Effect of Pressure and Temperature on Liquid Oleoresin Capsicum Extraction

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ABSTRACT

According to the possibility to fractionate selectively different pigments present in oleoresins, such as carotenoids and capsaicinoids, in this work the effect of pressure (140-300 bar) and temperature (313-333K) on the extraction of liquid Oleoresin Capsicum (OR_{Cap}) was analyzed. A 2^2 factorial design with centered points was proposed and different statistical analyses were carried out to study the effect of these parameters on the selected responses: extraction yield, colour, pungency and concentration of different pigments in the extract and raffinate. Moreover solubility parameters were experimentally obtained from the extraction curves and correlated using semi-empirical models.

INTRODUCTION

Despite some previous researches [1-4] based exclusively on the application of Supercritical Fluid Extraction (SFE) to paprika powder in order to produce liquid oleoresin without using organic solvents, the results obtained were not enough to determine the economical feasibility of this technique compared to the traditional process. In addition, the importance of some compounds present in oleoresin, which can be used with different applications in pharmaceutical industries, has focused the attention in the possibility to fractionate the liquid oleoresin.

According to the interesting compounds present in oleoresin, they can be classified as colorant pigments or carotenoids and pungency pigments or capsaicinoids. Within the first ones the more representative compounds can be seen at Figure 1 where *(a)* and *(b)* are representative from the red fraction and *(c)* and *(d)* are representative from the yellow one. All of them are important in human beings because they can serve several important biological activities, although the most widely studied and well understood nutritional role for carotenoids is their provitamin A activity [5].

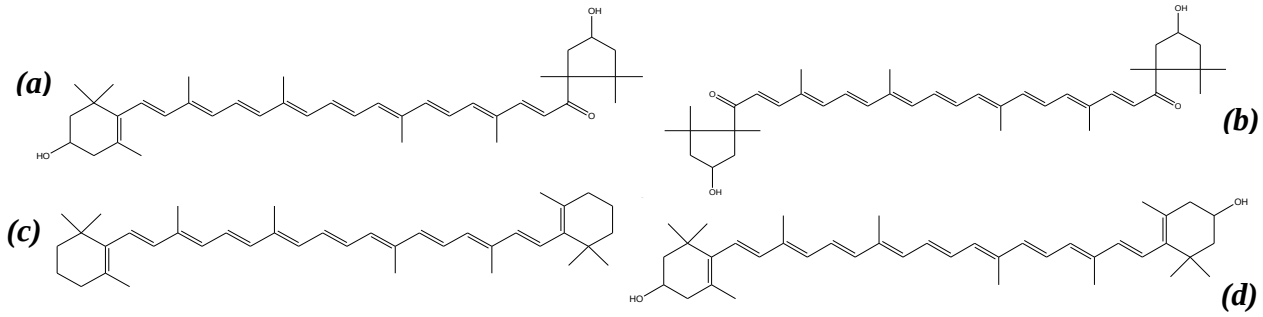

Figure 1. Main carotenoids present in oleoresin. *(a)* Capsanthin; *(b)* Capsorubin; *(c)* *β*-carotene; *(d)* Zeaxanthin.

In the case of capsaicinoids, these are alkaloids which have been intensively investigated for their physiological and pharmaceutical importance [6-7]. Figure 2 shows the main structures of the capsaicinoids present in oleoresin.

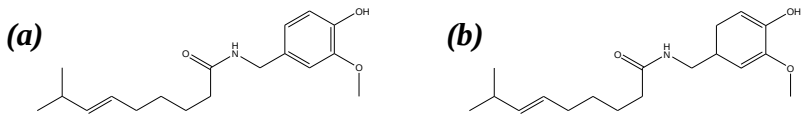

Figure 2. Main capsaicinoids present in oleoresin. *(a)* Capsaicin; *(b)* Dihydrocapsaicin.

Topical capsaicin was described as a counter-irritant in lumbago, neuralgia, and rheumatic disorders but, recent interest has centred on the use of capsaicin as a topical analgesic for a variety of conditions characterized by pain not responsive to classical analgesics [8].

Attending to the importance and potential applications of carotenoids and capsaicinoids, this work has been focused in the use of supercritical carbon dioxide to fractionate the different pigments present in oleoresin. A 2^2 factorial design was proposed to carry out the experiments and statistical analysis used to determine the effect over the responses. Besides, solubility was experimentally determined and correlated using semi-empirical models.

MATERIALS AND METHODS

Chemicals

A commercial oleoresin Capsicum (OR_{Cap}) was provided by R. Sabater S.A (Murcia, Spain) with a pungency capacity of $\sim 1.0 \cdot 10^6$ Scoville Heat Units (SHU) and a colour capacity of 2500 Standard Colour Units (SCU). CO_2 with 99.8% of purity was supply by Carburos Metálicos (Madrid, Spain). All organic solvents grade HPLC were supplied by Sigma Aldrich, S.A. (Barcelona, Spain) and standards used for identification and quantification were also supplied by Sigma Aldrich, S.A.

Equipment and experimental procedure

The experiments were carried out in a batch-type apparatus, as shows in Figure 1. Liquid CO_2 from a stainless-steel cylinder is filtered (F-1), cooled (E-1), and compressed by a positive-displacement pump (P-1). The pressure is regulated by a back-pressure regulator (BPR) and checked by a manometer (PI-1). The compressed fluid passes through a 100 mL stainless-steel cylinder (C-1) from the bottom to the top. At the top and at the bottom of the extractor a layer of glass wool is placed between two metallic meshes of 0.2 mm to prevent loss of particles. To keep the temperature of the extractor at the desired value, a digital controller (TIC) regulates the electric current through a resistance which is placed around the extractor cylinder. The oleoresin-laden gas from the extractor passes through a heated metering valve (V-7) where the supercritical CO_2 is depressurized, and the extracted oleoresin is collected in a cooled container (T-1). The gas flow through the extractor was measured by a turbine flow meter (Fl-1).

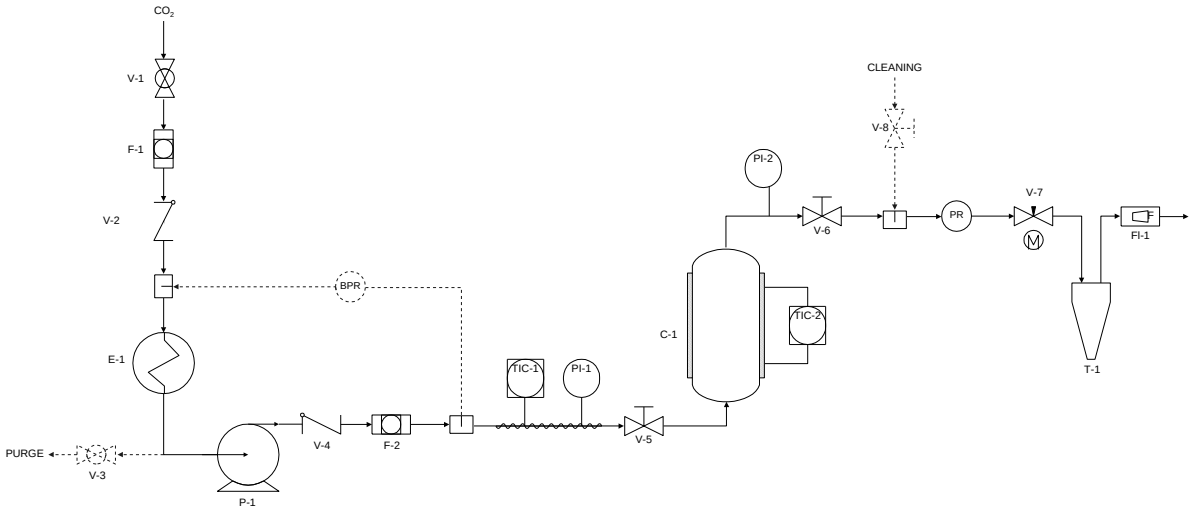

Figure 1. Schematic diagram of the experimental extraction system.

Colouring capacity

The colour capacity was determined through the measurement of parameters ASTA and SCU in the extracted oleoresin. ASTA value of paprika oleoresin samples was evaluated according to the ASTA $_{20.1}$ method [9] and using a Thermo Scientific spectrophotometer model Helios-Zeta UV-Vis (Madison, US). The ASTA colour value was then calculated as follows:

$$ASTA_{20.1}\ units = \frac{A_\lambda \cdot 164 \cdot I_f}{m_S} \quad (2)$$

where A_λ is the absorbance of the sample at 460 nm, I_f is a correction factor of the spectrophotometer and m_s is the mass of raw material in grams.

Once ASTA units are obtained Standard Colour Units (SCU) can be calculated from the relationship between both [10]:

SCU Units = 40.24 × ASTA Units (3)

Carotenoids concentration

According to a previous research [11], the concentration of the different carotenoid fractions from oleoresin can be calculated based on equations (5) and (6), where the absorbance (A_λ) at two different wavelengths are applied.

$$C^R = \frac{A_{508}.2144 - A_{472}.403{,}3}{270{,}9} \quad (5)$$

$$C^Y = \frac{A_{472}.1724{,}3 - A_{508}.2450{,}1}{270{,}9} \quad (6)$$

C^R and C^Y are respectively the concentrations of red and yellow fractions of carotenoids expressed in μg/mL.

Capsaicinoids concentration and pungency capacity

The concentration of capsaicinoids was determined by HPLC analyses. A known amount of sample was weighed and diluted with acetone. Prior to the injection into the HPLC column, the mixture was filtered through a 0.45 μm filter. The chromatographic analyses were carried out using a Jasco Chromatograph (Essex, UK) equipped with a quaternary pump PU-1580, an autosampler AS-1550 and an in-line degasser DG-1580-53. For the determination of the capsaicinoids content in the samples the mobile phase used was water/acetonitrile (45:55, v/v) with a flow rate of 1 mL/min and a Discovery C18 column (15 cm × 4.6 mm, 5 μm) purchased from Analisis Vinicos (Tomelloso, Spain). A Diode Array MD-1510/1515 detector was used and the identification and quantification of peaks was made by comparison with standards.

Once capsaicinoids concentration is known, pungency capacity expresses as Scoville Heat Units can be calculated based on the following relationship [12]:

1 ppm of capsaicinoids ~15 SHU (7)

RESULTS

2^2 Factorial Design

A 2^2 factorial design with two centered points was established to carry out all the experiments. For selecting the pressure and temperature intervals specifications of the experimental equipment and degradation of carotenoids were taken into account. Flow rate was kept constant in all experiments at 5 L/min to assure saturation conditions for CO_2. Table 1 reports the coded and uncoded matrix of the factorial design proposed.

Table 1. Coded and uncoded matrix of the 2^2 factorial design.

Run no.	*P*	*T*	*P (bar)*	*T (K)*
1	0	0	220	323
2	-	-	140	313
3	0	0	220	323
4	-	+	140	333
5	+	+	300	333
6	+	-	300	313

When a supercritical extraction of liquid oleoresin capsicum takes place, a fractionation of the different kind of pigments present in this type of oleoresin occurs. In this sense it is possible to obtain a raffinate with a high concentration of colorant pigments and an extract with low amounts of carotenoids and high concentrations of capsaicinoids. Nevertheless, the yield of the process is an important factor which should be considered if economical feasibility wants to be obtained.

In order to study the influence of temperature and pressure over these responses, the results of the statistical analysis, carried out using the software Statgraphics Plus 5.1, will be focused on

concentration of carotenoids in the raffinate, concentration of capsaicinoids in the extract and extraction yield.

Figure 2 shows the Pareto charts calculated for these variables. In these charts, the length of each bar is proportional to the standardized effect, which is the estimated effect divided by its standard error. The vertical line on the plot judges the effects that are statistically significant, so bars that extend beyond the line correspond to effects that are statistically significant at the 95 percent confidence level. As can be seen from figure, pressure has a statistical significance influence over extraction yield and concentration of capsaicinoids in the extract. Temperature only affects the concentration of capsaicinoids in the extract. Hence, concentration of carotenoids in the raffinate will be independent on pressure and temperature and will be always concentrated in the raffinate among the whole interval studied, whereas extraction yield and concentration of capsaicinoids in the extract will depend on those factors.

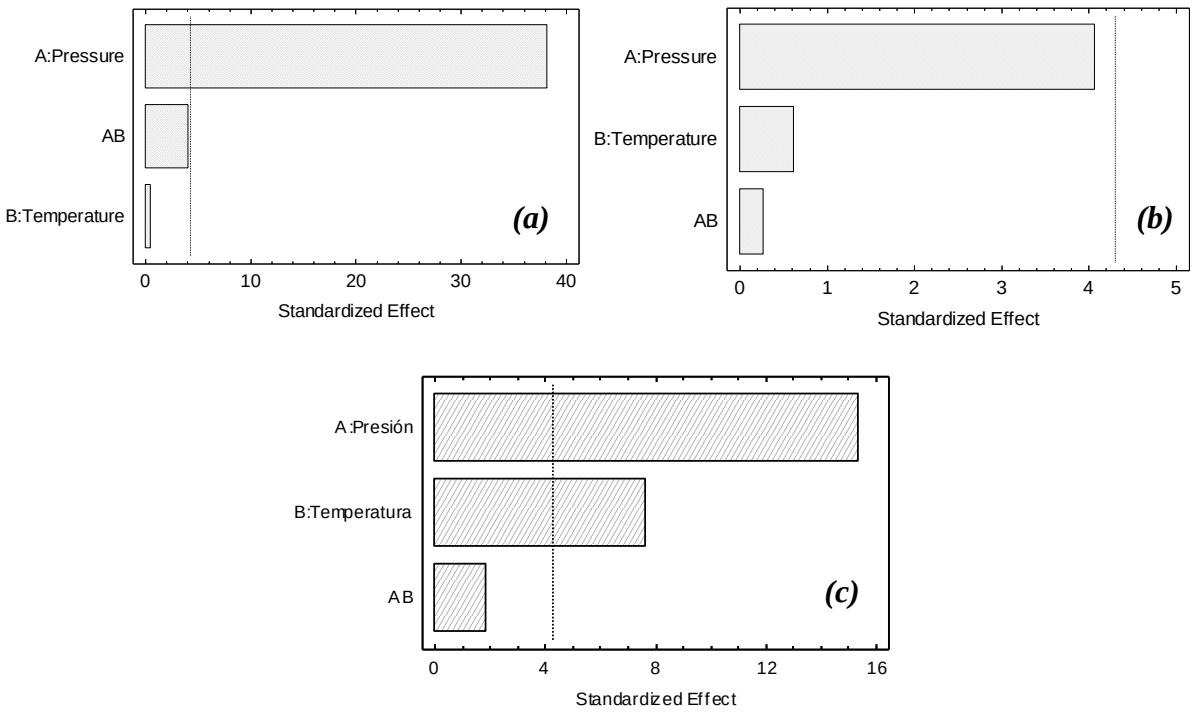

Figure 2. Pareto charts of effects obtained from the 2^2 factorial design. *(a)* Extraction Yield; *(b)* Concentration of carotenoids in the raffinate; *(c)* Concentration of Capsaicinoids in the extract.

Figure 3 shows the results of the response surfaces for the variables analyzed. In the case of extraction yield (a), an increase in pressure at constant temperature acts increasing the density of the supercritical solvent and enhancing the extraction yield. The effect of temperature is more complex because at low values of pressure an increase in temperature decreases the density of CO_2 and therefore the extraction yield decreases but at high pressures an increase in temperature increases the vapour pressure of the compounds to be extracted and therefore extraction yield increases. Depend on which effect would be dominant extraction yield will decrease or increase.

Figure 3 (b) shows that an increase in pressure increases the response of the concentration of carotenoids in the raffinate. Low values of temperature are preferred to avoid degradation processes.

In the case of concentration of capsaicinoids in the extract (Figure 3 (c)), the higher value of this response is collected with the lower value of pressure and the higher value of temperature probably due to the fact that when these conditions are employed the amount of lipids which accompanies capsaicinoids in the extract decreases, decreasing also the effect of dilution which exists when higher pressures are used.

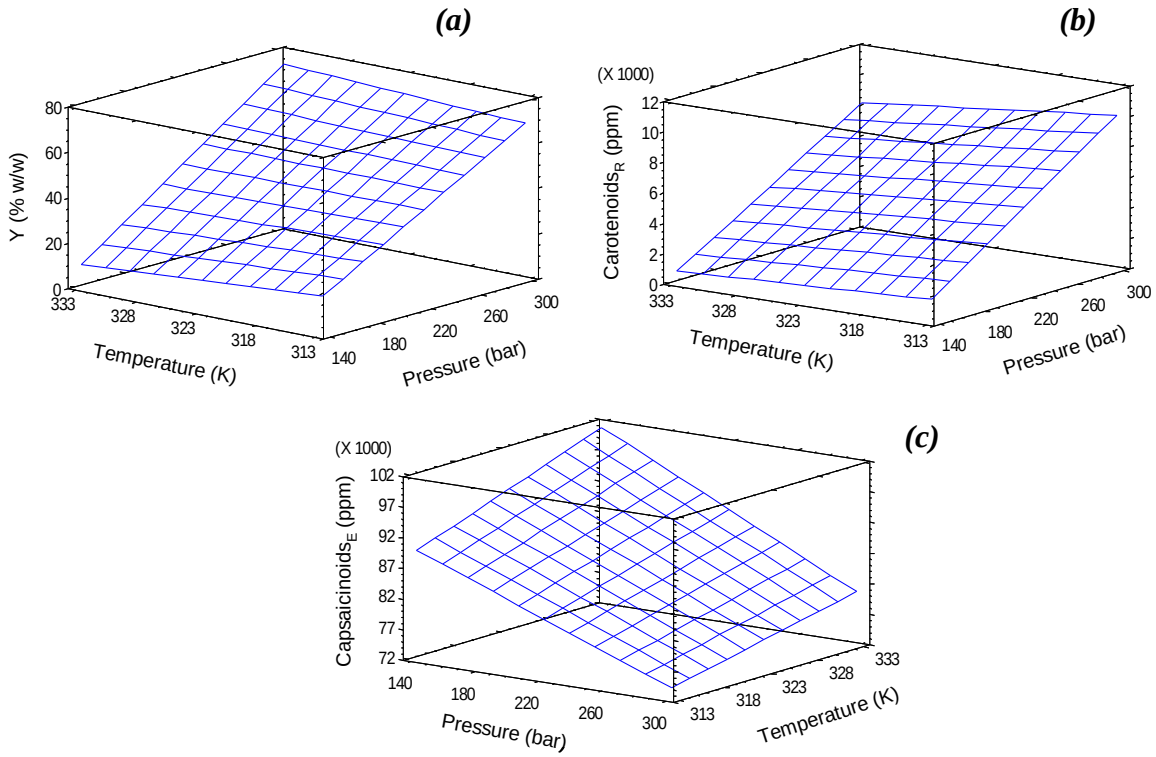

Figure 3. Response surfaces for extraction yield *(a)*, concentration of carotenoids in the raffinate *(b)* and concentration of capsaicinoids in the extract *(c)* as a function of pressure and temperature

The conclusion of the statistical analysis can be summed up saying that depends on the desired properties of the final products pressure and temperature should be selected. If low values of pressure and high values of temperature are used, a small amount of extract will be obtained but it will contain high concentration of capsaicinoids. On the other hand, when high pressures are used the solubility of oleoresin will increase and therefore the extraction yield increases too. As a consequence, the raffinate will be enriched in carotenoids pigments.

Solubility calculations

Additional information about solubility are required if economical analyses or scale up processes to determine the feasibility of the OR_{Cap} extraction using supercritical CO_2 are studied.

In this work, the solubility of OR_{Cap} in supercritical CO_2 at each condition was determined from the slopes of the linear parts of extraction curves [13] and can be seen at Table 2.

Table 2. Experimental solubility (S) of OR_{Cap} in supercritical CO_2

P (bar)	*T (K)*	ρ_{CO2} *(kg/m^3)*	*S (mg/g CO_2)*
140	313	763.27	1.72
300	313	909.89	8.10
220	323	806.61	4.73
140	333	561.37	1.13
300	333	829.71	9.07

According to the multicomponent character of OR_{Cap} there is a lack of information about its parameters which are requested for equilibrium estimations, such as its critical constants, so a theoretical modeling of solubility in dense fluids is difficult to obtain. For this reason, some semi-empirical models previously reported by other authors such as Chrastil [14], del Valle and Aguilera [15] and Adachi and Lu [16] were used to correlate the experimental results.

Figure 5 shows the comparison between experimental and theoretical solubility data. As can be seen all the semi-empirical models employed fit pretty well the experimental results but, according to deviations calculated the model of Adachi and Lu is the best of them. This is probably due to the fact that this model considered a strong dependency with density.

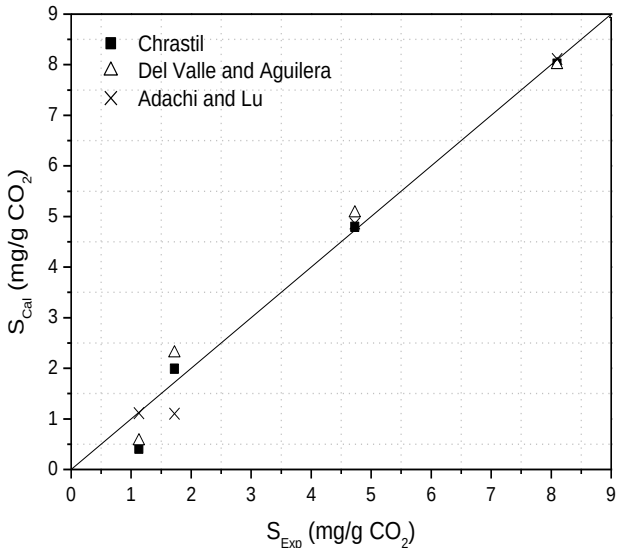

Figure 5. Comparison of experimental and correlating solubility data.

Equation (8) report the equation obtained to calculate the solubility of OR_{Cap} in CO_2 according to the model of Adachi and Lu. The standard deviation obtained by the use of this equation is 0.0780 mg/g $_{CO2}$.

$$lnS = -2.17 - \frac{466.28}{T} + \left(333.85 + 1039.90\rho - 813.29\rho^2\right)\ ln\rho \qquad (8)$$

CONCLUSION

The fractionation of different pigments present in oleoresin using supercritical carbon dioxide is possible but it depends on the conditions employed. The statistical analysis shows the dependency of extraction yield and concentration of capsaicinoids with pressure. As higher the pressure, higher the extraction yields reached and lower the concentration of capsaicinoids in the extract because of the effect of dilution which lipid matter causes. Moreover concentration of capsaicinoids in the extract is also affected by temperature. However concentration of carotenoids in the raffinate is independent of those variables, being always the raffinate enriched in carotenoids.

According to OR_{Cap} solubility in CO_2, solubility increases with an increase in pressure. An increase in temperature has not any significance effect on solubility when pressure is high because the variation in solvent density is small. Nevertheless, for low pressures an increase in temperature produces an increase in solvent density and therefore solubility enhances. Correlations of solubility using semi-empirical models fit fairly good experimental results.

ACKNOWLEDGMENTS

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