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Optimization of broccoli microencapsulation process by complex coacervation using response surface methodology

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ABSTRACT

This work is intended to optimize the microencapsulation process of broccoli particles preserving their chemical healthy composition, in terms of chlorophylls and polyphenol contents and antioxidant activity, increasing its chemical stability and hiding the characteristic broccoli odor that might have a negative impact in consumer's acceptance. Thus, the microencapsulated broccoli could be easily added to processed foodstuff increasing their healthy properties without altering their sensory attributes. Experimental design and response surface methodology (RSM) was applied to optimize operating conditions and the variables that affect broccoli microencapsulation. Based on that, the optimum process conditions determined by RSM were as follows: pH value 4.5; broccoli–wall material ratio 50% and concentration of wall material 4%, where the theoretical and practical encapsulation efficiency was 60% and 58%, respectively.

Industrial relevance: Nowadays, consumers are increasingly interested in beneficial effects of vegetables on health. In this sense, broccoli has been highly valued for their chemopreventive effects, attributed to its composition in glucosinolates, flavonoids, carotenoids, ascorbic acid and amino acids. All these substances are easily degraded by the action of oxygen, thus reducing their potential health benefit. Microencapsulation process has the advantage of reducing the reactivity to factors such as water, oxygen or light, while reducing evaporation or transmission rate to the outside environment.

1. Introduction

Broccoli, *Brassica oleracea* L. var. italica, is a floral green vegetable highly valued due to its richness in vitamins, antioxidants, anticarcinogenic compounds (Bachiega et al., 2016) and health-promoting phytochemicals (Yuan, Sun, Yuan, & Wang, 2010). Epidemiological studies have shown an inverse association between the consumption of Brassica vegetables and the risk of cancer (Day et al., 1994). The potential protective effects of cruciferous vegetables have largely been attributed to the complement of phytochemicals, which include vitamins C and E, the flavonols quercetin, kaempferol, the carotenoids b-carotene, lutein, and glucosinolates (Podsedek, 2007). The presence and abundance of the bioactive compounds and the alteration of the external parameters after harvest have been described as being dependent on genetic (cultivar), physiological (organ and age) and abiotic factors (Domínguez-Perles et al., 2011; Fernández-León et al., 2011). Therefore, microencapsulation of broccoli can be a useful alternative to preserve these compounds.

Microencapsulation is defined as a process in which tiny particles or droplets are surrounded by a coating wall or embedded in a homogeneous or heterogeneous matrix, to give small capsules (Calvo, Castaño, Hernández, & González-Gómez, 2011; Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007) and therefore building a barrier between the component in the capsule and the environment. So that, the capsule holding, the process and the wall materials should be suitable.

Wall materials used in encapsulation processes can be selected from a wide variety of polymers, both synthetic and natural, depending on the material to be encapsulated, the encapsulation process and the characteristics desired in the final product. In general, encapsulating agents can be classified into two groups: hydrophilic (carbohydrates and proteins) and hydrophobic materials (lipids). Carbohydrates are generally used in food encapsulation, although they do not have good interfacial properties, and they must be chemically modified. Proteins have amphiphilic characteristics, which conferred them the physico-chemical and functional properties necessary to encapsulate hydrophobic, while lipids are used in the encapsulation of hydrophilic substances (Calvo, 2012).

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The techniques developed to produce microcapsules may be classified as physical or chemical methods. The process used depends on the properties of the wall materials, the substance to be encapsulated, the desired release mechanism and the cost (Montes, De Paula, & Ortega, 2007). Coacervation is classified as a chemical process and is considered to be the original and the true microencapsulation process since the coating material completely surrounds the core with a continuous coating (Risch, 1995; Soper, 1995).

The concept behind coacervation microencapsulation is the phase separation of one or many hydrocolloids from the initial solution, and the subsequent deposition of the newly formed coacervate phase around the active ingredient suspended or emulsified in the same reaction media (Gouin, 2004). Microcapsules produced by coacervation are water-insoluble and heat-resistant, possessing excellent controlled-release characteristics based on mechanical stress, temperature and sustained release. Complex coacervation is an interaction driven by electrostatic force generated from two oppositely charged components. An increasing number of researchers have focused their attention on the study of this system, especially the mixture of protein and polysaccharide (Doublier, Garnier, Renard, & Sanchez, 2000; Harnsilawat, Pongsawatmanit, & McClements, 2006; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003).

In order to establish the operation conditions of the microencapsulation process, an experimental design (ED) together with response surface methodology (RSM) are proposed in this research work. ED–RSM is an effective statistical technique for optimizing complex processes. Instead of varying one variable at a time and keeping the rest constant, RSM reduces the number of experimental trials required to evaluate multiple parameters and their interactions, being less laborious and time-consuming than other approaches.

Thus, the aim of this work was to establish the operating conditions that influence the microencapsulation process of broccoli by complex coacervation and to optimize the process by means of experimental design and response surface methodology (ED–RSM) in order to obtain the highest yield.

2. Material and methods

2.1. Plant material

A total of 10 broccoli heads of the same cultivar (*B. oleracea* L. var. italica cultivar 'Parthenon') were purchased in a local grocery. They were grinded, frozen at −80 °C and freeze-dried in a VIRTIS lyophilizer, Mod. Génesis 25 LL Hücoa-Herlos. After lyophilization the product was powdered and sieved selecting a particle size between 65 and 125 μm.

2.2. Reagents

For the microencapsulation process arabic gum and gelatin were supplied by Panreac (Spain). All other chemicals were obtained from Thermo-Fisher Spain.

2.3. Microencapsulation process

The first stage to achieve broccoli microencapsulation was the formation of a fine and stable emulsion of the core material (broccoli) in the wall solution. Wall materials, gelatin and arabic gum (1:1), were dissolved separately in warm water (50 °C). After that, they were mixed and broccoli particles were added. The emulsion was prepared at room temperature (22 °C) using a lab blender (Fisher Scientific PowerGen Model 1800 Homogenizer, at 10,000 rpm) during 5 min. pH was adjusted by adding lactic acid and the microcapsules suspension was stored at 4 °C under stirring conditions for 12 h. 1 g of silica per 3 g of wall material was added to harden the microcapsule walls and to foster particle disaggregation after the final lyophilizing process. After 1 h of hardening at 4 °C under agitation, the microcapsule suspension was frozen at −80 °C and freeze-dried by a VIRTIS lyophilizer, Mod. Génesis 25 LL Hücoa-Herlos. Once the lyophilization process was concluded, microcapsules were grinded and transferred to double layer plastic bags, where they were stored until analysis. An optical microscope (Leica DML) equipped with a digital camera (Leica DC100) was used to check the microcapsules formation.

2.4. Optimization of microencapsulation process

Experimental design (ED) and response surface methodology (RSM) were proposed for designing and optimizing the independent variables that affect the microencapsulation process of broccoli particles. For this purpose The Unscrambler Software Version 9.8 (Camo Software AS, Norway) was used to generate the experimental design, statistical analysis and regression model. Firstly, an experimental design was built considering all the variables that might have influence in the system, thus pH, broccoli:wall material ratio, and wall material concentration were evaluated to estimate their effects on the microencapsulation process. A Box–Behnken design (BBD) was used to establish the experimental conditions and the effects were evaluated considering the design variables and their interactions (Table 1). In all cases, the microencapsulation yield, calculated in terms of chlorophylls content, was used as response variable.

In order to evaluate the suitability of the optimized model, an analysis of variance (ANOVA) test was completed, indicating that a quadratic polynomial function describes the optimized model, being the equation of the model as follows:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1x_2 + b_5x_1x_3 + b_6x_2x_3 + b_7{x_1}^2 + b_8{x_2}^2 + b_9{x_3}^2$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1, b_2 and b_3 (linear effect) and b_7, b_8 and b_9 (quadratic effect) and b_4, b_5 and b_6 (interaction effects).

Internal validation of prediction accuracy of the Box–Benhken model was based on statistical evaluation of the bias index. Practical evaluation of the model was based on a comparison of the responses observed with the response predicted (Tefas et al., 2015).

$$\text{Percentage bias} = 100[(\mu_{\text{experimental}} - \mu_{\text{predicted}})/\mu_{\text{predicted}}]$$

Table 1
Box–Behnken design of the experimental variables (independent variables): pH (A), broccoli percentage respects to total solid content in the microcapsule (B), and wall material percentage (C) and their effect onto the microencapsulation yield (response).

Design samples	Independent variables			Dependent variable
	A	B	C	Yield (%)
1	3.5	10	2.5	28.00
2	4.5	10	2.5	28.00
3	3.5	50	2.5	28.40
4	4.5	50	2.5	40.40
5	3.5	30	1	2.00
6	4.5	30	1	34.67
7	3.5	30	4	53.92
8	4.5	30	4	53.33
9	4	10	1	1.00
10	4	50	1	28.00
11	4	10	4	61.75
12	4	50	4	64.50
13	4	30	2.5	5.73
14	4	30	2.5	10.40
15	4	30	2.5	14.13

2.5. Encapsulation yield

Encapsulation yield was calculated by applying the following formula:

$$\text{Yield}(\%) = \frac{\text{initial broccoli}(g) - \text{non encapsulated broccoli } (g)}{\text{initial brocoli}(g)} \times 100$$

The amount of non encapsulated broccoli was determined by analyzing the chlorophyll content of broccoli particles that were not inside the microcapsule wall. For this purpose, a calibration curve was constructed by simulating the process of encapsulation; the formation of the microcapsules was induced without the addition of broccoli, which was added after the microcapsules were formed. Thus, the entire broccoli particles remained outside of the microcapsule, but in the same chemical conditions as in the final process, allowing the establishment of a relationship between the amount of non encapsulated broccoli and the chlorophylls content.

2.6. Microscopy and scanning electron microscopy

An optical microscope (Leica DML) equipped with a digital camera (Leica DC100) was used to check the microcapsules formation in the solution. The microcapsules final morphology was evaluated with a scanning electron microscope (Quanta 3D FEG, FEI Company).

2.7. Analytical methodologies

2.7.1. Total antioxidant activity

2,2-Azinobis(3-ethylbenzothiazoline)-6-sulfonic acid, diamonium salt $(ABTS)^{\bullet+}$ method was used for radical scavenging determination (Cano, Hernández-Ruiz, García-Cánovas, Acosta, & Arnao, 1998). Briefly, the radical was generated using phosphate buffer 50 mM. Once the radical was formed, 1 mL of $ABTS^{\bullet+}$ was mixed with 20 μl of broccoli extract in phosphate buffer, and the absorbance was measured at 730 nm during 20 min. The results were expressed as a TEAC (antioxidant equivalent to trolox) value as mmol trolox/g of freeze-dried broccoli, determined by interpolating the fall in absorbance (taking into account the adequate dilution and the linear range of calibration line) on a calibration curve using different trolox concentrations. All the measurements were performed in a Shimadzu UV spectrophotometer (Kyoto, Japan).

2.7.2. Total phenolics content

Total phenolic compounds (TPCs) of the freeze-dried broccoli were determined using a UV–vis 1204 Shimadzu spectrophotometer (Kyoto, Japan) according to Lima et al. (2005) modified procedure. The phenolic compounds were quantified from a calibration curve using 3,4-dihydroxybenzoic acid (Sigma-Aldrich, Spain) and data were expressed as mg of 3,4-dihydroxybenzoic acid per gram of freeze-dried broccoli ($n = 3$).

2.7.3. Chlorophyll pigments determination

Chlorophyll content was determined according to Fernández-León, Lozano, Ayuso, Fernández-León, and González-Gómez (2010) methodology. Briefly, an accurate amount of microcapsules was weighed and 15.0 ml of acetone was mixed into a centrifuge flask placed in an ice bath. After 1 min of homogenization using an Omni Mixer homogenizer (Omni International, GA, USA), samples were centrifuged for 15 min at 14,000 rpm at 4 °C. Samples were double extracted and supernatants were then filtered into a 200.0 mL volumetric flask using acetone as solvent to complete the final volume. Absorbance was measured at 600–700 nm and the highest value was recorded.

In order to avoid pigment degradation, chlorophyll extractions were performed under dark conditions and extracts were kept on ice until analysis. Chlorophylls extracts were analyzed as soon as they were extracted.

2.7.4. Odor evaluation of microcapsule particles

The presence of a group of volatile compounds, mostly glucosinolates derivative, in broccoli plants is responsible for its characteristic odor and taste (Krumbein, Schonhof, & Brückner, 2006). These organoleptic properties might have a negative impact in consumers acceptance, and hence the marketability of this product.

For microcapsules odor evaluation, a solid-phase microextraction coupled to a gas chromatography was used to qualitatively evaluate the aromatic profile alteration of broccoli particles after the microencapsulation process. A DVB/CAR/PDMS fiber was used for the volatile compounds extraction by exposing the fiber for 5 min at 25 °C to the broccoli samples. Volatile compounds were desorbed directly in the injection inlet of the gas chromatographic instrument. The inject temperature was set at 280 °C in a splitless mode. The chromatographic separation was achieved in a DB-23 (60 × 0.250 mm) capillary column (Agilent Technologies) with a temperature gradient from 200 to 230 °C at 10 °C/min, using nitrogen as carrier gas.

2.8. Statistical analysis

For the experimental design and test, The Unscrambler 9.8 software (CAMO Process AS, Oslo, Norway) was used. An analysis of variance (ANOVA) test was completed in order to evaluate the suitability of the optimized model.

3. Results and discussions

3.1. Optimization of microcapsule process

A Box–Behnken design was employed to evaluate the influence of the experimental variables on the encapsulation yield. In this design the experimental combinations were at the midpoints of edges of the process space and at the center, and required 3 levels for each factor. The variables considered for the experimental design were pH (3.5, 4.0, 4.5), broccoli percentage respect to total solid content in the microcapsule (10%, 30%, 50%) and the concentration of wall material components (1%, 2%, 4%), making a total of 15 experiments as it is shown in Table 1. The encapsulation yield, measured as indicated above, was used as response variable in order to optimize the model by means of RSM. The ranges of the experimental variables were established based on preliminary experiments and also taking into account the constraints of the complex coacervation process to encapsulate broccoli particles.

The optimized encapsulation model fitted to a second order polynomial equation shown in Table 2. The ANOVA results indicated that the quadratic model was significant ($p < 0.01$) and was adequate to describe the encapsulation yield, since the optimized model did not have significant lack of fit, together with very satisfactory values of determination coefficient (R^2) and multiple correlation coefficient (r) (Little & Hills, 1978; Mendenhall, 1975).

Table 2
Statistical parameters obtained after the ANOVA analysis of the modeled response surface and the mathematical surface equations for the encapsulation yield (%).

Determination coefficient (R^2)	0.967
Multiple correlation coefficient	0.983
Model p value	0.003
Quadratic p value	0.008
Model lack of fit	0.241
Mean square pure error (MSPE)	17.714
$Y = 10.089 + 11.021 * A + 0.266 * B + 13.986 * C + 1.714 * AB - 4.750 * AC - 3.464 * BC + 5.222 * A^2 + 6.841 * B^2 + 9.572 * C^2$	

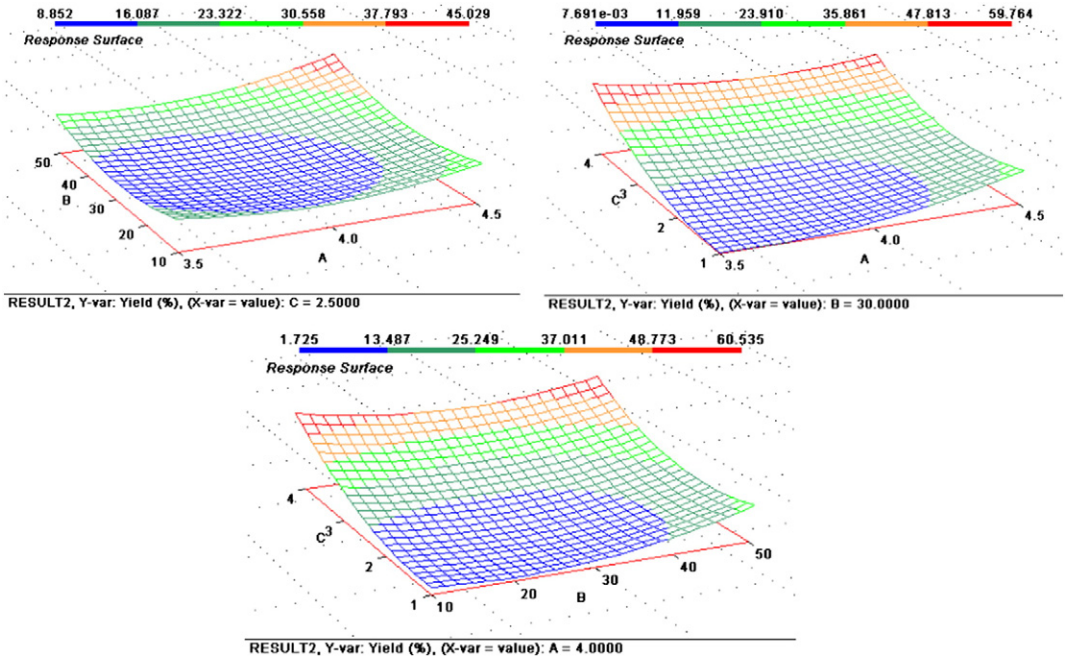

Fig. 1. Response surface for the microencapsulation yield of broccoli particles as function of the experimental conditions considered in the experimental design (A = pH, B = broccoli percentage respect to total solid content in the microcapsule and C = wall material percentage (*w*/*v*)).

The value of the multiple correlation coefficient (r) was found to be 0.983, indicating good fit. The yield values measured for the different batches showed wide variation (ie, values ranged from a minimum of 1.00% to a maximum 64.50%). These results clearly indicate that the yield value is strongly affected by the variables selected for the study. This is also reflected by the wide range of values for coefficients of the terms of equation in Table 2. The main effects of A, B, and C represent the average result of changing 1 variable at a time from its low level to its high level. The interaction terms (AB, AC, BC, A^2, B^2, C^2) show how the dependent variable changes when 2 variables are simultaneously changed. The positive coefficients for all 3 independent variables indicate a favorable effect on the yield, while the negative coefficients for the interactions between 2 variables (AC, BC) indicate an unfavorable effect on the yield.

The individual evaluation of the equation coefficients indicated that not all the studied variables and their interactions had a significant influence in the system, being the concentration of wall material (variable C) the one showing the highest significance ($p < 0.01$) while pH (variable A) and broccoli:wall material ratio (variable B) presented p-values > 0.05, so, variations in these variables values do not affect significantly the encapsulation yield. Variable C seems to have a direct relationship with yield.

The surfaces obtained after the model evaluation are summarized in Fig. 1. According to these figures, when C is at the high level, almost the same yield is obtained, regardless B or A values. Besides, the evaluation of the modeled surfaces indicated that the lower encapsulation yields were obtained when the pH was 4.2 or lower, the ratio broccoli:wall material was 40% or lower and the concentration of wall material was 2.5% or lower. On the other hand, outside of these areas, acceptable encapsulation yield values were obtained (>45%). Therefore the optimum conditions for the encapsulation process (the highest encapsulation yield) were obtained when the experimental variables were fixed at their maxima: pH 4.5 (A), broccoli–wall material ratio at 50% (B) and concentration of wall material at 4% (C). In these conditions, the theoretical encapsulation yield is around 60% (Fig. 1). Experimental and theoretical values are close, with a percentage bias under ± 15% set point; therefore the developed model could be used accurately for predicting the responses, confirming that the proposed response surface was

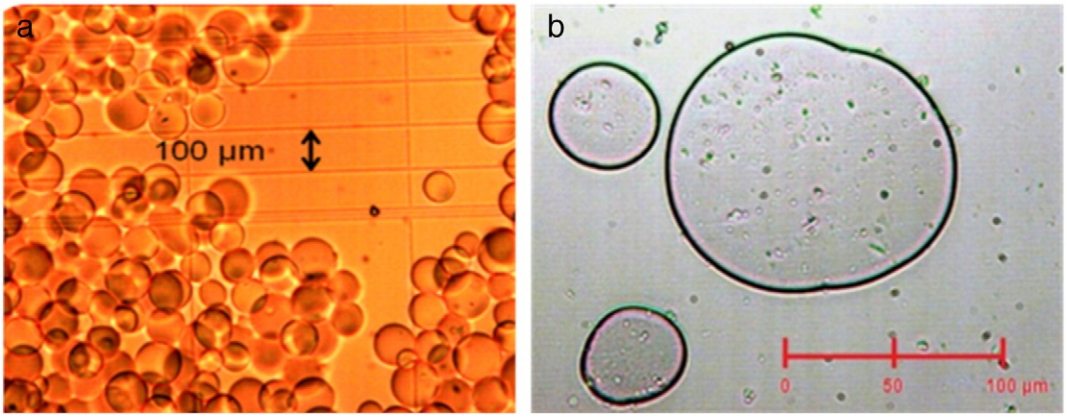

Fig. 2. Optical image of microcapsules emulsion taken by means of an optical microscope equipped with a digital camera.

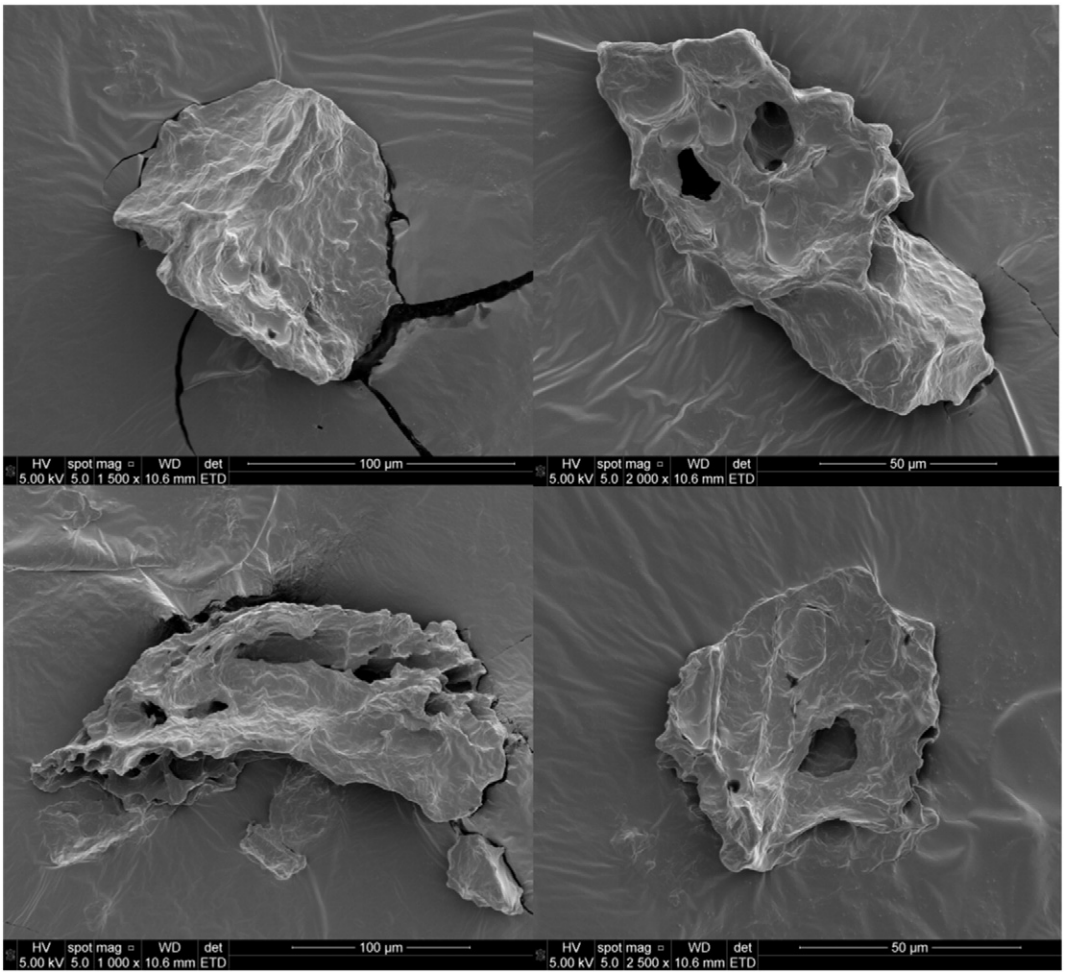

Fig. 3. Electron microscope scanning images taken to evaluate the microcapsules morphology.

adequate to predict the encapsulation yield of the process. Table 2 also includes the value of mean square pure error (MSPE) for this model that can be defined as the usual experimental error or design variance ($\sigma 2$) (Box & Draper, 1978). We can expect experimental values different to those predicted by the model but ranging within $\pm 2\sigma$. Taking into account this error, the difference between the yield obtained in the design sample (Table 1) and the experimental one could be explained.

3.2. Microcapsules formation

An empty microcapsule suspension and a broccoli microcapsule suspension prepared in optimum conditions were deposited in Neubauer chambers to check microcapsules formation (Fig. 2).

The average size of microcapsules was between 50 and100 μm. Yi, Xiaoming, Haiyan, Shabbar, and Eric (2013) found that as the pH value increases, from 3.5 to 4.5, the particle size decreases using the same concentration of wall material, although they obtained smaller particles (around 20 μm). Moreover, the particles were spherical across all the pH chosen, but some irregular objects appeared at pH 4.5, 3.9 and 3.5.

Optical microscopy images show that the coacervate microcapsules were predominantly spherical. However, Fig. 3 shows their final morphology and it can be observed that they present an irregular shape with folds and cracks, which may be due to the loss of water during the lyophilization process (Santos et al., 2014; Xiao-Ying, Zhi-Ping, & Jian-Guo, 2011).

3.3. Chemical characterization and odor evaluation

Broccoli heads were chemically characterized in terms of chlorophyll content, antioxidant activity (AA), and total phenolics content (TPCs). These results are summarized in Table 3.

The amount of chlorophyll (10.00 ± 0.13 mg/kg) in the lyophilized broccoli was similar to those found by other authors (Fernández-León, Fernández-León, Lozano, Ayuso, & González-Gómez, 2013; Garcia-Parra, 2010) for broccoli of same cultivar subjected to blanching treatment or packaged under controlled atmosphere. Antioxidant activity of lyophilized broccoli was found to be 21.65 ± 0.88 mg trolox/g. This value was comparable to those found by Garcia-Parra (2010) and Fernández-León, Lozano, Ayuso, Fernández-León, and González-Gómez (2012). Concerning the TPCs, the values found were similar to those reported by other authors (Domínguez-Perles et al., 2011; Fernández-León et al., 2011).

Table 3
Chemical characterization liophylized broccoli.

	Chl[a] ± SD[b]	AA[a] ± SD[b]	TPC[a] ± SD[b]
Broccoli	10.0 ± 0.13	21.65 ± 0.88	4.33 ± 0.23

Chl: chlorophyll (mg/kg), AA: antioxidant activity (mg trolox/g), TPC: total phenolics compounds (mg 3,4-dihydroxybenzoic acid/g).
[a] Expressed in dried weight.
[b] $n = 3$.

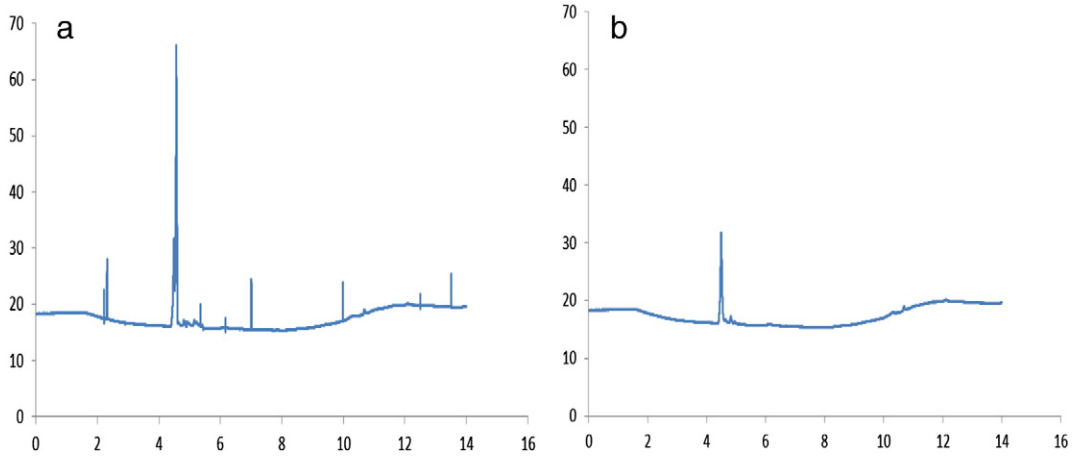

Fig. 4. Odor evaluation chromatograms corresponding to (a) fresh lyophilized and (b) microencapsulated broccoli.

Sulphur compounds are the most abundant volatile in broccoli as reported by Vidal-Aragon et al. (2009). Fig. 4 shows the volatile profile of lyophilized and encapsulated broccoli. It can be observed that the encapsulation process is effective to reduce the height and number of volatile compounds. Yeo, Bellas, Firestone, Langer, and Kohane (2005) found that complex coacervation using gelatin and arabic gum decreased drastically bake flavor odor of frozen foods. Thus, microencapsulation could be a good alternative not only to protect the encapsulated material but also to reduce the characteristic broccoli off odor, which is not pleasant to many consumers.

4. Conclusions

The Box–Behnken design is adequate to identify the most significant variables involved in the development of lyophilized broccoli encapsulation. Based on the empirical model, the variable that has a more pronounced effect on the process of encapsulation is the encapsulating agent concentration in the solution (gelatin/gum arabic). The microencapsulation process allows maintaining chlorophyll content and masking broccoli off odor.

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