Behaviour of Oryzanol and Tocotrienols during the Rice Bran Oil Deacidification Process by Liquid-liquid Extraction, at (283.2 to 333.2) K

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The edible oil deacidification by liquid-liquid extraction is an alternative route to obtain vegetable oils with low content of free fatty acids. The difference in polarity between the triacylglycerol, the main component of vegetable oils, and the solvent, ethanol, guarantees the formation of two phases and permits the removal of free fatty acids. Phase equilibrium data are important for the design of extraction process. This work presents experimental data for the system degummed rice bran oil + free fatty acids + ethanol + water + oryzanol + tocotrienols, at (283.2 to 333.2) K. The addition of the water to the system improves the heterogeneous area. This effect permits the processing of oils with high free fatty acid contents and reduces the loss of neutral oil. In the present work the effect of temperature was also evaluated on the partition of free fatty acids and of oryzanol and tocotrienols.

1. Introduction

Johnson and Lusas (1983) studied alternative solvents for vegetable oil extraction, including ethanol, which, during extraction at high temperature and subsequent cooling, formed two phases (alcohol and oil). The use of ethanol as solvent has been studied since the beginning of the 20th century. Undoubtedly, ethanol has shown advantages compared to hexane and other alternative solvents. However, it is necessary to assess the impact of ethanol in the subsequent stages of refining and on the final quality of oil produced. After extraction, it is necessary to conduct the subsequent steps of the refining process to ensure that the oil is sufficiently purified and that undesirable substances like phospholipids and free fatty acids (FFA) are removed. Several studies have shown that liquid-liquid extraction using ethanol can be used as alternative route in obtaining vegetable oils with acceptable levels of FFA. This new process, performed under milder conditions, avoids the formation of waste products such as soaps, and minimizes the loss of neutral oils and nutraceutical compounds (Rodrigues et al., 2006; Cuevas et al., 2009; Chiyoda et al., 2010). In fact, it is possible to associate the steps of extraction and deacidification if ethanol is used as solvent. To allow for appropriate design of such a coupled process, it is necessary to study the phase equilibrium properties of fatty system at different temperatures. In the present paper, equilibrium

data for systems containing rice bran oil and ethanol at 283.2, 308.2, and 333.2 K are reported. The experimental data were favorably correlated using the UNIQUAC model.

2. Materials and Methods

2.1 Materials

The solvents used in this work were absolute ethanol, from Merck, with purity greater than 99.5%, and aqueous solvents with different water contents (6.34, 18.87, and 28.29 mass %), prepared by the addition of deionized water to absolute ethanol. Degummed rice bran oil (RBO) (Irgovel, Brazil) was used in the equilibrium experiments. This oil underwent the degumming process at the refinery after oil extraction and solvent stripping and can be qualified as semi-processed. It is important to emphasize that this oil naturally possesses the measured acidity value, as described in the Methods section.

2.2 Methods

RBO was mixed with solvent, in the mass ratios oil:solvent (1:1, 1:2, 2:1). These data were used to adjust UNIQUAC interaction parameters. Liquid-liquid equilibrium data were determined using polypropylene centrifuge tubes (50 mL) (Corning Inc.). The components were weighed on an analytical balance with readability of 0.0001 g (Adam, UK). The tubes were vigorously stirred for at least 15 min, centrifuged for 10 min at 4500 g (Centrifuge Jouan model BR4i) and left to rest for 6 h in a thermostatic bath in a pre-set value (283.2, 308.2, 333.2 ±0.1 K) (Tecnal, Brazil). After this treatment, the two phases became clear, with a well-defined interface, and the composition of both phases was measured. The concentration of free fatty acids (FFA), present naturally in the degummed oil and in the alcohol and oil phases, was determined by titration (IUPAC, 1979). The total solvent concentration was determined by evaporation at 333.2 K in a vacuum oven (Tecnal, Brazil). The water concentration was determined by Karl Fischer titration (AOCS, 1998). For the UNIQUAC parameters adjustment process, the system was considered as a pseudoquaternary one composed only by triacylglycerols, fatty acids, ethanol and water. In this case, having determined the concentration of fatty acids, solvent and water, the triacylglycerol concentration can be obtained by difference. The concentration of γ-oryzanol was determined by spectrophotometry at 314.5 nm, as suggested by Seetharamaiah and Prabakar (Rodrigues et al., 2006). The quantification of tocols (tocopherols and tocotrienols) was determined by spectrophotometry at 520 nm according to the methodology developed by Emmerie-Engel (Rodrigues et al., 2006). In this work all measurements were performed at least in triplicate.

2.3 Modeling approach

The UNIQUAC model with mass fraction as the concentration unit was used due to the large difference in molar mass of the components in the system (Cuevas et al., 2009; Chiyoda et al., 2010). Adjustments were made by treating the systems composed of degummed RBO + FFA + ethanol + water as pseudoquaternary. For adjustment, the RBO was treated as a single triacylglycerol using the average molar mass of the oil. The same approach was extended to the free fatty acids. This approach assumes that the different triacylglycerols present in the rice bran oil behave in a similar way in the related liquid-liquid system. In this case, such components can be adequately replaced by pseudocompounds having corresponding average physical-chemical properties. The

same assumptions were made for the fatty acid mixture. The values of r_i' and q_i', volume and area parameters necessary for the UNIQUAC model, were calculated via eq 1, where x_j is the mole fraction of the triacylglycerols of the rice bran oil or the fatty acids, $v_k^{(j)}$ is the number of k groups in molecule j, $\overline{M}_i$ is the average molar mass of the vegetable oil or fatty acid, C is the number of compounds in the oil or in the fatty acid, G is the total number of groups and R_i and Q_i are van der Waals parameters (Cuevas et al., 2009).

$$r_i' = \frac{1}{\overline{M}_i} \sum_j^C x_j \sum_k^G v_k^{(j)} R_k; \qquad q_i' = \frac{1}{\overline{M}_i} \sum_j^C x_j \sum_k^G v_k^{(j)} Q_k \qquad (1)$$

In this work the data were correlated using the UNIQUAC equation with temperature dependent binary interaction parameters. The temperature dependencies of A_{ij} and A_{ji} are given by eq 2, where T is absolute temperature.

$$A_{ij} = A^{(0)}{}_{ij} + A^{(1)}{}_{ij}T; \qquad A_{ji} = A^{(0)}{}_{ji} + A^{(1)}{}_{ji}T \qquad (2)$$

Interaction parameter estimation was performed by minimizing the objective function of compositions (eq 3), following the procedure developed by Stragevitch and d'Ávila (1997).

$$S = \sum_m^D \sum_n^N \sum_i^{K-1} \left[\left(\frac{w_{inm}^{OP,ex} - w_{inm}^{OP,calc}}{\sigma_{w_{inm}^{OP}}} \right)^2 + \left(\frac{w_{inm}^{AP,ex} - w_{inm}^{AP,calc}}{\sigma_{w_{inm}^{OP}}} \right)^2 \right] \qquad (3)$$

where D is the total number of data groups, N is the total number of tie lines, K is the total number of components or pseudocompounds in the group of data (m), w is the mass fraction, i is the component subscript, n is the tie line subscript, m is the group number subscript, and OP and AP represent the oil and alcohol phases, respectively. ex and $calc$ refer to experimental and calculated compositions, respectively. $\sigma_{w_{inm}^{OP}}$ and $\sigma_{w_{inm}^{AP}}$ are the standard deviations observed in the compositions of the two liquid phases. The average deviations between experimental and calculated compositions in both phases were calculated according to eq 4.

$$\Delta w = 100 \sqrt{\frac{\sum_n^N \sum_i^K \left[\left(w_{i,n}^{OP,ex} - w_{i,n}^{OP,calc} \right)^2 + \left(w_{i,n}^{AP,ex} - w_{i,n}^{AP,calc} \right)^2 \right]}{2NK}} \qquad (4)$$

In the present work, the pseudocomponents used in the experimental data and in the UNIQUAC parameter fitting are denoted as follows: degummed rice bran oil (1), free fatty acids (2), ethanol (3), and water (4).

3. Results and Discussion

The fatty acid composition of the RBO is presented in Table 1. The results shown in Table 1 allow us to calculate the average molar masses of FFA naturally present in the oil and of degummed RBO (275.92 g·gmol^{-1} and 862.64 g·gmol^{-1}, respectively). It was considered that the free acidity has the same fatty acid composition as the oil.

Table 1: Fatty Acid Composition of Degummed Rice Bran Oil

Symbol - Fatty Acid		M (g/mol)[b]	% Mol	% Mass
C14:0[a]	Miristic	228.38	2.54	2.10
C16:0	Palmitic	256.43	20.38	18.94
C18:0	Stearic	284.49	1.67	1.72
C18:1	Oleic	282.47	36.49	37.36
C18:2	Linoleic	280.45	35.10	35.68
C18:3	Linolenic	278.44	2.00	2.02
C20:0	Arachidic	312.54	0.51	0.58
C20:1	Gadoleic	310.52	0.36	0.40
C22:0	Behenic	340.59	0.42	0.52
C22:2		336.56	0.22	0.27
C24:0	Lignoceric	368.65	0.30	0.41

[a] In Cx:y. x=number of carbons and y=number of double bonds; [b] M=molar mass

The RBO had a free fatty acid content of (4.90 ± 0.02) % by mass. It is important to emphasize that this calculated fatty acid level takes into account the average molar mass of FFA shown in Table 1. The volume and area parameters (eq 1) obtained for oil, free fatty acids, ethanol and water are (r_i' and q_i'): 0.04447 and 0.03606, for oil; 0.04504 and 0.03707, for free fatty acids; 0.05591 and 0.05618, for ethanol; and 0.05107 and 0.07771, for water.

Figures 1a and 1b show the experimental data points and calculated tie lines for the systems: rice bran oil + ethanol with 6.34% mass percent of water at 283.2 K and, rice bran oil + ethanol with 18.87% mass percent of water at 308.2 K, respectively.
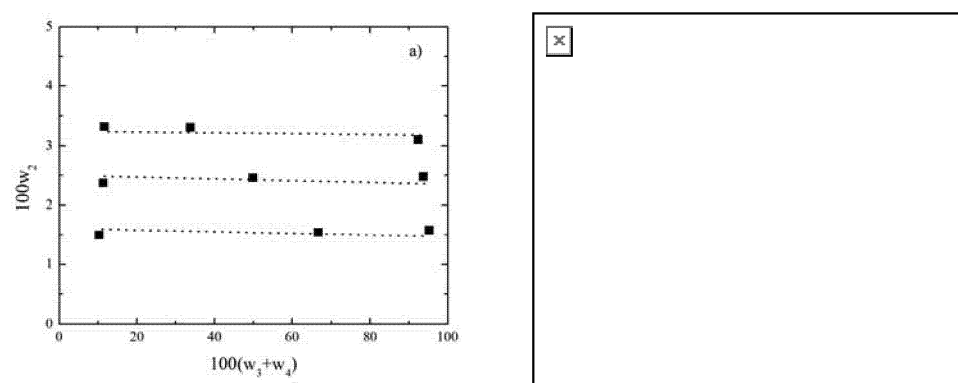

Figure 1: System of RBO (1) + FFA (2) + solvent [ethanol (3) + water (4)]: (■) experimental; (. . .) UNIQUAC model. (a) 6.34 % of water in the solvent, at 283.2 ± 0.1 K; (b) 18.87 % of water in the solvent, at 308.2 ± 0.1 K.

These equilibrium diagrams are plotted in triangular coordinates. To represent the pseudoquaternary systems in triangular coordinates, ethanol + water were included as a mixed solvent for graphical representation purposes only. Analysis of Figures 1a and 1b

indicates that the addition of water to the ethanol solvent causes an increase in the biphasic region. Water content reduces the solubility between the oil and solvent, and consequently, the composition of solvent in refined oil decreases with the addition of water, facilitating subsequent removal. The results shown in these Figures indicate that the addition of water reduces the solvent capacity for extracting FFA. This is suggested by the inversion of the tie lines for solvent with high water content. In a general, the results shown in Figures 1a and 1b indicate that the UNIQUAC model accurately describes the phase compositions for this system (see adjusted parameters in Table 2 and mean deviations in Table 3). It is also possible to observe that the temperature does not affect the solubility of the fatty system.

Table 2: UNIQUAC Parameters

Pair ij[a]	$A^{(0)}_{ij}$/K	$A^{(0)}_{ji}$/K	$A^{(1)}_{ij}$	$A^{(1)}_{ji}$
12	71.88	-436.21	-2.21	0.70
13	-54.85	-66.86	1.10	0.01
14	-361.5	208.01	3.47	-1.08
23	-1759.3	-843.05	7.07	-1.05
24	-2464.3	-28398	10.63	175.29
34	-2107.9	-400.44	9.73	0.030

[a] Rice bran oil (1), Free fatty acids (2), Ethanol (3) and Water (4). [b] See eq 2 where A_{ij} and A_{ji} are energy parameters expressed in Kelvin.

Table 3: Mean Deviations in Phase Compositions for the Fatty System

Water in the solvent (mass %)	T (K)	$100\Delta w$
0.62		0.73
6.34	283.2	0.53
18.87		0.22
6.34		0.22
18.87	308.2	0.16
28.29		0.29
6.34		0.54
18.87	333.2	0.35
28.29		0.26
Global Deviation of the correlation		0.35

Figure 2 shows the behavior of oryzanol and tocols (tocopherols and tocotrienols) as function of temperature. In this figure the distribution coefficient, k, is defined as $k_i = w_i^{AP}/w_i^{OP}$, where w_i is the mass fraction of nutraceutical compound, and the superscripts *OP* and *AP* represent the oil and alcohol phases, respectively.

It can be observed that the partition coefficients of oryzanol and tocotrienols are not function of temperature. On the other hand, these coefficients are highly suppressed by the increasing of water level in the solvent.
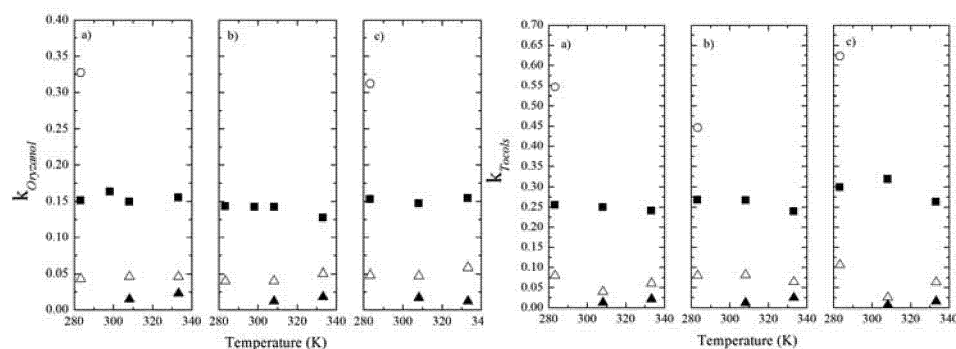
Figure 2: Distribution coefficients of Oryzanol and Tocols as function of temperature for different mass ratios oil:solvent: (a) 1:1; (b) 1:2; (c) 2:1.Water in the solvent: (○) 0.62 %; (■) 6.34 %; (△) 18.87 %; (▲) 28.29 %.

4. Conclusions

The obtained parameter set enables the simulation of liquid-liquid extractors aiming the deacidification of vegetable oils. The distribution coefficients of nutraceutical compounds are only function of water level in the ethanolic solvent.

Acknowledgements

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