PHOSPHOLIPIDS RECOVERY FROM DEOILED SOYBEANS USING SUPERCRITICAL CARBON DIOXIDE EXTRACTION

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ABSTRACT

The main objective of this dissertation was to evaluate the extraction process of phospholipids after the de-oiling phase of soybeans. The extraction technique was carried out with a supercritical extraction device of the type Spe-ed SFE, Applied Separations, model 701. The qualitative and quantitative analysis of the phospholipids was done through high-performance liquid chromatography (HPLC) using an equipment Shimadzu, model SPD-10AVvp. The independent variables evaluated during the extraction process were: pressure (bar), temperature (^{o}C) and static time (min). The percentage of phospholipids extracted was used as a response variable. A 2^3 factorial design was carried out in order to determine the variables of influence during the extraction process and from that evaluation and the application of an experimental response surface, the most favorable conditions of the study were determined. The eminently good conditions for the extraction led to the following results: the highest proportion of phosphatidylethanolamine (PE) $0,3264 \pm 0,0001\%$ is obtained at a pressure of $206,2 \pm 0,1$bar, at a temperature of 121 ± 1 ^{o}C and at a static time of $24,6 \pm 0,1$ min; the highest proportion of phosphatidic acid (PA) $0,1736 \pm 0,0001$ % is obtained at a pressure of $86,2 \pm 0,1$ bar, at a temperature of $121 \pm 1^{o}C$ and at a static time of $19,2 \pm 0,1$ min; the highest proportion of phosphatidylcholine (PC) $0,03009 \pm 0,00001$ % is obtained at a pressure of $79,0 \pm 0,1$ bar , at a temperature of 121 ± 1 ^{o}C and at a static time of $10,0 \pm 0,1$ min.

INTRODUCTION

Phospholipids (PL) are polar conjugated lipids which have been the object of several studies at the National Center for Agricultural Research in Peoria, Illinois, (USA) showing how effective supercritical carbon dioxide (SC- CO_2) was for extracting oil from seed matrices

without their PLs content . SC-CO$_2$ can remove oil from soybeans but PLs are left behind and in other studies they have been made more soluble with the help of ethanol (1,2).

The objective of this project was to evaluate the factors which affect the SC- CO$_2$ extraction of PL present in de-oiled soybeans with added ethanol and to determine the optimal values of these factors.

The recovery of PLs is important because they have a wide range of applications, such as emulsifying agents, stabilizers, dispersing agents, homogenizing and solvents in the cosmetic, textile, pharmaceutical and paint industries and in the food industry. Another important role of PLs is in medicine as a dietary complement, due to the benefits to health because they are a mixture of fats, fatty acids and phosphorus essential components of life.

Supercritical fluids extraction has the advantage of producing a mixture of phospholipids free of solvent residue whose physical properties permit their use without further purification (3).

MATERIALS AND METHODS

Raw material preparation for extraction

Soy beans were supplied by the firm Coposa C.A. they were selected, washed and dried at room temperature, ground and sieved through a 1.5 mm mesh. The experimental extractions were performed with a commercial apparatus described in a previous paper (4).

The soybean oil SC-CO$_2$ extraction was carried out using the procedure suggested by Teberikler et al (5) who used a pressure of 400 bar, temperature of 70°C and a dynamic time of 6 hours for de-oiling of soybeans. A total of seven de-oiling extractions were carried out using samples of 40 g. of ground soybeans. After de-oiling, 5 g. of de-oiled soybeans and 4 g. of ethanol were used in each of the experiments of the study

Extraction with SC- CO$_2$.

To determine which were the most influential variables in the extraction of PL from de-oiled soybeans a 2^3 (2x2x2) factorial experimental design was selected. 2 means the level under study (low and high) and 3 the studied variables: pressure (200,300 bar); static time (0, 30min) and temperature (62,72°C). The response variable was the percentage of PLs extracted. This factorial design produced 8 combinations of treatments and to be able to estimate the associated experimental error the experiments were duplicated, giving 16 in all. Table 1 presents the matrix of experiments used.

Table 1 Factorial Design 2^3 Structure

Combined treatments	Natural Variables			Codified Variables		
	P (bar)	T (°C)	t (min)	P (bar)	T (°C)	t (min)
1	200	62	0	-	-	-
2	300	62	0	+	-	-
3	200	72	0	-	+	-
4	300	72	0	+	+	-
5	200	62	30	-	-	+
6	300	62	30	+	-	+
7	200	72	30	-	+	+
8	300	72	30	+	+	+

Once the most influential variables were known, a new experimental design was performed to determine the combination of variables to maximised production of the evaluated PLs, (PE,PA and PC).

Analysis of PLs.

The analysis were realized in an HPLC equipment, SHIMADZU SPD-10AVvp with an UV detector at a wavelength of 220nm, an oven CTO-10A, a pump LC-10AD and a manual injection valve of 20µL. To construct a calibration curve, standards of PE at 98%,PA at 98% and PC at 99% were used. The mobile phase was hexane / isopropilic alcohol, acetic acid (0.2M)/ sodium acetate (0.2M) buffer in a proportion 8:8:1 vol:vol:vol, elution was isocratic at room temperature and at a wavelength of 220nm and a flow rate of 0.5 and 2.0 mL/min.

RESULTS AND DISCUSSION

The analysis of variance produced the results presented in tables 2,3 and 4.

Tabla 2. Analysis of variance of factorial design 2^3 for fosfatidiletanolamine (PE).

Variation Source	Sum of squares	Degrees of freedom	Mean squares	F_0
A: Pressure	0,00438327	1	0,00438327	36,54
B: Temperature	0,0157926	1	0,0157926	131,65
C: Time	0,00489997	1	0,00489997	40,85
AB	0,00036006	1	0,00036006	3,00
AC	0,0011454	1	0,0011454	9,55
BC	0,00318308	1	0,00318308	26,54
ABC	0,0000014406	1	0,0000014406	0,01
Error	0,00095966	8	0,000119958	
Total	0,0307255	15		

Tabla 3. Analysis of variance for factorial design 2^3 for fosfatidiletanolamine (PA).

Variation Source	Sum of squares	Degrees of freedom	Mean squares	F_0
A: Pressure	0,00148009	1	0,00148009	29,76
B: Temperature	0,00258034	1	0,00258034	51,89
C: Time	0,0011541	1	0,0011541	23,21
AB	7,50822 x 10-7	1	7,50822 x 10-7	0,02
AC	0,000196967	1	0,000196967	3,96
BC	0,000602236	1	0,000602236	12,11
ABC	0,000063841	1	0,000063841	1,28
Error	0,000397826	8	0,0000497282	
Total	0,00647615	15		

Tabla 4. Analysis of variance for factorial design 2^3 for fosfatidiletanolamine (PC).

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F_0
A: Pressure	0,0000561226	1	0,0000561226	24,48
B: Temperature	0,0000993311	1	0,0000993311	43,32
C: Time	0,0000285904	1	0,0000285904	12,47
AB	0,0000134102	1	0,0000134102	5,85
AC	0,00000375778	1	0,00000375778	1,64
BC	0,0000118508	1	0,0000118508	5,17
ABC	0,00000241958	1	0,00000241958	1,06
Error	0,0000183432	8	0,0000022929	
Total	0,000233826	15		

The results of the analysis of variance for the PLs in this study show that pressure, time and temperature have Fo values higher than the Fo (0.01,1,8) (theoretical) of 11.26 which indicates that all the evaluated factors have a statistically significant influence on the
SC- CO_2 process of de-oiled soybeans in the presence of a cosolvent. One these variables are known, the next step was optimizing the conditions to get the best response in the extraction of the evaluated PLs. To achieve this objective another experimental design was used; type 2^{3+star} in such a way the number of levels was increased, adding two central points and two axial points., giving 16 combinations of treatments, shown in Table5.

Tabla 5. Structure of the experimental design2$^{3\ +star}$ used.

Combination of treatments	Natural Variables			Codified Variables		
	P (bar)	T (°C)	t (min)	P (bar)	T (°C)	t (min)
1	250	36	50	0	0	1.68179
2	250	104	15	0	-1.68179	0
3	250	121	15	0	1.68179	0
4	200	72	0	-1	1	-1
5	300	62	0	1	-1	-1
6	300	72	30	1	1	1
7	336	36	15	-1.68179	0	0
8	250	36	0	0	0	-1.68179
9	300	72	0	1	1	-1
10	200	62	0	-1	-1	-1
11	250	36	15	0	0	0
12	504	36	15	1.68179	0	0
13	200	72	30	-1	1	1
14	250	36	15	0	0	0
15	300	62	30	1	-1	1
16	200	62	30	-1	-1	1

With these experimental results, the mathematical models to explain the effect of each of the variables on the percentage of extraction for each of the PLs studied, were determined

Model of PE:
PE = 0,286274 + (0,00980642 * pressure) + (0,0223476 * temperature + 0,0153225 * time) - (0,0786199 * pressure^2) + (0,00495625 * pressure * temperature) - (0,00886575 * pressure * time) - (0,00515564 * temperature^2) + (0,0141875 * temperature * time - 0,0228885 * time^2).

Model of PA:
PA = 0,16268 + (0,00695376 * pressure) + (0,00940447 * temperature) + (0,00930242 * time) - (0,0492377 * pressure^2) - (0,0016795 * pressure * temperature) - (0,003261 * pressure * time) - (0,00389207 * temperature^2) + (0,00573275 * temperature * time) - (0,0147218 * time^2).

Model of PC:
PC = 0,0250739 + (0,0012855 * pressure) + (0,00155952 * temperature) + (0,000961567 * time) - (0,00685551 * pressure ^{2}) + (0,00140063 * pressure * temperature) - (0,000203875 * pressure * time) + (0,000584693 * temperatura^2) + (0,000388875 * temperature * time) - (0,00234946 * time^2).

With these mathematical models representing the obtained response surfaces and which allows the expression of the percentages of the extracted PLs as a function of the most important factors in the process (pressure, temperature and time) the optimal conditions for the

extraction of the PLs were found:1. For PE a response of 0,3264±0,001 % at a pressure of (206,2±0,1) bar, a temperature of (121±1) and a time of (24,6 ±0,1)min.2. For PA a response of (0,1736± 0,0001)% at a pressure of (86,2±0,1) bar, a temperature of (121±1) °C and a time of (19,2±0,1)min.3.For PC, a response of 0,03% at a pressure of (79,0±0,1) bar, a temperature of (121±1) °C and a time of (10,0±0,1).

REFERENCES
1.Montanari,L;King,J.W;List,G.R;Rennick,J.A Selective Extraction and Fractionation of Natural Phospholipids Mixtures by Supercritical CO_2 and Cosolvent Mixtures. Proceedings of the Third International Symposium on Supercritical Fluids,vol.2,1994,p.p.497
2.Dunford, N. and Temelli, F. (1994) Extraction of Canola Phospholipids with Supercritical Carbon Dioxide and Ethanol. Proceedings of the Third International Symposium on Supercritical Fluids, tome 2,p.p471.
3.Marquina-Chidsey,G; Grosse P.,R; Figueroa, Y. and Martinez, J.L. (2000) Supercritical Carbon Dioxide Extraction and Characterization of Murraya Paniculata Essential Oil . Proceedings of the VII Meeting on Supercritical Fluids,vol.2. p.p.667.
4. Teberikler,L; Koseaglu,S and Akgerman,A (2001) Deoiling of Crude lecithin using Supercritical Carbon Dioxide in the Presence of Co-solvents. Journal of Food Science,vol.66,#6.p.p 85