STUDY OF THE YIELD AND FATTY ACID PROFILE OF COFFEE (*Coffea arabica*) OIL FROM ROASTED BEANS OBTAINED WITH SUPERCRITICAL CARBON DIOXIDE

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Abstract. The extraction of coffee oil from roasted beans with supercritical carbon dioxide under different conditions of pressure and temperature was studied to measure the influence of these parameters on the yield and fatty acid content in the oil. For this purpose a 2^2 factorial experimental design using the response surface methodology was realized. The extraction was performed in a pressure range from 150 to 300 bar and temperatures between 40 and 60 ° C with a constant CO_2 flow of 70 $g.min^{-1}$ and extraction time of 2 hours. The yield was expressed in percentage as g aceite/100 g coffee bean and the fatty acids were analyzed by Gas Chromatography GC-FID and Kovats index. It was found that the pressure, temperature and temperature-pressure interaction had statistically significant effect ($P \leq 0.05$) on the yield and the optimal extraction conditions were 331,06 bar and 35,86 ° C, where the yield achieved 8.89%. The fatty acids identified were palmitic, linoleic, oleic, stearic, arachidic and linolenic acid, being the palmitic and linoleic the major fatty acids with an average relative percentage of 46.07% and 32.89% respectively. The influence of the temperature and the extraction pressure were statistically significant for linoleic acid, achieving a maximum content of 37.75% in the oil under similar conditions to optimal yield (331,07 bar and 35,07°C). Only the temperature had a statistically significant effect on the content of palmitic acid. The compositional feature of the coffee oil obtained makes it special to be used in the food, pharmaceutical and cosmetic industry.

Keywords: Supercritical fluid extraction, Coffee oil, Response surface methodology.

1. Introduction

Coffee (*Coffea arabica*) is one of the most agricultural products consumed in the world, from which a popular beverage is prepared for its particular aroma, taste and composition [1]. In recent years, Colombia has positioned third worldwide in coffee production, is renown as the world's largest producer of mild coffee and especially, coffee from Nariño (Colombia) is internationally recognized for its high quality in aroma and flavor. Despite this, Colombia hasn't been unable to become an exporter of coffee with a higher level of processing. Meanwhile, international market demands more and more processed coffee products with high added value and especially of commercial concentrates of aroma and flavor of roasted coffee, whose market value rises every day, due to its increasing use in so-called fancy food [2].

Coffee oil is considered as an important vehicle to concentrate aroma and flavor of roasted coffee [3]. The coffee bean roasting significantly reduces levels of diterpene, increasing its stability and sensorial profile [4].

According to Martín *et al.,* [3] the coffee oil composition, specifically the fatty acids content can be considered as chemical descriptor to differentiate between coffee varieties. Studies carried out in obtaining oil from roasted coffee with different extraction methods, reported the presence of saturated and unsaturated fatty acids, mainly palmitic acid, stearic, oleic, linoleic, linolenic and arachidic [5, 6].

Generally, the use of oils depends on the constituents that compose them. The components presence or absence depends on characteristics such as: geobotany conditions (soil, light, humidity and altitude), cultivation method, harvest time, plant material storage, oil extraction method (distillation, maceration, pressing, solvent extraction, solvent type, etc) [7].

Among different techniques to obtain coffee oil, there is the extraction with organic solvents, which leaves an inherent residue on the oil, unacceptable in products for human consumption [8]. The roasted coffee extrusion allows to obtain a fixed oil with high volatile load [9], although Reglero [2] refers that coffee oil obtained by this method, has a lower antioxidants content than the oil obtained with supercritical fluids. This technique is characterized by being a flexible process since it is possible to optimize the extraction selectivity varying fluid density, allows almost complete absence of waste, low energy consumption, the possibility of thermolabile compounds extraction and easy disposal of the utilized fluid [6, 10].

Supercritical fluid extraction has proved to be an excellent technique to obtain extracts with functional activity based on different sources [8, 10], is also used to coffee decaffeination, extracting coffee oil diterpenes [5] and obtaining extracts rich in coffee aroma [2, 11].

In this context, the study was carried out in order to determine the pressure and temperature effect of extraction with supercritical carbon dioxide (CO_2-SC.) on yield and composition of fatty acids present in roasted coffee oil, as an agroindustrial use alternative of the special organoleptic characteristics of coffee from Nariño (Colombia) for the oil obtention which can be used in food industries such as soluble coffee, confectionery, milk products, liquor, beverages and pastry making among others, besides cosmetic and pharmaceutical industry.

2. Materials y Methods

2.1 Coffee sample

The roasted and ground coffee used in this study was supplied by Empresas de Nariño Ltda. Roasted coffee samples (*Coffea arabica*) were obtained from a mixture of beans variety Colombia, Caturra and Castilla, taken randomly from the north-west area of Nariño department with 11.5% of humidity, Coffee-excelso 1.5% UGQ mesh 14. The coffee beans were subjected to 200 °C, for 13 minutes of roasting time (half roasting) reaching an Agtron color classification / SCAA No. 45 (Middle dark). The cupping score obtained in this coffee was of 80 points.

2.2 Extraction Method

Oil extraction of roasted and ground coffee, was carried out using extraction technology with supercritical CO_2 on an equipment SFE-1000 F-2-BASE THAR, schematically shown in Fig. 2. The equipment consists of a regulation system and pressure control, temperature and CO_2 flow operated through the ICM software (Instrument Control Modules). The CO_2 was contained in cylinders with siphon of 25 kg with a 99,8% of purity.

The extraction of oil roasted and ground coffee with supercritical carbon dioxide was carried out in a pressure range of 150 to 300 bar and temperatures between 40 and 60°C using a constant CO_2 flow of 70 $g.min^{-1}$ and an extraction time of 2 hours. In each experiment was used a sample of roasted and ground coffee of 200 g of size. The extracts were stored in amber bottles at 4°C for further analysis. The yield was determined based on the coffee oil amount obtained per unit mass of the material (roasted and ground coffee) in percentage terms.

2.3 Quantification of variables

The weighing of samples, yield and humidity determination were carried out using an analytical balance (Mettler Toledo. New Classic mf ml204/01) of 3000 g with ± 0.0001 g. of accuracy.

2.4 Fatty acids determination by GC- FID gas chromatography

To determine the fatty acids present in coffee oil, samples were derivatized according to Couto *et al.*, methodology [6] with some modifications. A Methanol and hydrochloric acid mixture were used and were

subjected to a water bath at 50°C for 8 hours; the fatty acid methyl esters were separated with n-Hexane, HPLC grade and was carried out a gas chromatography (GC-FID) analysis.

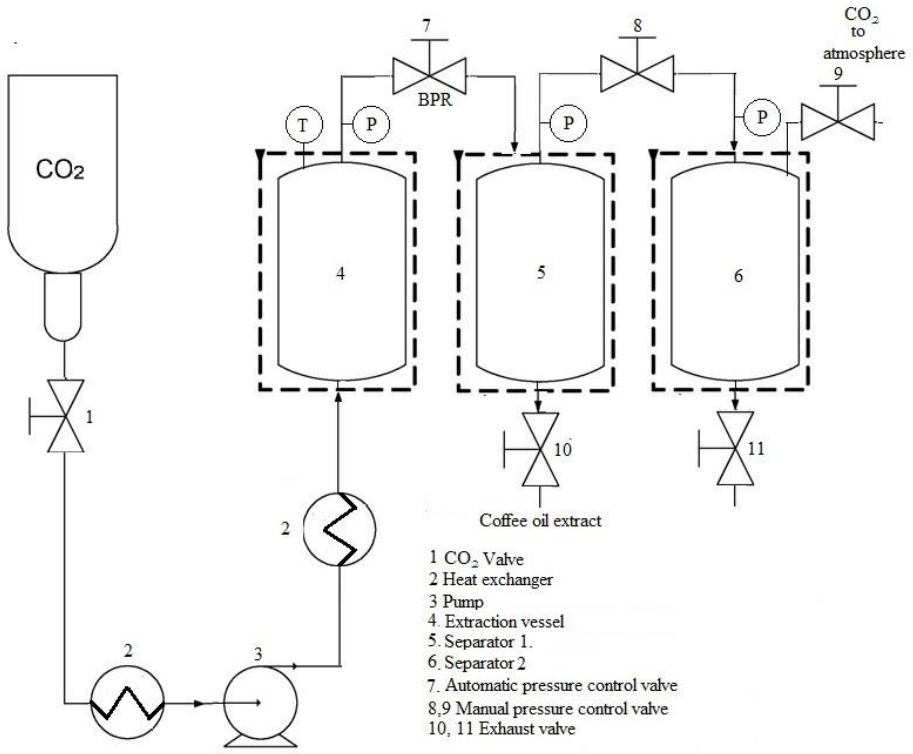

Figure 2. Process diagram of coffee oil extraction from roasted beans with CO_2-SC.

2.5 Analysis Conditions

The chromatographic analysis of the oils was carried out in the University of Nariño laboratories, at the main campus Torobajo Pasto (Nariño) at an altitude of 2.527 m.a.s.l., in a gas chromatograph Shimadzu GC 17A versión 3, equipped with a split/splitless injector, at 250°C of temperature, 1:10 split ratio and a flame ionization detector (FID) (280 °C). The chromatographic data were acquired and processed with the software Shimadzu Class VP 4.3. To the mixtures separation was used a capillary column DB-WAX (J&W Agilent Scientific. 30m*0.25mm I.D 0,25µm) of 30 m x 0,25 mm I.D. and 0,25 µm of a stationary phase of 5% phenyl-polymethylsiloxane. The oven temperature was programmed from 40°C (5 min) to 250°C at 5°C min^{-1}. The carrier gas and auxiliary gas employed was helium (99.99%, *Aga-Fano* S.A) with a flow rate of 1 ml min^{-1}, flow rates for combustion gases FID were 300 mL min^{-1} for air and 30 mL min^{-1} for hydrogen, the extract volume injected was 1,0 µL. Methyl esters patterns of Fatty acids were used to the respective comparison. The compounds identification was carried out using Kovats retencion index, using a set of n-alkanes (C_6-C_{32}) [12] and through the comparison of mass spectra obtained with the Wiley mass spectra library. Quantification was carried out through the calculation of the chromatographic relative percentage area.

2.6 Experimental design.

A factorial design of experiments using the response surface methodology (RSM) were applied, in order to determine the effect of the pressure and temperature of extraction over the response variables: yield and fatty acids present in coffee oil, using 4 factorial points, 4 star points and 4 central points for a total of 12 experiments. The experiments were carried out in duplicate and randomly. The experimental design matrix was carried out with the Statistical Software STATGRAPHICS Centurion XV.

The analysis of variance was carried out for the essential fatty acids with nutricional, pharmaceutical or cosmetic value.

3. Results and Discussion

3.1 Extraction Yield

Table 1 shows the experimental conditions of extraction of roasted and ground coffee oil with CO_2-SC pilot-scale, as well as the obtained yield for each experiment (%, oil g/ 100 g roasted coffee).

Table 1. Coffee oil yield based on roasted beans with CO_2-SC.

No.	Temperature (ºC)	Pressure (Bar)	Yield (p/p %)
			Experimental
1	40	150	1,62
2	40	300	7,38
3	60	150	1,79
4	60	300	3,49
5	50	119	0,59
6	50	331	4,62
7	36	225	5,32
8	64	225	2,28
9	50	225	4,36
10	50	225	3,60
11	50	225	3,37
12	50	225	3,28

The statistical significance of factors on the response variable was carried out using an analysis of variance, as ANOVA (table 2) indicates. Three factors were statistically significant ($P \leq 0,05$) on yield; pressure (A), temperature (B) and the pressure-temperature interaction (AB), other interactions did not show significant effects on the evaluated levels of the response variable. Behavior also observed by Couto *et al.*, [6] on oil extraction with CO_2-SC based on coffee residues.

Table 2. ANOVA coffee oil extraction yield based on roasted beans with CO_2-SC.

Source	Sum of Squares	Gl	Mean Square	F- Ratio	P-Value
A:Pressure	21,6458	1	21,6458	91,37	0,0025
B:Temperature	8,03846	1	8,03846	33,93	0,0103
AA	1,1937	1	1,1937	5,04	0,1123
AB	4,1209	1	4,1209	17,39	0,0256
BB	0,175564	1	0,175564	0,74	0,4558
Lack of fit	0,699564	3	0,233188	0,97	0,5100
Total Error	1,42144	3	0,240625		
Total (corr.)	36,8437	11			

On the main effects diagram on yield (Figure 2), due to the slope inclination is seen that the pressure has a greater influence on the yield in the evaluated range and being a positive slope, shows a direct ratio between pressure and yield, result of density increased of solvent, this translates into a solubility increased of CO_2-SC on the oil [13, 14, 15].

On the other hand, temperature, besides of showing a minor influence over yield, provides an inverse ratio (negative slope) with regard to yield. It was observed that when the temperature value increases this one actually decreases, because increases of this variable may decrease the CO_2 density and thus its solvating capacity [6, 7].

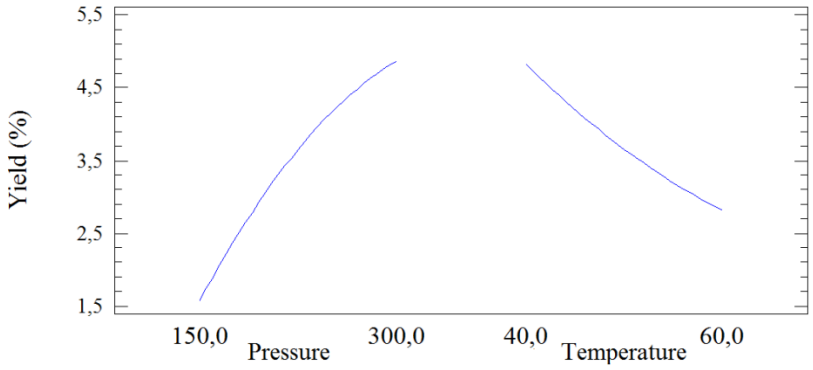

Figure 2. Main effect diagram for yield on the roasted coffee oil extraction with CO_2-SC**.**

Response surface analysis of Figure 3, indicates that optimun pressure and temperature are presented to 331,06 bar and 35,86 °C respectively, with which is obtained 8,89% of yield; these values are verified on the optimal response table (table 3), where the optimal points produced by the statistical program to the extraction process in the studied experimental range are shown. Similar results by other authors were obtained [2], under conditions close to those used in this study. Couto *et al*., [6] showed a 12,9 % of yield on roasted coffee residues at 50 °C and 200 bar for 3 hours of extraction. This difference respect our result may be due to the increase of extraction time.

In contrast to other techniques used to extract roasted coffee oil such as the soxhlet extraction, have been obtained yields between 6,10 and 9,83% to a light and dark roast level, respectively, whereof is estimated that the oil amount also depends on the roasting temperature and the time spent, this is the stage at which by chemical reactions within the grain, volatile components are developed [4]. Couto *et al.*, obtained 18.3% of yield from roasted coffee residues [6]. Even though it seems that this technique offers a better yield, oils with solvent traces were obtained and also requires long extraction times (8-16 h). López realized the roasted coffee oil extraction by extrusion method, obtaining 8.78% of yield [9]; Ramírez obtained 6,02 and 7,05% of yield for light and dark roasted coffee, respectively [5]. Regarding this technique, the CO_2–SC use shows differents advantages, because generally the supercritical extraction allows the obtention of high purity oils and low temperatures use without chemical changes of the extracted components.

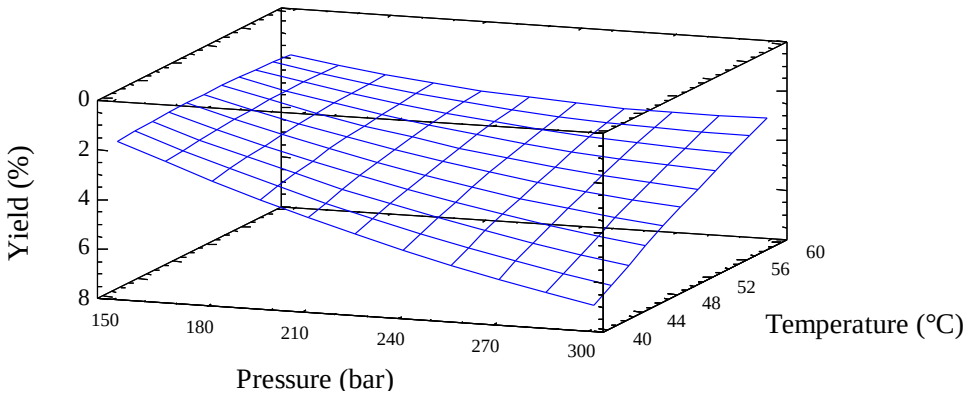

Figure 3. Response surface on yield of coffee oil extraction from roasted grain with CO_2-SC

Table 3. Yield optimal response on coffee oil extraction from roasted grain with CO_2-SC

Factor	Low	High	Optimal
Pressure (bar)	118,93	331,06	331,06
Temperature (°C)	35,86	64,14	35,86

3.2 Analysis of fatty acids profile

Table 4 shows the fatty acid profile of roasted coffee oil extracted with CO_2-SC under different conditions of pressure and temperature. Main fatty acids presents in all samples were palmitic acid (C16: 0) with an average percentage of 46.07%, linoleic (C18: 2) with 32.89%, followed by oleic acid (C18: 1) with 8.02%, stearic acid (C18: 0) with 6.61% and other fatty acids on minor proportion as arachidic acid (C20: 0) with 1.89% and linolenic (C18: 3) with 1.23%. Similar results were obtained on roasted coffee oil and coffee residues extracted with CO_2-SC [5, 6] and roasted coffee oil obtained by pressing [4, 11]. In addition, it was found that the coffee oil obtained has a 52.68% of saturated fatty acids, 8,02% of monoinsaturados fatty acids and 36,01% of polyunsaturated fatty acids.

Table 4. Fatty acids in roasted coffee oil extracted with CO_2-SC

Pressure (Bar)	Temperature (°C)	Chromatographic Area %					
		C16:0	C18:0	C18:1	C18:2	C18:3	C20:0
150	40	46,03	6,27	8,66	33,45	1,19	2,13
300	40	44,59	6,52	7,81	35,74	1,09	2,38
150	60	43,37	9,85	9,02	29,45	0	0
300	60	47,51	6,4	7,99	33,81	1,29	2,13
119	50	46,22	6,67	9,28	33,17	1,20	1,80
331	50	44,92	7,02	8,30	34,95	1,30	2,22
225	36	40,20	7,06	7,65	37,75	1,43	2,09
225	64	50,42	6,65	4,30	34,60	1,07	2,10
225	50	46,39	6,72	6,44	32,11	1,22	1,96
225	50	44,39	6,61	7,95	33,91	0,98	2,03
225	50	45,39	6,49	9,69	33,00	1,06	1,89
225	50	48,12	6,62	8,00	32,55	1,65	1,70

Analysis of variance (Table 5) clearly shows that factors: pressure (A), temperature (B) and temperature quadratic effect (BB), had a statistically significant incidence ($P \leq 0,05$) over the linoleic acid content on the roasted coffee oil at the evaluated levels.

Table 5. ANOVA for relative percentage of linolenic acid present on roasted coffee oil.

Source	Sum of Squares	Gl	Mean Square	F- Ratio	P-Value
A: Pressure	10,3929	1	10,3929	17,55	0,0248
B:Temperature	13,608	1	13,608	22,98	0,0173
AA	0,0468516	1	0,0468516	0,08	0,7968
AB	1,12254	1	1,12254	1,90	0,2623
BB	8,36214	1	8,36214	14,12	0,0329
Lack of fit	10,3171	3	3,43904	5,81	0,0912
Total Error	1,77647	3	0,592158		
Total (corr.)	45,7156	11			

Influence of pressure and temperature factors, individually, respect to relative percentage of linoleic acid present on coffee oil is observed in the main effects diagram (Figure 4), where temperature is the factor that exerts a greater influence, decreasing this percentage when the temperature is increased from 40 to 50°C where there is a lineal ratio, while at raising the pressure, the linoleic acid percentage rose only slightly.

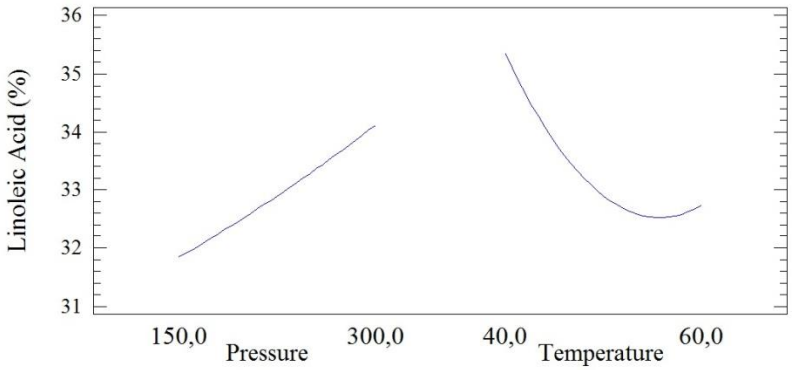

Figure 4. Main effects diagram for relative percentage of linoleic acid.

The response surface diagram (Figure 5) for linoleic acid, indicates that the optimum extraction conditions are presented at 331,07 bar and 35,07°C, with which a 37.75% is obtained and are similar to the supercritical extraction conditions, with which the highest oil yield are obtained. The linoleic acid content on coffee oil, gives a special compositional characteristic, since this is an essential polyunsaturated fatty acid and therefore essential in human nutrition because of its participation in the prostaglandin synthesis and other biological processes related to cell regeneration and its absence may cause dermatological disorders [16]. A daily dose between of 3 and 4 g aproximately, appears to have the most beneficial results [17]. Linoleic acid is useful in dry skin treatments, therefore oils rich in this fatty acid and others as palmitic, stearic and oleic acids are an excellent natural raw material for cosmetics [18, 19]. According to values reported by Restrepo and Vinasco, the linoleic acid content obtained of coffee oil is comparable to the present in vegetable sources such as cherimoya and soursop seeds and lower than the one found in corn oil, soybean and sacha inchi [20].

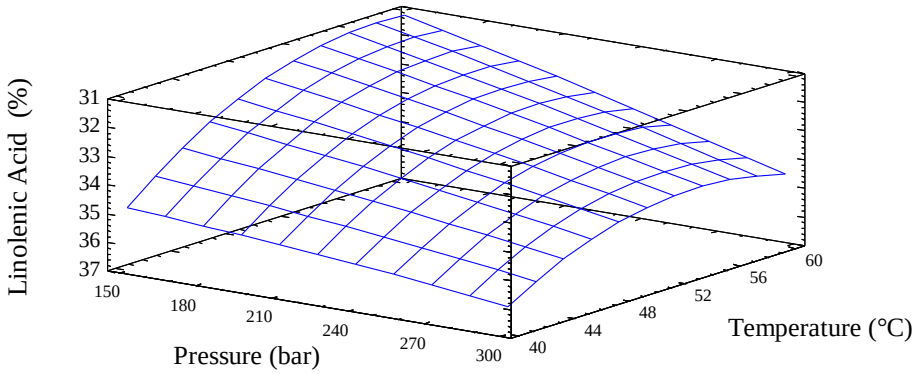

Figure 5. Response surface for relative percentage of linoleic acid on roasted coffee oil.

Moreover, the only one that had a statistically significant effect was the temperature (B) ($P \leq 0,05$) on the palmitic acid content, as shown on the analysis of variance (Table 6).

Table 6. ANOVA for relative percentage of palmitic acid present in roasted coffee oil.

Source	Sum of Squares	Gl	Mean Square	F- Ratio	P-Value
A: PRESSURE	0,0934249	1	0,0934249	0,04	0,8599
B:TEMPERATURE	27,071	1	27,071	10,70	0,0467
AA	0,458601	1	0,458601	0,18	0,6990
AB	7,77573	1	7,77573	3,07	0,1779
BB	1,01219	1	1,01219	0,40	0,5720
Lack of fit	27,7573	3	9,25243	3,66	0,1576
Total Error	7,58967	3	2,52989		
Total (corr.)	71,5353	11			

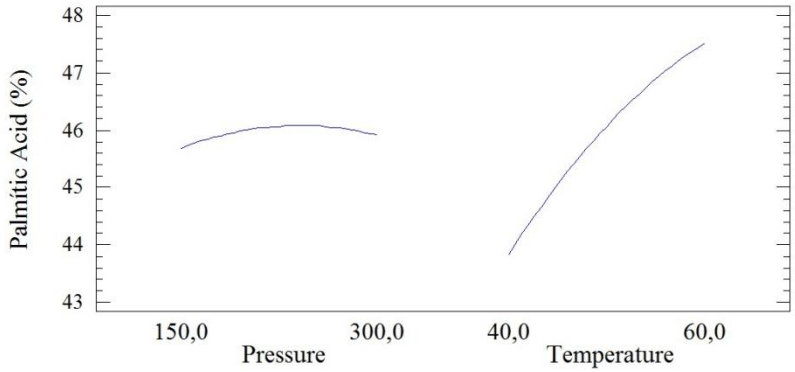

Figure 6. Main effects diagram for relative percentage of palmitic acid in roasted coffee oil.

Figure 6 shows that temperature is the most influential factor, presenting a positive and inclined slope, which indicates that when this factor increased, the relative percentage of palmitic acid on coffee oil also increases, opposite to what happens with the linoleic acid content, the relative percentage decreases when this variable increases (Figure 4), which indicates its temperature sensitivity.

Optimal extraction conditions where achieve a 50.28% of relative percentage of palmitic acid on coffee oil are: 331,07 bar and 64,14°C as the response surface diagram shows (Figure 7), which only differ in the optimal conditions obtained on variable temperature with regard to the yield of the linoleic acid content of the roasted coffee oil.

Palmitic acid is an abundant saturated acid in human diet by its presence in foods such as meat, butterfat and in palm and coconut oils. Its consumption is unhealthy because it increases blood cholesterol levels, but is a fatty acid widely used in a variety of cosmetics and hygiene products. In addition, studies have identified that this fatty acid may be responsible for antimicrobial activity of some extracts [10].

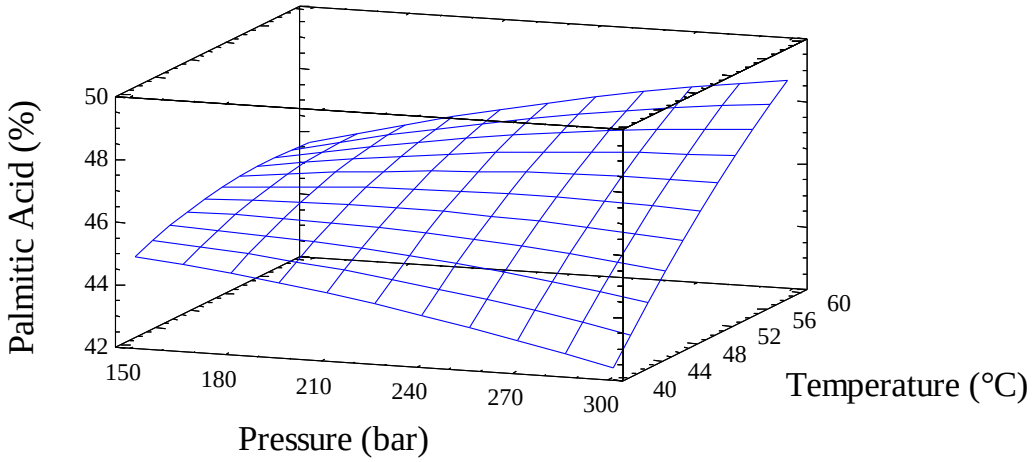

Figure 7. Response surface for relative percentage of palmitic acid on roasted coffee oil.

4. Conclusion

Pressure and temperature variables of supercritical extraction had a statistically significant effect on coffee oil yield, being 331,06 bar and 35,86°C the optimal conditions, with which it is possible to achieve 8.89% of yield. On the other hand were identified as main components on its lipid panel to the palmitic and linoleic acid, in addition to the other fatty acids presence such as: oleic acid, stearic acid, arachidic acid and linolenic acid. The influence of pressure and temperature extraction was statistically significant for linoleic acid, for which optimal conditions were similar to those identified with regard to yield, obtaining with them a 37.75% of yield. While temperature was the only factor which influenced over the palmitic acid content in the oil, thus obtaining under optimal extraction conditions of 331,07 bar and 64,14°C a 50,28% of this fatty acid.

The high linoleic acid content along with other acids of interest present in coffee oil, give a functional compositional characteristic, particularly to be used in food, pharmaceutical and cosmetic industry. For this reason it is established that the favorable conditions in terms of linoleic acid content and roasted coffee oil yield are 331,06 bar and 35,86°C.

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