Electronic Supplementary Data

Material and Methods

Experimental design (Process Optimization)

Response surface methodology (RSM) was used to model the isolation of betalains form cactus pear fruits and optimize extraction conditions. 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. Isolation of betalains through High Pressure Carbon Dioxide (HPCD) assisted-water extraction were carried out following a Central Composite Face-Centered Design (CCFC), as a function of three factors: temperature, pressure and volume ratio of (solid-liquid mixture)/(pressurized CO_2) ($R_{S\text{-}L/CO2}$). A total of 17 experiments were performed (Table 1).

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

Variable, factors, unit	**Levels**		
	-1	**0**	**+1**
Temperature, X_1 (°C)	40	55	70
Pressure, X_2 (bar)	100	175	250
CO_2 ($R_{S\text{-}L/CO2}$), X_3 (%)	20	50	80

The pressure varied from 100 to 250 bar, the temperature from 40 to 70 ^{o}C and the volume ratio of (solid-liquid mixture)/(pressurized CO_2) from 20 to 80 %, 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 methodology described in the Materials and Methods section.

Table 2. The CCFC design for the three independent variables.

Experiment	Temperature, X_1 (°C)	Pressure, X_2 (bar)	$R_{S\text{-}L/CO2}$, X_3 (%)

number			
1	40 (-1)	100 (-1)	20 (-1)
2	40 (-1)	100 (-1)	80 (+1)
3	40 (-1)	250 (+1)	20 (-1)
4	40 (-1)	250 (+1)	80 (+1)
5	70 (+1)	100 (-1)	20 (-1)
6	70 (+1)	100 (-1)	80 (+1)
7	70 (+1)	250 (+1)	20 (-1)
8	70 (+1)	250 (+1)	80 (+1)
9	70 (+1)	175 (0)	50 (0)
10	70 (+1)	175 (0)	50 (0)
11	55 (0)	100 (-1)	50 (0)
12	55 (0)	250 (+1)	50 (0)
13	55 (0)	175 (0)	20 (-1)
14	55 (0)	175 (0)	80 (+1)
15 (C)	55 (0)	175 (0)	50 (0)
16 (C)	55 (0)	175 (0)	50 (0)
17 (C)	55 (0)	175 (0)	50 (0)

Results and discussion

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 betalains yield during the extraction process. For total phenolic content, ORAC, HOSC, colour strength and L*, 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 (A), Pressure (B) and $R_{S\text{-}L/CO2}$ (C)] and interactions on betalains yield and HORAC values.

Factors	Betalains yield		HORAC	
	Effect	p value	Effect	p value
A (L)	-2.422	0.266[b]	-206	0.715
A (Q)	4.957	0.241	3817	0.008
B (L)	-4.915	0.044[a]	312	0.581
B (Q)	-2.221	0.584[b]	-651	0.552[b]
C (L)	2.692	0.221[b]	-480	0.403[b]
C (Q)	6.092	0.160[b]	-3162	0.019[a]
A x B	5.741	0.038[a]	-1231	0.081[b]
A x C	2.559	0.291[b]	-237	0.707[b]
B x C	5.684	0.039[a]	503	0.432

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

For the results obtained for betalains yield, a linear negative effect ($p<0.05$) of pressure on betalains extraction indicated that higher pressure values, within the tested range, correspond to a lower extraction of betalains. This effect was more pronounced at lower temperatures and lower $R_{S\text{-}L/CO2}$. This result can be explained by the pigment degradation under these conditions.

In addition, the recovery of betalains was significant affect ($p<0.05$) by some interactions between factors, pressure with temperature and pressure with $R_{S\text{-}L/CO2}$. As the pressure and temperature values decreased, the yield of betalains increased. This result may be explained by the negative impact of higher pressure and temperature on the pigments stability during the extraction process. When the pressure and $R_{S\text{-}L/CO2}$ values decreased the recovery of betalains increased. This effect can be explained by the increase of the volume of pressurized CO_2 (lower $R_{S\text{-}L/CO2}$), which possibly plays a crucial role during the betalains extraction.

Concerning the HORAC values, the positive quadratic effect ($p<0.05$) of temperature indicated that the experimental results on HORAC values can be fitted to a four-dimensional concave surface. Moreover, it also presented a negative quadratic effect (p<0.05) of $R_{S\text{-}L/CO2}$ indicating that the experimental results on HORAC values can be fitted to a four-dimensional convex surface.

The response surfaces (Figure 1) fitted to betalains yield and HORAC values can be described by second-order polynomial models as a function of temperature, pressure and $R_{S\text{-}L/CO2}$ (Table 4).

Table 4. Model equations for the response surfaces fitted to the values of betalains yield (BY) and total phenolic content, as a function of Temperature (A), Pressure (B) and $R_{S\text{-}L/CO2}$ (C), and respective R^2 and R_{adj}^2.

Polynomial model equations	**R^2**	**R_{adj}^2**
$BY = 163.6 – 0.237A – 1.701B + 0.009B^2 – 0.634C + 0.003C^2 + 0.003AB + 0.001\ AC +$	0.817	0.581
$HORAC = 8964 + 0.306B^2 – 40.57C – 0.891C^2 – 0.204AB + 0.232\ AC$	0.736	0.617

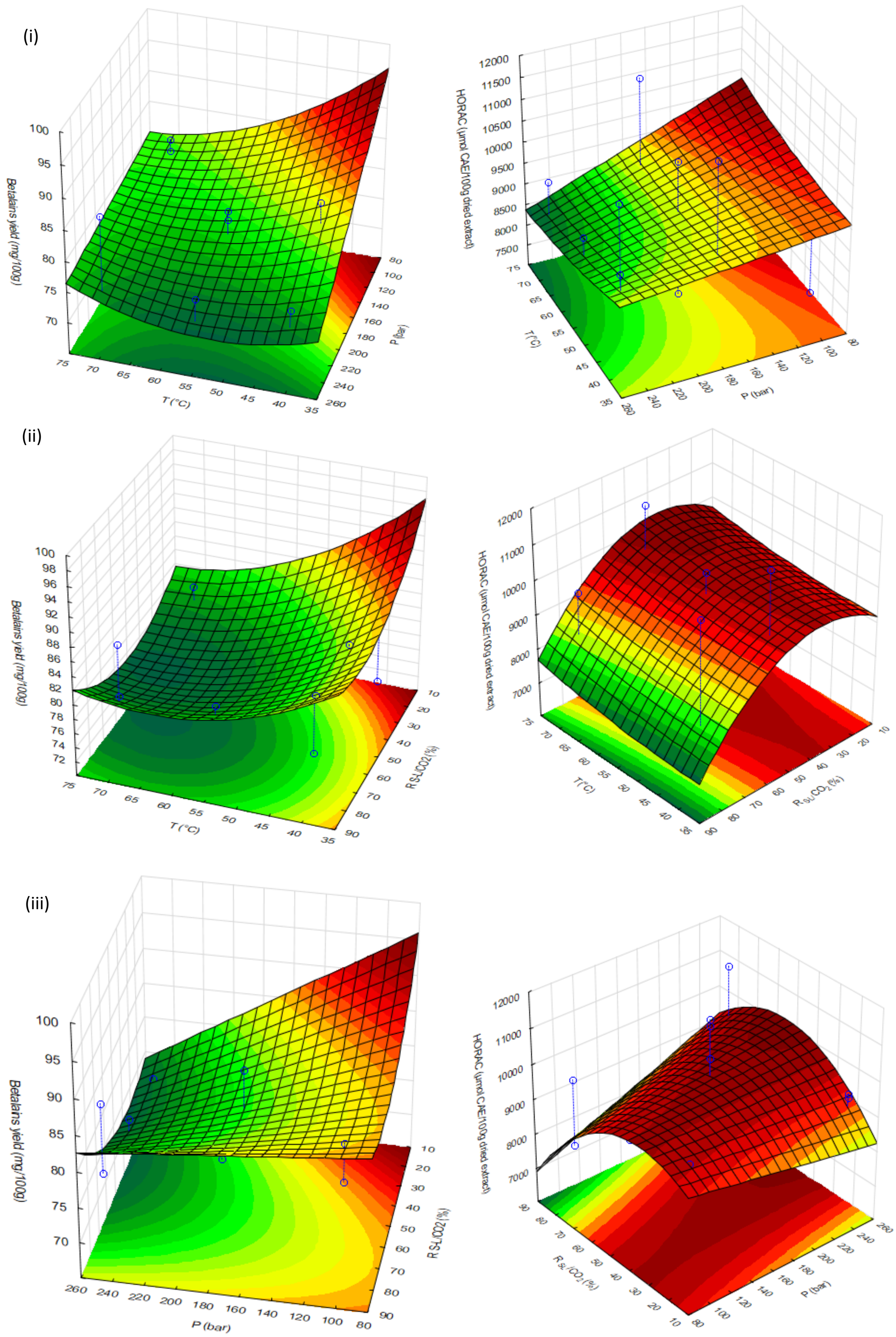

Figure 1. Response surfaces fitted to the betalains yield and HORAC as a function of (i) temperature and pressure, (ii) temperature and $R_{S-L/CO2}$ and (iii) pressure and $R_{S-L/CO2}$.