Electronic Supplementary Data

Materials and Methods

Encapsulation

Response Surface Methodology (RSM) consists of a set of mathematical and statistical methods developed for modelling phenomena and finding combinations of a number of experimental factors (variables) that will lead to optimum responses. With RSM, several variables are tested simultaneously with a minimum number of trials, using special experimental designs that enable to find interactions between the variables which cannot be identified with classical approaches. The encapsulation experiments were carried out following a Central Composite Face-Centred Design (CCFC), as a function of two factors: temperature (T) and solids concentration [solids].

Table 1. **Actual values of the variables for the coded values.**

Variable, factors, unit	Levels		
	-1	0	+1
Temperature (T, °C)	110	150	180
Solids concentration ([solids] (g/mL)	0.01	0.02	0.04

A total of 10 experiments were performed coded levels as high cubic (+1), low cubic (-1) centre points (0) (Table 1). The temperature varied from 110 to 180 ^{o}C and the solids concentration from 0.01 to 0.04 g/mL, according to the experimental design (Table 2). The ranges were chosen previously taking into account preliminary studies (data not shown). The repetitions of the centre points are used to determine the experimental error, which is assumed to be constant along the experimental domains.

Table 2. The Composite Face-Centred Design (CCFC) for the two independent variables

Experiment number	Variables		Responses
	Temperature, T (˚C)	Solids concentration ([solids] (g/mL)	
1	110	0,01	Yield of collected particles (%) Encapsulation load (mg TOC/mg particles) Encapsulation Efficiency (%) Oxygen radical absorbance capacity –ORAC (µmol TEAC/g part.)
2	110	0,04	
3	180	0,01	
4	180	0,04	
5	110	0,02	
6	180	0,02	
7	150	0,01	
8	150	0,04	
9	150	0,02	
10	150	0,02	

Experimental design analysis / Statistical Analysis

The results of the CCFC, concerning the Yield of collected particles (YCP), Encapsulation load (EL), Encapsulation Efficiency (EE) and Oxygen radical absorbance capacity (ORAC) were analysed using the software Statistica™, version 10, from Statsoft (Tulsa, USA). Both linear and quadratic effects of each factor under study, as well as their interactions were calculated. Their significance was evaluated by analysis of variance. A surface, described by a second-order polynomial equation, was fitted to each set of experimental data points. First- and second-order coefficients of the polynomial equation were generated by regression analysis.
The fit of the models was evaluated by the determination coefficients (R^2) and adjusted R^2 (R_{adj}^2). [1,2] The R^2 value provides a measure of how much of the variability in the observed response values can be explained by the experimental factors and their interactions. However, the R^2 should be used with caution since it always increases with the inclusion of a new variable in the model. The use of R_{adj}^2 is preferred and is related with R^2 by the following equation[3]:

$$R_{adj}{}^2 = 1 - \frac{n-1}{n-p}(1 - R^2)$$

Where n is the number of experiments and p is the number of variables (factors) in the model. The R_{adj^2} takes into account the fact that the number of residual degrees of freedom in the polynomial regression changes as the order of the polynomial changes. R_{adj^2} is a unbiased estimate of the coefficient of determination and is always smaller than R^2. In practice, R^2 should be at least 0.75 or greater; values above 0.90 are considered to be very good. [2]

Results and Discussion

Modelling of spray-drying process for the encapsulation of alpha-tocopherol in zein protein

The effects of each factor and the interactions between factors on the various responses were calculated. Table 3 shows the linear and quadratic effects of each variable and of their interactions on the ORAC during the encapsulation process. For the YCP, EL and EE, a lack of fit of the polynomial models exhibited by low values of R^2 and R_{adj^2} was observed.

Table 3. Linear (L) and quadratic (Q) effects and respective significance levels (p) of the tested variables [factors: Temperature (T), and solids concentration ([solids]) and interactions on ORAC

Factor	ORAC (µmol TEAC/g particles)	
	Effect	p value
[solids] (L)	-16,72	0,014 [a]
[solids] (Q)	-4,50	0,575 [b]
T (L)	-18,86	0,010 [a]
T (Q)	11,12	0,166 [b]
[solids] by T	0,76	0,882

[a] Significant effects with p ≤0.05
[b] Effects with p >0.05 considered in the model

The significant negative linear effect of [solids] and T, indicated that as the [solids] and T increased (independently), the ORAC value decreased, meaning that when the encapsulation process was conducted at higher temperatures or higher [solids], the antioxidant activity of the systems decreased.

The response surface (Figure 1) fitted to the ORAC can be described by second-order polynomial model as a function of [solids] and T (Table 4).

Table 4. Model equations for the response surfaces fitted to the values of ORAC as a function of temperature (T) and solids concentration ([solids]) and respective R^2 and R^2adj

POLYNOMIAL MODEL EQUATIONS	R^2	R_{adj}^2
$ORAC = 226.595 - 56.451[solids] - 992.932[solids]^2 - 1.587T + 0.005T^2$	0,92	0,85

In the fitted response surface, the significant effects p<0.05 and those having confidence range smaller than the value of the effect, or smaller than the standard deviation (data not shown), were included in the model equations of these surfaces. It is better to accept factor with p values higher than 0.05 rather than to take the chance of missing an important factor[26]. The good values for both R^2 and R_{adj^2} of these models (Table 4) suggest a close agreement between the experimental data and the theoretical values predicted by the model. Concerning ORAC, only the identification of the region corresponding to the best response could be achieved. The best response was achieved at a temperature of 110˚C and solids concentration of 0.01 g/mL

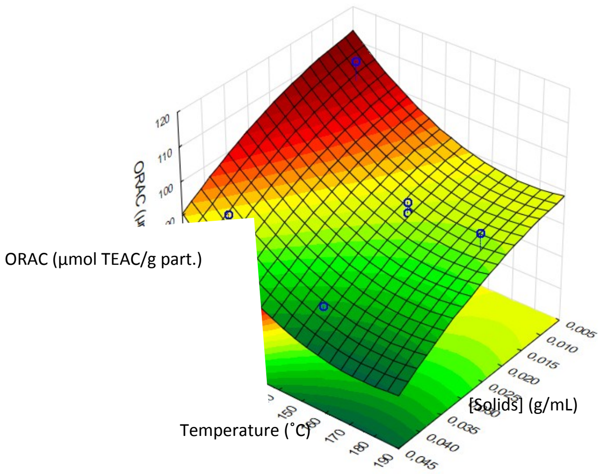

Figure 1. Response surface fitted to the ORAC as a function of temperature and solids concentration