Electronic Supplementary Information

EXPERIMENTAL

Experimental design (Process Optimization)

RSM was used to model the encapsulation of betacyanins and optimize encapsulation conditions. The encapsulation of betacyanins 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 centre 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, X_1 (MPa)	9	12	16	20	23
Temperature, X_2 (°C)	57	60	65	70	73
Equilibrium time, X_3 (min)	5	15	30	45	55

The pressure varied from 9 to 23 MPa, the temperature from 57 to 73^{0}C and the equilibrium time from 5 to 55min, according to the experimental design followed (Table 2). A total of 17 assays including three replicates of the centre points were generated. The repetitions of the centre points are used to determine the experimental error, which is assumed to be constant along the experimental domains. Experiments were conducted randomly, according to the methodology described in PGSS® technique.

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

Experiment number	Pressure, X_1 (MPa)	Temperature, X_2 (°C)	Equilibrium time, X_3 (min)
1	16 (0)	65 (0)	30 (0)
2	12 (-1)	60 (-1)	45 (+1)
3	16 (0)	65 (0)	30 (0)
4	20 (+1)	70 (+1)	45 (+1)
5	16 (0)	65 (0)	30 (0)
6	12 (-1)	60 (-1)	15 (-1)
7	20 (+1)	60 (-1)	15 (-1)
8	20 (+1)	60 (-1)	45 (+1)
9	20 (+1)	70 (+1)	15 (-1)
10	16 (0)	57 (-1.68)	30 (0)
11	12 (-1)	70 (+1)	45 (+1)
12	23 (+1.68)	65 (0)	30 (0)
13	12 (-1)	70 (+1)	15 (-1)
14	16 (0)	65 (0)	5 (-1.68)
15	16 (0)	65 (0)	55 (+1.68)
16	9 (-1.68)	65 (0)	30 (0)
17	16 (0)	73 (+1.68)	30 (0)

Oxygen radical absorbance capacity (ORAC)

ORAC assay was carried out by the method of Huang *et al*., 2002[22] modified for the FL800 microplate fluorescence reader (Bio-Tek Instruments, Winooski, VT, USA), as described by Feliciano et al., 2009[20]. This assay measured the ability of the antioxidant species in the sample to inhibit the oxidation of disodium fluorescein (FL) catalyzed by peroxyl radicals generated from AAPH. Briefly, 25 µL of the appropriate sample dilutions and 150 µL of disodium fluorescein (2×10^{-7} mM) were added to a 96-well microplate. The microplate was put in a fluorescent reader and allowed to incubat at 37 ºC, for 10 minutes. The reaction was started with 25 µL of AAPH (153 mM) added through the injector. Fluorescence emitted by the reduced form of FL was measured in an FL800 microplate fluorescent reader (Bio-Tek Instruments, Winooski, VT, USA) and recorded every 1 minute at the emission wavelength of 530 ± 25 nm and excitation wavelength of 485 ± 20 nm for a period of 40 minutes. Phosphate buffer (75 mM, pH=7.4) was used to prepare AAPH and FL solutions and as blank. Solutions of 5, 10, 20, 30, and 40 µmol/L of Trolox were used as control standards. Final ORAC values were calculated by EC (Effective Concentration) method to diminish the impact of the dilution effect as described by Bolling *et al*., 2012[19]. The results were presented as µmol of trolox equivalents antioxidant capacity (TEAC) per g of particles and were expressed as a mean of eight replicates.

Hydroxyl radical adverting capacity (HORAC)

The HORAC assay was based on a previously reported method[23] modified for the FL800 microplate fluorescence reader. This assay evaluates the hydroxyl radical prevention capacity of a sample using fluorescein as a probe.

Briefly, 30 µL of appropriate sample dilutions and 170 µL of FL ($9.28\text{x}10^{-8}$ M) were added to a black 96-well microplate. Then, 40 µL of hydrogen peroxide (H_2O_2), 0.206 M, were added to each well of the microplate. Finally, the reaction was started by adding 60 µL of cobalt (II) fluoride (CoF_2), 1.15 mM, to the mixture previously placed in the microplate. Sodium phosphate buffer (SPB), 75 mM, pH=7.4, was used to prepare the solution of FL, H_2O_2 and CoF_2 were prepared with Milli-Q water. Caffeic acid was used as a standard, and 50, 100, 150, 200 and 250 µM solutions in acetone:Milli-Q water (50:50, v/v) were used to create the calibration curve. Acetone:Milli-Q water (50:50, v/v) solution was used to prepare the samples and as a blank. The fluorescence emitted by the reduced form of FL was measured and recorded every 1 minute during 60 minutes, at 37 ºC. The FLx800 fluorescence microplate reader was controlled by software Gen5 and was used with fluorescence filters for an excitation wavelength of 485 ± 20 nm and an emission wavelength of 530 ± 25 nm. Final HORAC values were calculated by EC (Effective Concentration) method to diminish the impact of the dilution effect as described by Bolling *et al*., 2012[19]. Data was expressed as µmol of caffeic acid equivalents antioxidant capacity (CAEAC) per g of particles. Results were presented as a mean of eight replicates.

Hydroxyl radical scavenging capacity (HOSC)

The HOSC assay was performed according to Moore *et al*., 2006[24] and adapted for the FLx800 fluorescence microplate reader (BioTek Instruments, Winooski, VT, USA). This assay evaluates the hydroxyl radical scavenging capacity of a sample using fluorescein as a probe and a classic Fenton reaction with Fe (III) and H_2O_2 as a source of hydroxyl radicals.

Briefly, 30 µL of appropriate sample dilutions, 40 µL of H_2O_2 (0.1990 M) and 170 µL of FL (9.28x10-8 M) were added to a black 96-well microplate. The reaction was started by adding 60 µL of iron (III) chloride ($FeCl_3$), 3.43 mM, to the wells of the microplate. SPB, 75 mM, pH=7.4, was used to prepare the solution of FL, and the solutions of H_2O_2 and $FeCl_3$ were prepared with Milli-Q water. Trolox was used as a standard, and 5, 10, 15, 20 and 30 µM solutions in acetone:Milli-Q water (50:50, v/v) were used to perform the calibration curve. Acetone:Milli-Q water (50:50, v/v) solution was used to prepare the samples and as a blank. The fluorescence emitted by the reduced form of FL was measured and recorded every 1 minute, during 60 minutes, at 37 ºC. The FLx800 fluorescence microplate reader was controlled by software Gen5 and was used with fluorescence filters for an excitation wavelength of 485±20 nm and an emission wavelength of 530±25 nm. Final HOSC values were calculated by EC (Effective Concentration) method to diminish the impact of the dilution effect as described by Bolling *et al*., 2012[19]. Data was expressed as µmol of trolox equivalents antioxidant capacity (TEAC) per g of particles. Results were presented as a mean of eight replicates.

RESULTS AND DISCUSSION

The effects of each factor and the interactions between factors on the various responses were calculated. Table 1 shows the linear and quadratic effects of each variable and of their interactions on the betacyanin content, ORAC, HOSC and L* value during the encapsulation process. For HORAC and yield of collected particles, a lack of fit of the polynomial models exhibited by low values of R^2 andR_{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 betacyanin content, ORAC, HOSC and L*

Factor	Betacyanins		ORAC		HOSC		L*	
	Effect	p value	Effect	p value	Effect	p value	Effect	p value
P (L)	-19.81	0.019[a]	-5.97	0.032[a]	-6.14	0.005[a]	5.04	0.019[a]
P (Q)	7.48	0.335[b]	2.99	0.266[b]	3.41	0.018[a]	-1.91	0.330[b]
T (L)	-8.32	0.243[b]	-5.58	0.041[a]	-3.61	0.165[b]	5.14	0.017[a]
T (Q)	0.31	0.967	1.17	0.649	1.30	0.112	-0.02	0.991
t (L)	-1.20	0.860	0.67	0.775	-0.20	0.570	0.68	0.694
t (Q)	8.20	0.291[b]	2.79	0.294[b]	3.03	0.923[b]	-1.27	0.505
P x T	-9.95	0.282[b]	-3.35	0.289[b]	-2.03	0.207	-0.07	0.974
P x t	13.20	0.166[b]	5.80	0.088[b]	4.08	0.460[b]	-2.96	0.211[b]
T x t	-10.65	0.252[b]	-3.00	0.339[b]	-1.38	0.160	1.46	0.520

[a] Significant effects with $p \leq 0.05$.
[b]Effects with $p > 0.05$ considered in the model.

For the results obtained for betacyanins, a negative significant effect of P and T on betacyanin encapsulation indicated that higher P and T values, within the tested range, correspond to a lower encapsulation of betacyanins. This result can be explained by the emulsion instability at higher pressures or the pigment degradation under these conditions. The t has demonstrated to have lower effect on the encapsulation of betacyanins. The positive quadratic effect of t indicated that the experimental results on betacyanin encapsulation can be fitted to a four-dimensional concave surface. All the interactions between factors for the betacyanin content were important. As the P and T values increased, the betacyanin encapsulation decreased. When the P and t increased the betacyanin encapsulation increased. As T and t increased, the encapsulation of betacyanins decreased.

Concerning the ORAC values, significant linear negative effects of P and T were found, in contrast to the observations for the betacyanin content. As P and T increased, the ORAC values decreased. Also, the significant quadratic positive effects of P and t indicated that ORAC can be described by a four dimensional concave surface. When the P and t increased the ORAC increased and as T and t increased, the ORAC values decreased.

Regarding the HOSC values, significant linear negative effects of P and T were found, in accordance with the observations for the ORAC values. As P and T are increased the HOSC

values decreased. Also, the significant quadratic positive effects of P and t indicated that ORAC can be described by a four dimensional concave surface. When the P and t increased, the HOSC values increased, as well.

Finally, for the results of L^*, the same trend was verified as with the ORAC and HOSC values. As P and T are increased, the L^* values increased (lighter particles are obtained). Also, the significant quadratic positive effects of P and t indicates that the L^* value can be described by a four dimensional convex surface. When the P and t increased, the L^* values decreased.

The response surfaces fitted to ORAC, HOSC and L^* value are presented below (Figure 1).

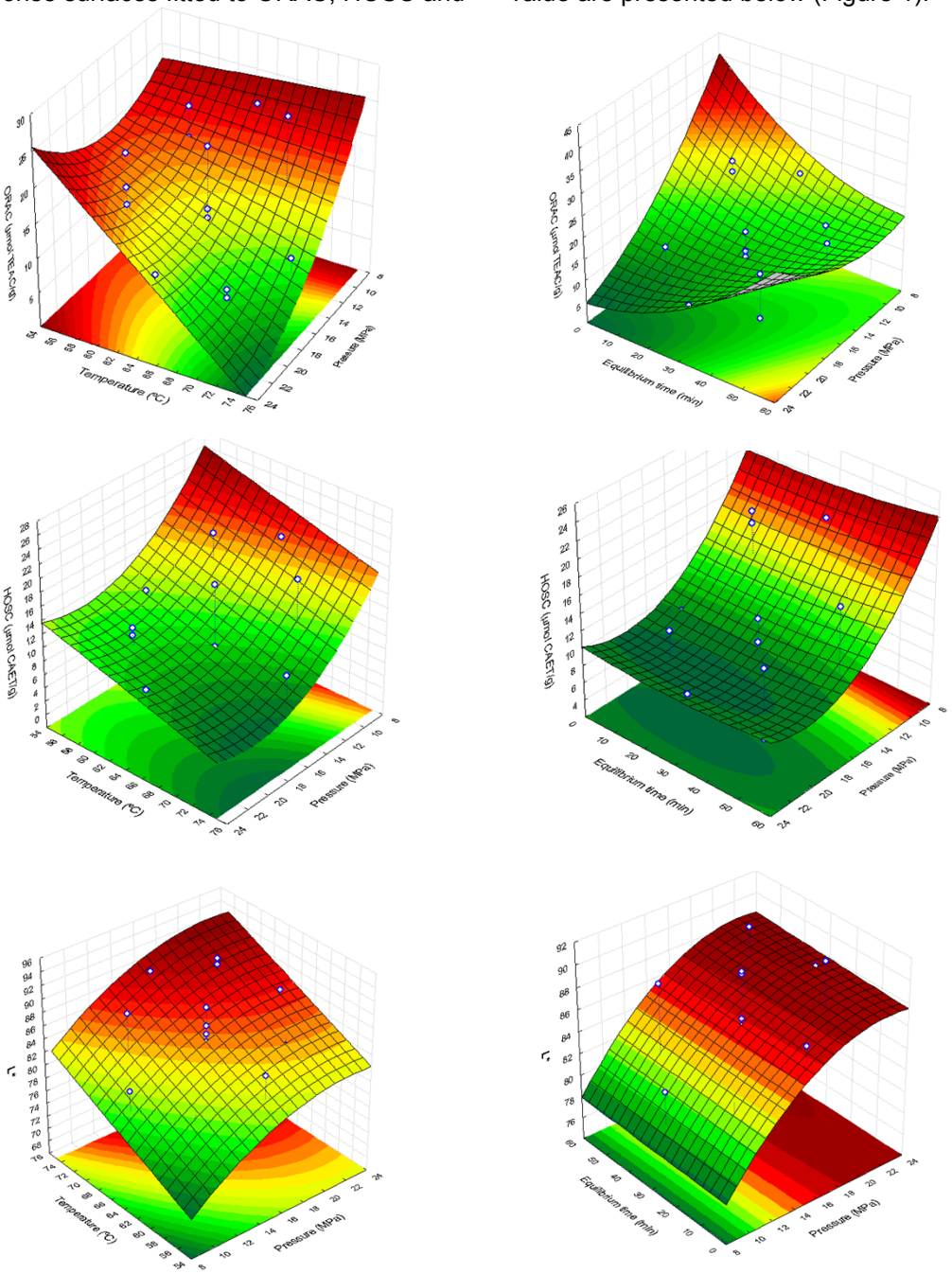

Figure 1. Response surfaces fitted to the ORAC, HOSC and L* value as a function of (i) temperature and pressure and (ii) of equilibrium time and pressure.