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Reduction of Cholesterol in Lard by Solvent Extraction

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The presence of high levels of cholesterol in lard has been a barrier to good acceptance of the use of the product in the formation of blends for formulating processed foods. This fact makes interesting the study of a process that allows the reduction of the cholesterol content in animal fats. Thus, the aim of this work was to evaluate the viability of using alcoholic solvents (ethanol + water) for reducing the cholesterol content in lard, through a process known as liquid-liquid extraction (LLE). The experiments were carried out in accordance with an experimental design, varying the concentration of free fatty acids from 0 to 10% and water in the solvent from 0 to 12%. The components were weighed in polypropylene tubes and left in water bath at 45 ° C. After 24 hours of rest, the liquid-liquid equilibrium was achieved and samples of the two phases were collected for analysis of cholesterol by spectroscopy. The response surface methodology (RSM) was used to analyze the effect of process variables on the partition coefficient of cholesterol. It was observed that the percentage of acidity initially present in fat does not have a significant influence on the partition of cholesterol between phases. Moreover, it appears that the increase in the percentage of water in ethanol decreases the partition coefficient, namely, it causes a reduction in the capacity of the solvent to extract the cholesterol.

1. Introduction

Oils and fats have always been part of human food, being essential for health. Industrially, they have played an important role in the development of different areas of chemical products, pharmaceutical, cosmetic, and most importantly, food. In the first 50 years of the twentieth century, the use of animal fats in food was very common. The lard (pig fat), for example, was the most used product for domestic frying as well as raw material in mass producing for breads and cakes. For the food industry, lard still serves as an important ingredient in the formulation of some food products, mainly embedded products. However, many studies on nutrition have shown the evil effects of some types of fat, like saturated fat (rich in saturated fatty acids), found primarily in animal products. In addition to have a high level of cholesterol, animal fat, when consumed in excess, can increase the concentrations of bad cholesterol, LDL-c, and lower the good

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cholesterol, HDL-c, thus increasing the development of heat diseases (Stewart et al., 2001).

For these reasons, animal fats were replaced in many formulations for industrial fats from vegetable sources, obtained through the hydrogenation of vegetable oils. Although they are naturally free of cholesterol, the hydrogenated vegetable fats have made the same terrible effects of fats from animal sources, although worst. This is because during the hydrogenation process *trans* fatty acids are produced (Eller et al., 2005).

The news that *trans* fat could lead health problems has forced the industries to seek new alternatives, such as the use of vegetable fat from palm, which is naturally saturated and does not require hydrogenation. Another alternative would be a return to the use of animal fats, such as lard, which has a high content of saturated fatty acids, but lower than that found in other animal fats and even in palm oil. However, due to changes in the current profile of the consumer, much more concerned with health, the presence of cholesterol continues being a barrier to good acceptance of the product. This fact turns interesting the study of a process that allows the reduction of the cholesterol content in fat in order to alleviate the "negative image" of it.

A process that could be used for this purpose is the liquid-liquid extraction (LLE), in which the cholesterol can be extracted with alcohol or other solvents that have affinity with the cholesterol.

Through the determination of liquid-liquid equilibrium data, this research aims to study the technical feasibility of using liquid-liquid extraction to decrease the level of cholesterol in lard, using ethanol with different levels of water.

2. Materials and Methods

2.1. Liquid-Liquid Equilibrium Experiments

For determination of the liquid-liquid equilibrium data, model fatty systems containing lard (obtained from a local slaughterhouse in Pirassununga, State of São Paulo/Brazil) and commercial oleic acid (Synth) were mixed with ethanolic solvents (mixtures of anhydrous ethanol (Merck, with a purity >99.5%,) and distillate water) in the mass ratio fat/solvent 1:1. The experiments were carried out in accordance with an experimental design, varying the concentration of free fatty acids from 0 to 10% and water in the solvent from 0 to 12%. The equilibrium data were determined using polypropylene centrifuge tubes (15 mL). The components were weighed on an Adam model AAA200 analytical balance, accurate to 0.0001 g. The tubes were vigorously stirred for at least 30 min and left to rest for 24 h in a thermostatic bath at 45 ºC. After this treatment, the two phases became clear, with a well-defined interface, and the cholesterol concentration of both phases was measured.

2.2. Cholesterol Determination

The concentration of cholesterol was determined by spectroscopy according to the methodology described by Saldanha et al. (2004), adapted to samples of this work. A detailed description of the methodology is as follows: Phase samples (around 2 g) were directly saponified with 8 mL of 50% KOH in water plus 12 mL of anhydrous ethanol for 15 min in a water bath at 60 °C. Water (10 ml) was added and after cooling to room temperature the unsaponifiable fraction was extracted twice with 10 ml of hexane.

Exactly 0,5 ml of the hexane extract obtained by direct saponification were dried and diluted in 1 ml of isopropyl alcohol in a test tube. After that, 3 ml of a reagent mixture (laboratory kit Laborlab) was added, and the samples incubated for 10 min in a 37 °C water bath, and then cooled to room temperature. The absorbance of the samples at 499 nm was determined in relation to a control containing only isopropyl alcohol and the reagent mixture. The cholesterol concentration of sample solutions was determined using a standard curve constructed by graphically plotting the absorbance *vs* mg/ml cholesterol (0.04 to 0.20 mg in isopropyl alcohol). The slope of the standard curve obtained was always greater than 0.98. Enzymatic reactions were measured using an UV-visible spectrophotometer (Shimadzu).

2.3 Results Analysis

The experiments were carried out in accordance with an experimental design, varying the concentration of free fatty acids from 0 to 10% and water in the solvent from 0 to 12%. The experimental set was planned to obtain a quadratic model, consisting of 2^2 trials plus a star configuration with three repetitions in central point. Surfaces were built using the quadratic model for the statistically significant variables. The response surface methodology (RSM) was used to analyze the effect of process variables on the partition coefficient of cholesterol (k_c), which is calculated according equation 1.

$$k_c = w_c^{II} \,/\, w_c^{I} \tag{1}$$

In eq 1, w_c is the cholesterol concentration, in mg/100g, and the superscripts *II* and *I* are alcoholic and fatty phases, respectively. The software Statistica (Statsoft, v.8.0) was used to analyze the results by non-linear multiple regression.

3. Results and Discussion

Table 1 shows the cholesterol partition coefficients (k_c) obtained from the liquid-liquid experiments.

Table 1- Experimental Design: 2^2 + star configuration + central points.

	Exp	Coded Variables		Real Variables		Response
		V1[a]	V2[b]	V1[a]	V2[b]	k_c
2^2	1	+1	+1	10.29	8.56	0.401
	2	+1	-1	10.29	1.45	0.447
	3	-1	+1	1.77	8.56	0.691
	4	-1	-1	1.77	1.45	0.718
Central Points	5	0	0	6.11	5.04	0.577
	6	0	0	6.11	5.04	0.584
	7	0	0	6.11	5.04	0.603
Star Configuration	8	-1.41	0	0	4.95	1.136
	9	+1.41	0	11.92	5.26	0.524
	10	0	+1.41	6.11	10.12	0.356
	11	0	-1.41	6.11	0	0.807

[a] V1 = water in the solvent in %; [b] V2 = free fatty acid in lard in %

The statistical analysis of the experimental results allowed to formulate a model (in coded variables) representing the k_c value as a function of variables statistically significant.

$$k_c = 0.588 - 0.178 \cdot \mathrm{V1} + 0.086 \cdot \mathrm{V1}^2 - 0.089 \cdot \mathrm{V2} - 0.039 \cdot \mathrm{V2}^2 \quad (2)$$

Figure 1 presents the contour curve generated by the model obtained in eq 2, representing the influence of the water content in solvent (V1) and of the free acidity in lard (V2) on the response studied (k_c).

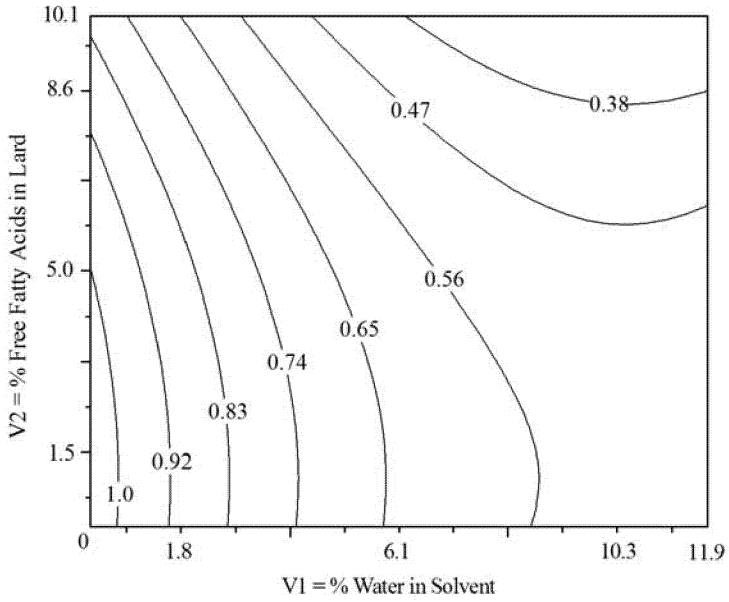

Figure 1- Contour curve of cholesterol partition coefficient (k_c) expressed as function of water content in solvent (V1) and free acidity in lard (V2).

As can be seen in Figure 1, increasing the water content in the solvent from 0 to 12 mass % significantly reduces the cholesterol partition coefficient, namely the water reduces the ability of the solvent to extract the cholesterol. The k_c values also show such behavior when the content of free fatty acids in lard is increased, however, this effect is more pronounced for solvents with higher levels of water.

Table 2 shows the analysis of variance (ANOVA) for the responses at 95.0% of confidence.

Table 2-Analysis of Variance (ANOVA)

Source of Variation	SS[a]	MS[b]	DF[c]	F[d]
Regression	0.3673	0.0918	4	5.99
Residual	0.0920	0.0153	6	
Total	0.4593		10	
Correlation Coefficient		0.81		

[a] Sum of squares; [b] Mean square; [c] Degrees of freedom; [d] F calc = $F_{0.95;\,4;\,6} = 4.53$

As can be observed in Table 2, the correlation coefficient (R^2) is not very high and the F-test shows that the respective model are not reliable since the calculated F values are less than 2 times greater than the value obtained from Box et al. (1978).
Despite the statistical model has not been adjusted to the experimental data, it was possible to analyze trends in k_c for variables studied. The use of anhydrous ethanol (0 % of water), for example, results in k_c values around 1, ie, the solvent can extract fifty percent of cholesterol in 1 stage of equilibrium.

4. Conclusions

The response surface methodology analysis has not provided a reliable model to predict k_c values. However, it was possible to conclude that the increase of water content in the solvent has a significant effect on the partition of cholesterol between phases in equilibrium. Finally, the liquid-liquid extraction can be used for the reduction of cholesterol, an undesirable component of lard, making it a more acceptable product for the food industry and the consumer.

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