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Electronic Supplementary Data

Materials and Methods

Particles from Gas Saturated Solutions (PGSS®)

Response Surface Methodology (RSM) was used to model the encapsulation of pea protein. RSM consists of a set of mathematical and statistical methods developed for modeling 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 of pea protein through PGSS® were carried out following a Central Composite Rotatable Design (CCRD), as a function of three factors: pressure, temperature and equilibrium time. A total of 17 experiments were performed: 8 factorial points (coded levels as (+1) and (-1); 6 star points (coded as (+α) and (-α)); 3 center points (coded as 0) (Table 1).

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

Variable, factors, unit	Levels				
	-α.	-1	0	+1	+α
Pressure, *P* (MPa)	7.3	10.0	14.0	18.0	20.7
Temperature, *T* (°C)	51.2	56.0	63.0	70.0	74.8
Equilibrium time, *t* (min)	3.0	10.0	20.0	30.0	36.8

The pressure varied from 7.3 to 20.7 MPa, the temperature from 51.2 to 74.8 °C and the equilibrium time from 3 to 36.8 min, according to the experimental design followed (Table 2). The repetitions of the center points are used to determine the experimental error, which is assumed to be constant along the experimental domains. A total of 17 assays including three replicates of the center points were generated. Experiments were conducted randomly, according to the PGSS® methodology described in the Materials and Methods section.

Table 2. The central composite rotatable design for the three independent variables

Run order	Pressure, *P* (MPa)	Temperature, *T* (°C)	Equilibrium time, *t* (min)
1	10.0 (-1)	56.0 (-1)	10.0 (-1)
2	10.0 (-1)	56.0 (-1)	30.0 (+1)
3	18.0 (+1)	56.0 (-1)	10.0 (-1)
4	18.0 (+1)	56.0 (-1)	30.0 (+1)
5	10.0 (-1)	70.0 (+1)	10.0 (-1)
6	10.0 (-1)	70.0 (+1)	30.0 (+1)
7	18.0 (+1)	70.0 (+1)	10.0 (-1)
8	18.0 (+1)	70.0 (+1)	30.0 (+1)
9	14.0 (0)	51.2 (-1.68)	20.0 (0)
10	14.0 (0)	74.8 (+1.68)	20.0 (0)
11	7.3 (-1.68)	63.0 (0)	20.0 (0)
12	20.7 (+1.68)	63.0 (0)	20.0 (0)
13	14 (0)	63.0 (0)	3.2 (-1.68)
14	14.0 (0)	63.0 (0)	36.8 (+1.68)
15	14.0 (0)	63.0 (0)	20.0 (0)
16	14.0 (0)	63.0 (0)	20.0 (0)
17	14.0 (0)	63.0 (0)	20.0 (0)

Experimental design analysis / Statistical Analysis

The results of the CCRD, concerning the Encapsulation Efficiency (EE), Yield of Collected Particles (YCP) and Inhibition of Pancreatic Lipase Activity (IPLA) were analyzed using the software Statistica^{TM}, 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 equations 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)[25, 26]. 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[27]:

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

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. $R_{adj}{}^2$ ia 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[26].

Results and Discussion

Modelling of PP encapsulation through PGSS®

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 EE and YCP during the encapsulation process. For the IPLA, 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: Pressure (P), Temperature (T) and Equilibrium time (t)] and interactions on EE and YCP

Factor	**EE (%)**		**YCP (%)**	
	Effect	p value	Effect	p value
T (L)	-7.22	0.20[b]	11.26	0.05[a]
T (Q)	5.75	0.66	-5.30	0.34[b]
P (L)	14.59	0.03[a]	12.45	0.03[a]
P (Q)	-6.60	0.28[b]	-6.80	0.23[b]
t (L)	-3.62	0.59	-9.01	0.10[b]
t (Q)	9.36	0.48	-16.62	0.01[a]
T x P	-15.89	0.05[a]	2.5	0.70
T x t	10.75	0.25[b]	-2.5	0.70
P x t	11.48	0.22[b]	3.5	0.59

[a] Significant effects with $p \leq 0.05$.

[b] Effects with $p > 0.05$ considered in the model.

For the results obtained for the EE, a negative effect of T on pea protein encapsulation indicated that higher T values, within the tested range, correspond to a lower encapsulation of pea protein. This result can be explained possibly by the denaturation of the protein at higher temperatures. The positive significant linear effect of P on the encapsulation of pea protein indicated that lower P values, lead to higher EE. The EE was not affected by the equilibrium time at linear level (p=0.53). The negative significant interaction between T and P shows that as T and P increased, the encapsulation of pea protein decreased. The interaction T x t and P x t showed a positive effect on EE.

The negative quadratic effect of P indicated that the experimental results on pea protein encapsulation can be fitted to a four-dimensional convex surface, as a function of P. The positive quadratic effects of both T and t indicate a concave curvature of this surface, as a function of T or t.

Concerning the YCP, a positive significant linear effect of P and T indicated that the increase in T or P values, within the tested range, correspond to an increase in the YCP. However, longer contact times conducted to lower YCP (negative linear effect of t). Also, the significant quadratic negative effects of T, P and t indicated that the YCP can be described by a four dimensional convex surface. The linear interactions showed not to have any significant effect on YCP. Thus, the interactions can be removed in the final polynomial model fitted to the experimental results.

The response surfaces (Figure 1) fitted to the EE and YCP can be described by second-order polynomial models as a function of pressure, temperature and equilibrium time (Table 4).

Table 4. Model equations for the response surfaces fitted to the values Encapsulation efficiency (EE) and yield of collected particles (YCP), as a function of Pressure (P), Temperature (T) and equilibrium time (t), and respective R^2 and R_{adj}^2

POLYNOMIAL MODEL EQUATIONS	R^2	R_{adj}^2
$EE = -54.355 - 1.481T + 0.044T^2 + 25.327P - 0.251P^2 + 0.023t^2 - 0.284TP - 0.032Tt + 0.069\,Pt$	0.77	0.54
$YCP = -282.173 + 7.621T - 0.054T^2 + 7.50P - 0.213P^2 + 2.872t - 0.083t^2$	0.78	0.65

In these fitted response profiles, 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 6) suggest a close agreement between the experimental data and the theoretical values predicted by the model. Optimum conditions were observed in the response profile for the yield of collected particles. Concerning the pea protein load, only the identification of the region corresponding to the best response could be achieved.

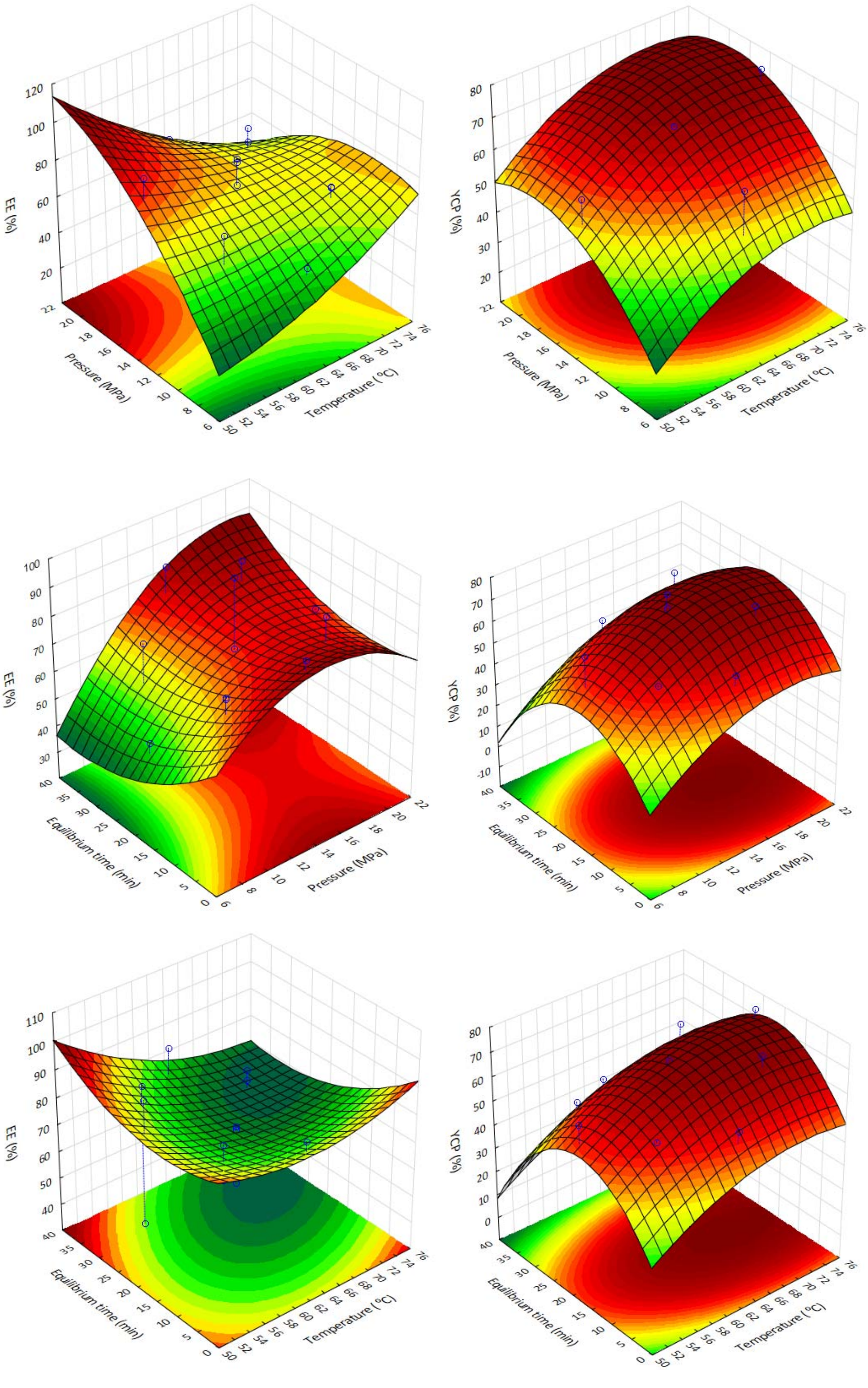

Figure1. Fitted response surfaces to the EE and YCP as a function of temperature, pressure and equilibrium time

Pancreatic Lipase activity of PP and SLPP

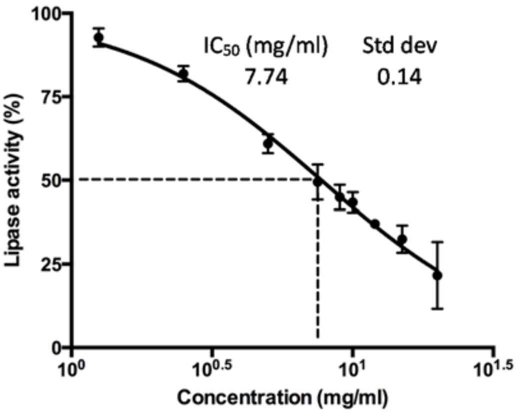

Figure 2. Concentration of pea protein that inhibits 50% of lipase (IC$_{50}$)