cookies
1.	SD	P1	15	75	52	111793	5.81	8.95	1648	0.66
2.		P2	25	125	57	147183	8.39	6.79	1453	0.58
3.		P3	50	250	63	204900	12.91	4.88	1325	0.53
4.	LY	P4	15	75	83	74181	7.79	10.,65	1293	0.52
5.		P5	25	125	89	104657	10.99	8.10	1078	0.43
6.		P6	50	250	105	152436	16.01	6.56	847	0.34
7.	-	P7	-	-	-	-	-	1.00	778	0.31
8.	-	M	-	-	-	-	-	-	0.00	0.00

Fig. 1. Effectiveness of the microencapsulation processes

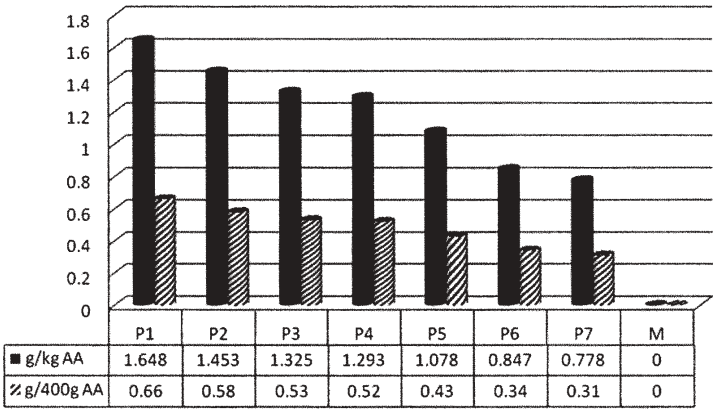

Fig. 1. Vitamin C in the gluten-free cookies

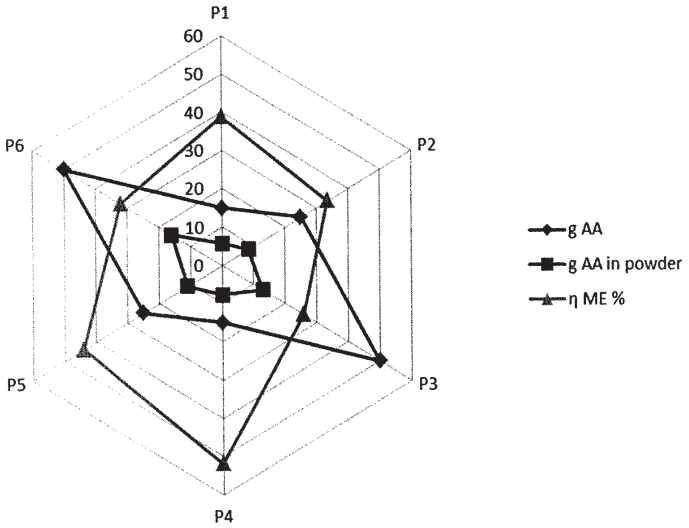

Fig. 2. Effectiveness of the microencapsulation processes

For the lyophilization ME technique a Christ ALPHA 1-2 LD *plus* liophylizator was used, at working conditions of -54 ºC and a pressure 0.048 mbar (these parameters were optimized in the Food Science and Nutrition laboratory, from the Catholic University of San Antonio, Murcia, Spain).

The content of AA was performed using an HPLC Agilent 1100 Series (Merck–Hitachi, Darmstadt, Germany), in a Lichrospher 100 RP-18 reverse-phase column (Merck, Darmstad, Germany) (25 x 0.4 cm, 5 µm particle size) at 245 nm using a Shimadzu SPD-M6A UV diode array detector (Shimadzu, Kyoto, Japan), as described by Schuep and Keck in 1990 [22].

After the determination of AA in the ME samples, the obtained powders were further used to make gluten-free (GF) cookies (P1-P6), two other batches were made, one with pure AA (P7), and the last one was the control sample (M). The weights of each powder were optimised in order to each batch of cookies will have the same concentration of (1g of AA) (table 1, g powder GF cookies). The same recipe and ingredients were used for all the batches of cookies (rice flour, sugar, margarine, eggs), that were purchased in a local market (Murcia, Spain). The cookies were cooked for approximately 20 min at 200ºC in a laboratory oven, each GF batch had approximately 400 g.

As can be seen in figure 1, for GF cookies the content of AA was also determined by HPLC using the same conditions described in matherials and methods.

Results and discussions

The aim of this study was to investigate the effect of different ME techniques on the protection/stabilization of ascorbic acid during processing and storage of different types of fortified gluten-free cookies. The highest efficiency (yield) of microencapsulation processes was obtained by lyophilization P4 and P5 (fig. 2), in addition, the amount of microencapsulated AA play an important role in the efficacy, since for P1 - SD and P4 - LY lower concentration of AA were used (table 1, *g AA*).

For the GF cookies, the highest values of AA were for the samples fortified with ME powder obtained by spray-drying (fig. 1), though there should have been similar results for all batches, because the same quantity of AA – dry basis-, was added at each batch of cookies (table 1, g).

Conclusions

In the present study two techniques for the microencapsulation of AA were compared by HPLC. Although both techniques had a significant protective effect, the microencapsulated powders obtained by lyophilization showed a higher % of AA than those obtained by spray dry. However, batches of GF cookies fortified with powders obtained by spray-drying technique offered the highest protection.

For both spray-drying and lyophilization techniques, the microencapsulations most effective were achieved with samples caontainig small quantity of AA (P1 and P4, 15g of AA). The smallest losses of AA were reported for ME samples obtained by lyophilization (P4, P5 and P6). Though the smallest losses were for ME samples obtained by lyophilization, when microencapsulated powders were used to fortify GF cookies, the spray-drying offered the highest protection for AA (P1,P2 and P3).

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