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INVESTIGACIÓN

Extraction of oil from pequi fruit (*Caryocar Brasiliense,* Camb.) using several solvents and their mixtures

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RESUMEN

Extracción de aceite del fruto pequi (*Caryocar Brasiliense,* Camb.) utilizando diversos disolventes y sus mezclas

En este trabajo fue estudiado el proceso de extracción de aceite de la pulpa de pequi utilizando diferentes disolventes (n-hexano, acetona y etanol) y sus mezclas, empleando diseño central simplex. Las extracciones fueron realizadas a 50°C, durante 16 horas de agitación (22 Hz). La proporción sólido:líquido empleada fue 1:10 (p/p). Los mayores rendimientos fueron obtenidos para las extracciones con acetona y con hexano, especialmente cuando fueron mezclados con etanol. El índice de yodo, el índice de saponificación y el índice de refracción no difirieron significativamente entre los tratamientos. Los mayores valores de acidez se obtuvieron en la extracción con etanol. Los mayores contenidos en carotenoides se obtuvieron en las extracciones con acetona y etanol como disolventes puros. El perfil de los ácidos grasos en las fracciones de aceite de los extractos no presentó variación entre los diferentes tipos de disolventes y sus mezclas.

PALABRAS CLAVE: Extracción sólido-líquido – Extracto de Pequi – Regla de mezclas

SUMMARY

Extraction of oil from pequi fruit (*Caryocar Brasiliense*, Camb.) using several solvents and their mixtures

In this study, the oil extraction process from pequi pulp using different solvents (hexane, acetone and ethyl alcohol) and their mixtures was investigated, using a simplex-centroid design. The extraction occurred at 50°C, under stirring (22 Hz), for 16 hours. The solid-liquid ratio used was 1:10 (w/w). Higher yield values were obtained for extractions with acetone and hexane, especially their mixtures with ethanol. Iodine value, saponification value and refractive index did not differ significantly among the treatments. A higher acid value was obtained for the extraction with ethyl alcohol. Higher carotenoid contents were obtained for the extraction with acetone and ethyl alcohol as pure solvents. The fatty acid profile in the oil fraction of the extracts did not vary among the different types of solvents and their mixtures.

KEY-WORDS: Mixing rule – Pequi extract – Solid-liquid extraction.

1. INTRODUCTION

Pequi (*Caryocar brasiliense*) is an oil-rich fruit cultivated in the Brazilian *cerrado* savanna and despite being little explored by the processing industry, it has many applications in the food, medicine and cosmetic industries as it is particularly rich in carotenoids and oleic and palmitic fatty acids (Silva *et al*., 1993; Facioli and Gonçalves, 1998; Azevedo-Meleiro and Rodriguez-Amaya, 2004; Lima *et al*., 2007).

Organic solvents are widely used as a technique to extract oil (solid-liquid extraction), as authorized by the Committee on Food Chemicals Codex (1996), including acetone, ethanol and hexane; and they must be eliminated in the final stage of processing.

Water can also be used for the extraction of pequi oil and indeed it is used in small-scale production (Facioli and Gonçalves, 1998). However, it produces a low yield besides the high risk of microbiological contamination and the use of high temperatures that can degrade compounds of nutraceutical interest, including carotenoids (Abu-Arabi *et al*., 2000).

Hexane is the most commonly used solvent in solid-liquid extraction, especially in the vegetal oil industry, due to its strong affinity with oils and fats and to its easy recovery (low vaporization temperature). However, other solvents, especially polar solvents, have been investigated as potential substitutes for hexane, a petroleum by-product that can potentially cause cancer. Studies have been performed by various authors (Kim and Yoon, 1990; Abu-Arabi *et al*., 2000; Lalas and Tsaknis, 2002;

Dunford and Zhang, 2003; Abdulkarim *et al.*, 2005) on the effect of extraction using different solvents and their mixtures on the yield and quality of oil, obtaining extracts with different components other than fatty acids, such as antioxidants, pigments, proteins and others. Different compositions may or may not affect the characteristics of the lipid extract (Moreno *et al.*, 2003, Dunford and Zhang, 2003).

The objective of this work is to evaluate the effect of using different types of solvent (hexane, acetone and ethyl alcohol) and their mixtures on the yield of pequi extract, physico-chemical characteristics, fatty acid composition and the total carotenoid content of the pequi extract.

2. MATERIALS AND METHODS

2.1. Materials

The pequi samples (*Caryocar brasiliense* Camb.) used in this study were obtained in the municipality of Cordisburgo, Minas Gerais state, during the harvest season of January 2006. Only undamaged ripe samples were selected.

2.2. Sample preparation

The pequi samples were washed under running water and immersed in a solution of sodium hypochlorite at 50 mg/kg for five minutes, then peeled. They were pulped manually with a stainless steel knife, then cut into slivers 0.3 cm thick, 0.5 cm wide and 1.5 cm long and frozen in plastic bags. The frozen pulp was dried in a Fanem incubator at 40°C for 19 hours according to previously optimized results (Aquino *et al.*, 2006). Next it was crushed in a Britania multiprocessor for 1 minute and the granulometry was determined using Granutest Tyler sieves 9 (2.00 mm), 20 (0.85 mm) and 60 (0.25 mm), for 10 minutes. The experiment used samples presenting Tyler granulometry of between 9 and 20.

2.3. Experimental design

The experimental design was entirely randomized, with two replicates for each variable studied, except for yield, which used six replicates. The treatments were based on the mixing rule using a simplex-centroid design for three components (Cornell, 2002). The treatments are specified in Table 1.

The extracts obtained from different treatments were confronted with a control sample obtained at the central market of Belo Horizonte (Minas Gerais state, Brazil), with the determination of acid value, refractive index, total carotenoid content and fatty acid composition being verified if the values of the control sample were inside of the limits of the values obtained for the treatments with organic solvents and mixtures. The iodine value and saponification value were calculated based on the fatty acid composition. The extraction of oil from the control sample used hot water as solvent.

Table 1
Statistical design of the mixtures with the levels of variables used in the simplex-centroid design

Test	Proportion[a]		
	X_1	X_2	X_3
T1	1	0	0
T2	0	1	0
T3	0	0	1
T4	1/2	1/2	0
T5	1/2	0	1/2
T6	0	1/2	1/2
T7	1/3	1/3	1/3

[a] X_1: mass fraction of hexane; X_2: mass fraction of acetone; X_3: mass fraction of ethyl alcohol.

2.4. Extraction of oil

Extraction was performed in a Marconi (MA830/A) cooling incubator-shaker. The pequi sample and solvent were placed in a previously tared 250 mL Pyrex® flask (N:4100J24/40) with a ground-glass cap. During the extraction process, the controlled variables were temperature (50°C), stirring (22 Hz), time (16 hours) and solid-liquid ratio at 1:10 (w/w). The time to achieve a practical equilibrium was 16 hours according to preliminary tests. A temperature of 50°C was selected for the extraction process as it is lower than the usual boiling temperatures of solvents (acetone, hexane and ethyl alcohol) used in this extraction stage.

After the extraction, the solution was vacuum filtered by a Primar pump (model 141, n.284, Type 2VC, air displacement of 37 L/min) and the extract was collected into a previously tared 250 mL Pyrex® beaker and transferred to a vacuum oven at 65°C until the solvent evaporated (constant weight). The solute mass (oil) was determined by gravimetry and the yield was calculated based on the ratio between the amount of extract obtained and the sample initially placed in the extractor (dry basis).

2.5. Physico-chemical analysis

The AOCS (1997) official method was used for the determination of the acidity of the lipid extracts (method Ca 5a 40). The refractive index (RI) was determined using an Abbé refractometer, according to the official method Cc 7-25 (AOCS, 1997).

Analysis of fatty acid composition

The fatty acid composition was determined by gas chromatography after derivatization to methyl esters. Methyl esters were prepared according to Hartman and Lago (1973) and analyzed by high-resolution gas chromatography using an HP-5890 chromatograph with a cyanopropyl siloxane fused

silica capillary column (60m × 0.32mm × 0.25 μm), with a programmed temperature of 150°C to 200°C, set to rise 1.3°C/min. Identification was made through comparison of the retention times with Nu-Chek Inc. standards (Elysian, IL) and quantification used internal normalization. Based on fatty acid composition, the iodine value and saponification value were calculated according to official methods Cd 1c 85 and Cd 3a 94 (AOCS, 2004).

Carotenoid content

The total carotenoid content was analyzed by reading the samples, in the 452 nm visible spectrum, using spectrophotometric grade hexane as solvent. The calculation of total carotenoids was based on the extinction value of 2500, as suggested by Davies (1976).

2.6. Statistical analysis

The response variables, oil yield, iodine value (IV), saponification value (SV), acidity, refractive index (RI) and carotenoid content, of each experiment were analyzed using the program Statistica (StatSoft Inc., 1999), with mixing rule analysis using a simplex-centroid design. An analysis of variance was applied to test the fit of the models. The validation of the models considered the significance of regression and lack of fit in relation to 90% confidence, and by the coefficient of determination R^2, an important evaluator of the predictive power of the models. In order to determine the effect of the independent variables on the responses evaluated, we constructed contour line graphs defined in the experimental area. The proposed model is thus illustrated in equation 1

$$Y = a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 + a_{123}X_1X_2X_3 \quad (1)$$

where Y is the response variable for each type of treatment, X_1 is the mass fraction of hexane, X_2, of acetone, X_3, of ethyl alcohol, and a_i (i=1, 2, 3), a_{ij} (j=1,2,3 and i<j) and a_{ijk} (k=1,2,3 and i<j<k) are the coefficients of regression. The coefficients a_i (a_1, a_2 e a_3) represent the expected response to extraction with pure solvent. The second-order coefficients a_{ij} describe the binary interactions between solvent pairs in the extraction process. A significant positive term of a_{ij} in this model indicates that a solvent binary mixture extracts an excess of material than would be expected by simply averaging to value using each solvent separately. This excess is called synergism. The opposite of synergism (a_{ij} is negative) is called antagonism of the binary mixture. The coefficients a_{ijk} will be important if ternary interactions among mixture solvents are important for the extraction process (Cornell, 2002; Garcia *et al.*, 2010).

3. RESULTS AND DISCUSSION

Table 2 provides the coefficients of the quadratic equation Q, (yield, iodine value, acidity, refractive index and carotenoid content) and cubic equation C, (saponification value) of the experimental results for a simplex-centroid design, fitted to the model with the best fit.

Table 2
Results of fitted models for response variables: yield, iodine value (IV), acidity, refractive index (RI), saponification value (SV) and carotenoid content[a]

	Yield	IV	SV	Acidity	RI	CC
Model	Q	Q	C	Q	Q	Q
R^2 (%)	69.41	62.27	46.59	67.19	29.23	66.42
Significance of regression	S	NS	NS	S	NS	S
a_1	60.17 ± 2.0	49.19 ± 0.49	197.49 ± 0.29	1.54 ± 0.97	1.458 ± 0.001	199 ± 16
a_2	61.07 ± 2.30	50.15 ± 0.49	197.20 ± 0.29	1.75 ± 0.97	1.459 ± 0.001	300 ± 16
a_3	39.78 ± 2.30	51.34 ± 0.49	197.22 ± 0.29	4.00 ± 0.97	1.457 ± 0.001	296 ± 16
a_{12}	−29.98 ± 10.61	0.39 ± 2.23 (NS)	1.03 ± 1.42 (NS)	4.00 ± 4.45 (NS)	−0.0034 ± .0046 (NS)	50 ± 74 (NS)
a_{13}	47.29 ± 10.61	−2.01 ± 2.23 (NS)	0.16 ± 1.42 (NS)	−0.77 ± 4.45 (NS)	−0.0014 ± 0.0046 (NS)	50 ± 74 (NS)
a_{23}	53.75* ± 10.61	−5.41 ± 2.23	1.15 ± 1.42 (NS)	−3.17 ± 4.45 (NS)	−0.0034 ± 0.0046 (NS)	−151 ± 74 (NS)
a_{123}	–	–	−21.78 ± 9.97 (NS)	–	–	–

[a] Q: quadratic model; C: cubic model; S: significant at 10% probability level; NS: not significant.

The results in Table 2 indicate that the regression was significant only for the variables' yield, carotenoid content and acidity. Hence the construction of contour lines for these variables only, given that the significance of these models led us to reject the hypothesis that the coefficients are significantly different from zero. The statistically insignificant terms were kept in the model because the exclusion of these terms reduces the value of coefficient determination R^2. The analysis of variance for yield and carotenoid content is illustrated in Table 3, where it can be noted that the quadratic model fit was significant ($P<0.1$) and the lack of fit was not significant ($P>0.1$), explaining 69.41% and 66.42% of variability in yield and carotenoid content, respectively. The analysis of variance for acidity (Table 4) indicates that the fitted model was significant ($P<0.1$), the lack of fit was not significant and explains 67.19% of variability.

Figure 1 provides contour lines for yield, obtained from the quadratic model fit.

For treatments using pure solvents (Treatments T1, T2 and T3 – Table 1) the maximum yield was 61.07% with acetone (T2), followed by 60.17% with hexane (T1) while the lowest yield was obtained using ethyl alcohol (39.78% - T3). From the model equation we observed a synergic effect of the solvents mixed with ethyl alcohol, with higher yields noted for 1:1 ratios of acetone-ethyl alcohol (63.86% - T6) and hexane-ethyl alcohol (61.80% - T5). The extract obtained using the acetone-hexane mixture also produced a good yield (53.13% - T4), although we noted an antagonist interaction between hexane and acetone, since the value of a_{12} coefficient is negative (Table 2).

The yield values using hexane, acetone and their mixtures with ethanol at a 1:1 ratio were higher than those found by Lima *et al.* (2007) in the extraction of pequi oil using petroleum ether as solvent (57% db).

Franco *et al.* (2007) observed an increased solubility of rubiginosa rose oil in ethanol with a rise in temperature. According to the authors, although solubility increases with a rise in temperature, it will be higher in hexane, with a low solid: liquid ratio being required in the extraction with ethanol to obtain the same performance. Freitas and Lago (2007) reported that Freitas *et al.* (2001) had obtained higher yield values in the extraction of coffee and sunflower oil with ethanol at 70°C to 75°C (sample ratio for ethanol 1:3) in relation to the lipid fraction extracted using petroleum ether. According to these authors, the use of extraction temperatures above 70°C may have favored the removal of wax by ethanol. Chien *et al.* (1990) observed that the yield of corn oil in the extraction using ethanol increased with a rise in temperature. According to the above works and judging by the effect of temperature on the yield of extract using

Table 3
Analysis of variance of quadratic regression models for yield and carotenoid content

Source	Yield				Carotenoid Content			
	SS	DF	MS	F	SS	DF	MS	F
Regression	2473.249	5	494.6499	15.46[a]	9791.17	5	1958.233	3.76[a]
Residual	1087.707	34	31.9914		4169.39	8	521.174	
Lack of fit	11.858	1	11.8584	0.364 (NS)	1024.01	1	1024.006	2.279 (NS)
Pure error	1075.849	33	32.6015		3145.38	7	449.341	
Total	3560.957	39	91.3066		13960.56	13	1073.889	

[a] Significant at the 10% probability level.
NS: not significant.

Table 4
Analysis of variance of the quadratic regression model for acidity

Source	Acidity			
	SS	DF	MS	F
Regression	31.01233	5	6.202466	3.28
Residual	15.14325	8	1.892906	
Lack of fit	0.12229	1	0.122288	0.057 (NS)
Pure error	15.02096	7	2.145851	
Total	46.15558	13	3.550429	

* Significant at the 10% probability level.
NS: not significant.

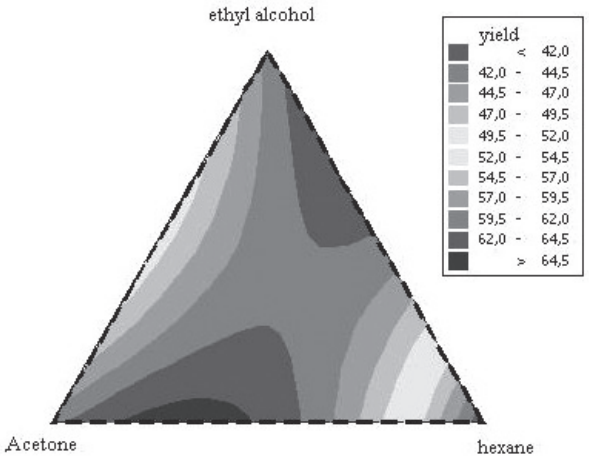

Figure 1
Contour line for extract yield (% dry basis) in different treatments.

ethanol, we expect to obtain a higher yield of pequi oil with ethanol if we raise the temperature to values near 70°C, yet under such condition the extract may present a different composition from that obtained in this work. That said, the use of ethanol as solvent in acetone and hexane mixtures is attractive to obtain pequi extract, due to the high expected yields, yet further studies should be done to investigate its feasibility as a pure solvent using higher temperatures. Franco *et al.* (2007) points to the advantage of using ethanol as a solvent in the extraction of oil due to it being a renewable fuel and due to the low solubility of oil in ethanol at low temperatures, facilitating the separation of oil from ethanol.

According to Lalas *et al.* (2002), higher yields of lipid extract were obtained from *Moringa Olifera* seeds using a mixture of polar solvents (1:1 chloroform-methanol) as opposed to the results with hexane. According to these authors, the extraction using polar solvents produced higher yields due to an increased ability of the polar solvent to overcome the forces that bind lipids within the sample matrix. Mani *et al.* (2007) studied the optimization of operating conditions (temperature, particle size, extraction time and solvent type) to extract oil from *Moringa Olifera* seed using hexane, petroleum ether and acetone as solvents. The extraction was performed using a Soxhlet apparatus, and the maximum yields were 33.1% for hexane, 31.8% for petroleum ether and 31.1% for acetone at a temperature of 60°C.

Higher yields of lipid extract were obtained by mixing polar and non-polar solvents to obtain oil from *Moringa Olifera* seeds (Lalas *et al*., 2002). In their study on the solid-liquid extraction of jojoba oil using different solvents, Abu-Arabi *et al.* (2000) observed higher yields for hexane, petroleum ether and benzene than for chloroform (polar solvent), which, according to them, was due to the chemical nature of the oil being extracted.

The iodine value (IV) reflects the fatty acid composition of oils and fats as it measures the amount of unsaturation present in the oil. The proposed quadratic model presented a low correlation coefficient, with the Iodine value not being affected by the use of different solvents (Table 2). Similar results were obtained by Lalas *et al.* (2002) for the extraction of oil from *Moringa Olifera* seeds using hexane, chloroform-methanol and cold pressing. This result was expected, since this analysis compares only the glyceride fraction present in the various extracts and it presents the same composition, regardless of the solvent used (Table 5). The iodine values were found to be between 49.19 and 51.34 g of iodine/100g of sample, which is close to the control sample results and results found by Facioli and Gonçalves (1998), that is, 50.69 and 50g of iodine/100g of sample, respectively.

The saponification value (SV) shows the relative amount of high and low molecular weight fatty acids,

Table 5
Fatty acid composition of pequi oil (%) in different treatments[a]

	Saturated fatty acids										Unsaturated fatty acids					
	C8:0	**C10:0**	**C12:0**	**C14:0**	**C16:0**	**C17:0**	**C18:0**	**C20:0**	**C22:0**	**C24:0**	**C16:1**	**C17:1**	**C18:1**	**C18:2**	**C18:3**	**C20:1**
T1	0.05	T	T	0.09	41.55	0.11	2.52	0.19	T	T	1.11	0.10	52.90	1.10	0.24	0.15
T2	0.04	Nd	T	0.08	40.48	0.10	2.58	0.20	T	T	1.07	Nd	54.09	1.11	0.21	0.16
T3	0.05	Nd	0.04	0.08	39.64	0.09	2.28	0.18	T	T	1.09	0.10	54.79	1.32	0.29	0.17
T4	T	Nd	T	0.18	40.87	0.10	2.52	0.20	T	T	1.10	Nd	53.50	1.13	0.27	0.17
T5	0.03	T	T	0.08	40.75	0.10	2.50	0.20	T	T	1.05	0.09	53.69	1.13	0.23	0.16
T6	0.05	T	T	0.09	41.41	0.10	2.41	0.20	T	T	1.05	0.09	53.17	1.12	0.26	0.17
T7	0.03	T	T	0.09	41.50	0.50	2.44	0.20	T	T	1.08	0.09	52.97	1.11	0.26	0.16
CS	0.04	T	T	0.10	40.71	T	1.73	0.16	T	T	0.96	0.09	54.70	1.11	0.25	0.22

[a] T= traces; Nd= not detected; CS= control sample.

being inversely proportionate to the molecular weight of fatty acids present in triacylglycerols. The proposed model for this index presented low variation among treatments using different solvents and their mixtures; and so the quadratic fit was poor, leading us to conclude that the saponification value was not affected by the use of different solvents, with the approximate value being 197 mg of KOH/g. The saponification value for the control sample was 197.23 mg of KOH/g. Facioli and Gonçalves (1998) obtained similar iodine values for pequi oil (200 mg of KOH/g). In a study on the extraction of oil from avocado using different solvents, Moreno *et al.* (2003) obtained lower values using acetone. In the extraction of oil from *Moringa* seeds this index did not vary when hexane and chloroform-methanol were used as solvents (Lalas *et al.*, 2002).

Acidity in oil results from enzymatic hydrolysis which in turn is related to the conservation state and humidity level of the raw material. When fruits have high humidity, hydrolytic enzymes (lipase) act rapidly on triacylglycerols, releasing free fatty acids.

Acidity presented a significant response variation in the treatment using ethyl alcohol, which is noticeable by the parameter estimates of the quadratic model fitted to the response variable acidity (Table 2) and by Figure 2, given that it is the most recommended solvent for the extraction of free fatty acids from oil because it is the most efficient. The levels obtained were in excess of 3.85% and this probably relates to the difficulty with which the oil-solvent solution separates, as the boiling temperature of alcohol is 78.4°C; or maybe because ethanol dissolves more fatty acid. A higher acidity value was verified for oil extracts from avocado using acetone (Moreno *et al.*, 2003).

The acidity of the control sample (1.52% of oleic acid) revealed that extraction with water did not affect its conservation state, as with the extract obtained with hexane (1.54% of oleic acid). Facioli and Gonçalves (1998) obtained a lower acidity value (0.27% of oleic acid) when pequi oil was obtained through a simplified small-scale process (using hot water).

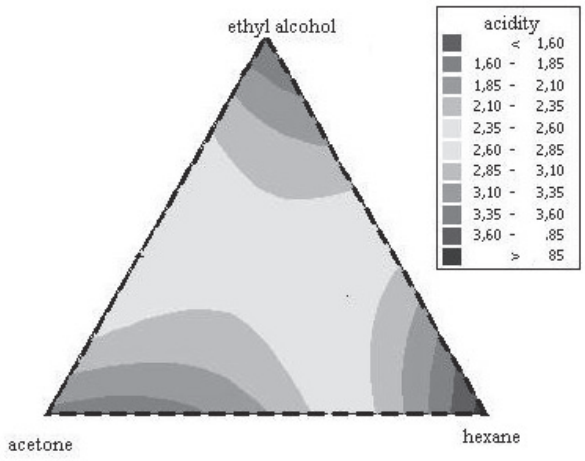

Figure 2
Contour line for acidity (expressed in % of oleic acid) in different treatments.

Comparatively, an analysis of oil extraction from soy using various solvents (hexane, water and chloroform-methanol mixture at 2:1 v/v) revealed that the iodine value (133-136 g I^2/100g of sample) and the saponification value (195-198 mg of KOH/g of sample) did not differ significantly. Acid value, on the other hand, presented a higher value in the solvent mixture (1.3% of oleic acid) in comparison to hexane and water, with 0.5% and 0.4% of oleic acid respectively (Kim *et al.*, 1990.

As to the refractive index, no significant difference occurred among the different types of treatment, the average value being similar to the control sample that is 1.46. This index can change not only as a function of the fatty acids (which may vary according to solvent type) but also with isomerization and conjugation which may result from the oil being badly handled (Elleuch *et al.*, 2006). Silva *et al.* (1993) found a similar refractive index for pequi of 1.46.

The fatty acid compositions of the pequi extract in each treatment are illustrated in Table 5. We noted a similarity among the different types of treatment, as with the results obtained by Dunford and Zhang (2003) when extracting oil from wheat germ using different types of solvent: polar group (ethanol, isopropanol and acetone) and non-polar group (n-hexane, high-purity hexane and isohexane). Pequi extract was found to contain more oleic acid (unsaturated fatty acid), also verified by Silva *et al.* (2003), and followed by palmitic acid (saturated fatty acid). Lima *et al.* (2007) observed that pequi oil is chiefly composed of unsaturated fatty acids, which attributes an excellent quality to the oil, especially for consumption.

Silva *et al.* (1993) points out the presence of oleic acid (52% to 54%), palmitic acid (39%), linoleic acid (2%) and stearic acid (1%) in pequi oil. According to the author, this composition is compatible with the epicutaneous natural coating, which justifies its use in cosmetic and pharmaceutical formulations.

The fatty acid composition in pequi oil, according to Lima *et al.* (2007), revealed a predominance of oleic, linoleic and palmitic acids and, to a lesser degree, the presence of stearic, linolenic, vaccenic, palmitoleic and arachidic acids. The values obtained reveal great disagreement with other works, leading the author to conclude that the oil had been adulterated, as, for instance, by the addition of soy oil.

Regarding carotenoid content, according to the contour lines illustrated in Figure 3, obtained through a quadratic model where R^2 is approximately 66%, we note that the most significant effects were produced by the treatments with acetone (T2) and ethyl alcohol (T3), where higher values are verified, 300 μg/g and 296 μg/g respectively. The carotenoid content in the control sample was 202.72 μg/g, followed by hexane (199 μg/g). Judging by the model equation, we verified

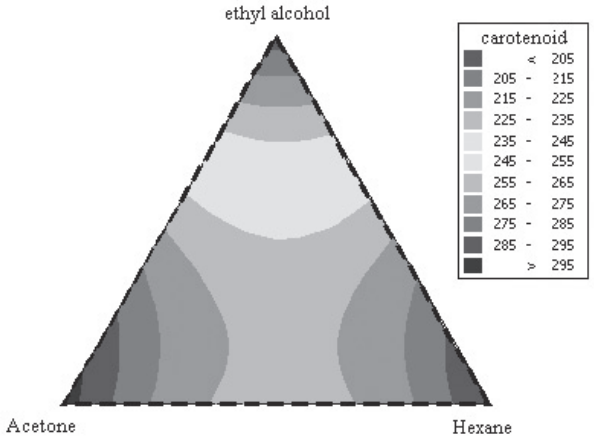

Figure 3
Contour line for carotenoid content (mg/g) in different treatments.

an antagonistic interaction between acetone and ethyl alcohol (coefficient $\alpha_{23} < 0$ - Table 2).

In a study on carotenoid recovery from shrimp waste using various solvents, Sachindra *et al.* (2005) concluded that a higher content was obtained with an isopropanol and hexane mixture (1:1), 43.90 μg/g of residue. As for extracts obtained with other solvents (hexane, acetone and ethyl alcohol), it was verified, similarly to this experiment, that acetone presented a higher value (40.6 μg/g), followed by ethyl alcohol (31.9 μg/g) and hexane (13.1 μg/g). The value obtained for the hexane-acetone mixture (1:1) was 38.5 μg/g. Based on these results, we can confirm that the carotenoid content was smaller when a non-polar solvent such as hexane was used.

Delgado-Vargus *et al.* (2000) reported on the advantages and disadvantages of using various organic solvents for the extraction of carotenoids and verified that the use of non-polar solvents is not recommended where humid samples are concerned.

De Ritter and Purcell (1981) verified that a full extraction of carotenoids should be done in low-humidity samples by using a mixture of non-polar and slightly polar solvents. This fact is in agreement with the results obtained in this work, considering the yield obtained for the different mixtures, that is, hexane-acetone and hexane-ethyl alcohol.

4. CONCLUSIONS

Higher oil yields were obtained using acetone and hexane, especially their mixtures with ethyl alcohol. The different types of solvent did not influence the iodine value, saponification value and refractive index. A higher acidity value was verified in the extract using ethyl alcohol. Higher carotenoid contents were found in the extract using acetone and ethyl alcohol as pure solvents. The profile of fatty acids in the oil fraction of the extracts did not vary among the different types of solvents and their mixtures. As for the oil fraction, oleic acid was the dominant unsaturated fatty acid (53%-54.8%) and palmitic acid was the dominant saturated acid (39.6%-41.5%).

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