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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