

Liquid liquid equilibrium for ethanolysis systems of fish oil.

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Abstract

Enzymatic alcoholysis of fish oil can produce monoacylglycerols (MAG) rich in polyunsaturated fatty acids at atmospheric pressure and low temperature. Biocatalysis in organic solvents helps to create a homogeneous reaction system for the reactants, ethanol and fish oil. This work presents liquid-liquid equilibrium at two different temperatures (303.2 and 323.2 K) and at atmospheric pressure for two solvent-systems in the ethanolysis of fish oil: Ethanol + oil + tert-pentanol and ethanol + oil + hexane. Experimental solubility (binodal) curves were obtained by the cloud point method and tie-line composition was obtained by density and high temperature gas chromatography (HT-GC). The consistency of experimental tie line data was checked by using the Othmer-Tobias equation. The experimental liquid-liquid data were correlated satisfactorily by the nonrandom two liquid (NRTL) model for activity coefficient calculation.

Keywords: Liquid-liquid equilibria; fish oil; ethanolysis; organic solvents.

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1. Introduction

Fish oil is one of the main sources of n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA). These compounds have been reported to have beneficial effects in human health (Ustun, 1997).¹ They are also widely used as ingredients in functional foods, mostly as their ethylester derivatives. However, their acylglyceride derivatives are preferred to achieve a better assimilation by the human body. From the different acylglycerides, MAGs are non-ionic surfactants and emulsifiers used in the food industry. Enzymatic alcoholysis of fish oil by using 1,3-specific lipases is a simple method to obtain 2-MAGs significantly enriched in EPA and DHA,^{2,3} providing a practical alternative to the conventional chemical methods in the production of MAGs. Many factors affect the lipase-catalyzed synthesis of MAG, including reaction conditions, type of enzyme^{4,5} and reaction media. To obtain the highest yield, all these factors should be optimized.^{6,7} By using organic solvents as reaction media, the mutual solubility of reactants, ethanol and fish oil could be achieved. Therefore, knowledge of the phase behavior for ethanolysis systems of fish oil in organic solvent as reaction media is necessary to optimize the amount of solvent and guarantee the full miscibility of the reaction mixture. As pointed out by Lanza et al.^{8,9} this type of studies are important for a correct design of the process since this can influence the reaction pathway as well as the further purification steps.

Despite the industrial interest, no equilibrium data were found in the literature for alcoholysis systems of fish oil in different organic solvents. In a previous work, liquid-liquid equilibrium for systems glycerol + sardine oil + tert-alcohols was determined.¹⁰ This work presents liquid-liquid equilibrium data for ethanolysis systems of fish oil in two organic solvents: ethanol + fish oil + hexane and ethanol + fish oil + tert-pentanol, at 303.2 K and 323.2 K.

Binodal curves were obtained by the cloud-point method. Compositions of tie-lines have been directly determined by using a high temperature gas chromatograph (HT-GC) and evaporation of the solvents. The results were compared with indirect measurements of tie lines composition through density measurement of the two phases. The consistency of the experimentally measured tie line data was verified by using the Othmer-Tobias equation. The experimental data were correlated by the nonrandom two-liquid (NRTL) activity coefficient equation, using the Simplex Nelder Mead minimization method with a weight composition-based objective function.

2. Experimental section

2.1. Materials

Ethanol was purchased from Merck KGaA with a purity of 99.9 % and a water content of 0.05 %. Tert-pentanol was obtained from Merck with a purity of $\geq 99\%$ and a water content of 0.065 %, Hexane was purchased from Lab-Scan Analytical Sciences with a purity of $\geq 99\%$ and a water content of 0.01%. Refined fish oil was kindly provided by AFAMSA and it is a mixture of tuna and sardine oil. Products were stored over activated 3Å molecular sieves to keep them dried.

Densities measurements were carried out by using an Anton Paar DMA 5000. Results for reactants and solvents are presented in Table 1 with some values found in the literature.^{11,12,13.}

2.2. Apparatus and procedure

Binodal curves determination

Solubility (binodal) curves of the two ternary systems studied in this work have been determined at 303.2 ± 0.5 K and 323.2 ± 0.5 K and atmospheric pressure by the cloud point method using the titration method. Temperatures investigated in this work were chosen since enzyme catalyzed

reactions are carried out under mild conditions. Different binary mixtures of fish oil + hexane (or tert-pentanol) and ethanol + hexane (or tert-pentanol) were prepared by using an analytical balance (Sartorius Basic, accurate ± 0.0001 g). These binary mixtures were titrated with the third component (ethanol or fish oil) by using a syringe needle until a change from transparent to turbid was observed by using a turbidimeter (Eutech Instruments TN-100). The temperature was controlled by a thermostatic bath with a precision of ± 0.5 K. To determine the mass added of the third component, the mixture was weighed again. The amount of the third component was also determined from the mass change of the syringe before and after the titration. The same results were obtained by these two measurements. The cloud point was considered to be a binodal curve point. Samples were collected for density analysis. This way, an expression for density as a function of weight fraction for the three components could be obtained. Each experimental point was replicated at least twice.

Tie lines determination

Experiments have been carried out in equilibrium cells of 20 cm^3 . The overall mixture was prepared directly inside the equilibrium cell by weighing known amounts of fish oil, ethanol and solvent, hexane or tert-pentanol, on an analytical balance (Sartorius Basic, accurate ± 0.0001 g). The cell temperature was controlled by a thermostatic bath (± 0.5 K). The ternary mixture was then vigorously stirred for at least 3 h to allow good contact between the two liquid phases and ensure equilibrium. After that, the mixture was allowed to stand for at least 24 h at constant temperature until two transparent liquid phases, with a defined interface, could be clearly observed. The upper phase was the ethanol-rich phase and the lower phase was the oil-rich phase. Samples of both phases were collected and composition was determined. Tie line experiments were replicated twice.

2.3. Analytical methods

Fatty acid profile of fish oil used in this work has been determined by gas chromatography by following the AOAC method.¹⁴ The fatty acid methyl esters were firstly prepared and then analyzed by gas chromatography (GC) in a Hewlett Packard gas chromatograph (6890N Network GC System) equipped with an auto-sampler (7683B series) and a flame ionization detector (FID). A fused silica capillary column (OmegawaxTM-320, 30m×0.32mm i.d.) was used. Most of the fatty acid methyl esters were identified by comparison of their retention times with those of chromatographic standards (Sigma Chemical Co.). Further details of the gas chromatograph method can be found elsewhere.¹⁵ Table 2 shows the fatty acid composition of the fish oil used in this work.

The free fatty acid (FFA) content of the fish oil has been determined according to AOCS Official Method Ca 5a-40 using an automatic titrator Methrom (Titrand 605).¹⁶ The FFA content was found to be 0.23 ± 0.015 % expressed as percentage of oleic acid. Due to the low free fatty acid content, FFA were not taken into account in the studied systems.

High-Temperature Gas Chromatography (HT-GC) has been used to determine the composition of the tie lines. A Hewlett Packard (HP 6890 Series GC System) gas chromatograph equipped with a flame ionization detector (FID), a fused silica capillary column of 30m×0.25mm i.d., coated with a 0.25 mm film thickness of 65% Phenyl Methylpolysiloxane (65HT) as a stationary phase, and an Agilent Technologies 7683B Series automatic injector was used. Helium (1 mLmin^{-1}) was used as carrier gas. The initial oven temperature, 80°C , was kept for 2 min and raised to 340°C at a rate of $15.0 \text{ }^\circ\text{Cmin}^{-1}$. Then, it was raised to $365 \text{ }^\circ\text{C}$ at a rate of $1.5 \text{ }^\circ\text{Cmin}^{-1}$ and held isothermally for 4 min. Split injection mode was used with a ratio of 1:40. The injector temperature was kept at 380°C , while the detector temperature was at 400°C .

For the ethanolysis system of fish oil in tert-pentanol, all components could be satisfactorily quantified by HT-GC. In the literature, it has been reported that HT-GC could thermally degrade species that contain PUFAs such as fish oils.¹⁷ To check the reliability of HT-GC to quantify the fish oil used in this work, composition of the tie line was also determined by density measurements. A density calibration curve was obtained from the previous cloud point determination as a function of composition of the three components of the mixture.¹⁸ This way, for tie line measurements, density and just one composition (tert-pentanol) must be known to determine the composition of the other two components since the third component can be written in terms of the other two to satisfy the material balance. For the ethanolysis system of fish oil in hexane, the HT column was not able to separate ethanol and hexane and only fish oil composition could be determined by HT-GC. For this system, fish oil composition was also measured by evaporation at 323 K. The time necessary to remove completely the other two components was determined by weighing samples every hour until constant mass was reached. The oil composition was obtained by difference. Similar procedure was followed by Lanza et al.⁸ in the LLE study of systems of interest in biodiesel production. The composition of the other two components could be determined by density measurements, as it has been described for the ethanolysis system in tert-pentanol.

3. Results and discussion

3.1 Experimental data

Tables 3 and 4 present the binodal curve and the density data at 303.2 K and 323.2 K for the systems ethanol + fish oil + tert-pentanol and for ethanol + fish oil + hexane, respectively. A direct

fit of the density data, similar to the approach used in the correlation of the boiling points of ternary mixtures without using binary data suggested by Tamir,¹⁹ has been used in this work:

$$\rho = \sum_{i=1}^N w_i \rho_i + \sum_{i=1}^{N-1} \sum_{j=i+1}^N w_i w_j \left[A_{ij} + B_{ij} (w_i - w_j) + C_{ij} (w_i - w_j)^2 + \dots \right] \quad [1]$$

The coefficients of the empirical Eq. 1 were determined by using the Marquardt algorithm and are listed in Table 5.

Figures 1 and 2 show the binodal curves for the systems determined in this work. The information provided by the binodal curve is important to optimize the amount of solvent used to create a homogeneous system between reactants. From Figures 1 and 2 can be observed that the two-phase region is slightly bigger when using tert-pentanol as solvent than when using hexane. As expected, an increase in temperature, in the range covered in this work, decreases the biphasic region for both solvents.

Tie line data for the system ethanol + fish oil + tert-pentanol or hexane are presented in Tables 6 and 7 respectively and Figures 3 to 6. For the system ethanol + fish oil + tert-pentanol composition of tie lines has been determined by HT-GC as explained in section 2.2. Additionally, mass fraction of two of the components of the mixture was determined by density calibration using tert-pentanol as the key component. This way, by using parameters from Table 5, composition of fish oil and ethanol could be also determined. Composition of fish oil and ethanol calculated by density calibration and by HT-GC were similar. For the system ethanol + fish oil + hexane, oil composition was determined by HT-GC and evaporation. The composition of the other two components was determined from density measurements with parameters from Table 5.

Tie lines show that, for the system with tert-pentanol, the ethanol phase is richer in tert-pentanol than the oil phase, however for the ethanol + fish oil + hexane system, the content of hexane is very similar in both phases.

The reliability of the tie-line data were tested by the Othmer–Tobias equation:²⁰

$$\ln\left(\frac{1-w_2^{OP}}{w_2^{OP}}\right) = A + B \ln\left(\frac{1-w_1^{EP}}{w_1^{EP}}\right) \quad [2]$$

where w_2^{OP} is the mass fraction of fish oil in the oil rich phase and w_1^{EP} is the mass fraction of ethanol in the ethanol-rich phase. The Othmer-Tobias plot for the studied systems is shown in Figure 7. The linearity of the plot indicates a good degree of consistency of the experimental data. The correlations are given in Table 8 at 303.2 K and 323.2 K.

3.2 Data correlation

In this work, it has been assumed that the different triacylglycerols present in the fish oil behave in a similar way in the liquid-liquid system. Therefore, the fish oil has been treated as a single compound. This approach has been suggested in the literature when dealing with different types of oil.²¹ To verify this assumption, fatty acid composition has been determined for the oil and ethanol rich phase. Table 1 shows the fatty acid composition of the fish oil used in this work, together with the fatty acid composition of the corresponding oil rich phase and ethanol rich phase for a tie line obtained at 303.2 K in the system ethanol (1) + fish oil (2) + hexane (3). A similar distribution of the fatty acids in both phases can be observed. This result supports the previous assumption of considering the fish oil as a single compound. Similar results were obtained for the other tie lines and systems studied in this work. Minority fatty acids in the fish oil (see Table 1), such as α -linolenic and steric acid, could not be properly detected in the ethanol phase due to the low concentration of fish oil in this phase (see Tables 6 and 7).

NRTL model has been used to correlate the experimental tie line data. In this work, mass fraction was used as composition unit instead of mole fraction due to the large difference in molar mass of the components of the system. This fact has been suggested in the literature dealing with liquid-liquid equilibrium systems including different kinds of vegetable oils.⁸ The isoactivity criterion of phase equilibrium can be expressed as a function of mass fraction units as follows:

$$(\gamma_i^w w_i)^{EP} = (\gamma_i^w w_i)^{OP} \quad [3]$$

The mass fraction-scale activity coefficient γ_i^w must be related to the NRTL activity coefficient γ_i by the following equation:^{8, 21.}

$$\gamma_i^w = \frac{\gamma_i}{M_i \sum_j^n \left(\frac{w_j}{M_j} \right)} \quad [4]$$

where M_i and M_j are the molecular weight of component i and j respectively and w_j is the mass fraction of component j respectively.

Rodriguez et al.²¹ present an expression for the activity coefficient, γ_i , for the NRTL model using mass fractions as unity of concentration:

$$\ln \gamma_i = \frac{\sum_{j=1}^K \tau_{ji} G_{ji} w_j / M_j}{\sum_{j=1}^K G_{ji} w_j / M_j} + \sum_{j=1}^K \left[\frac{w_j G_{ji}}{M_j \sum_{l=1}^n G_{lj} w_l / M_l} \times \left(\tau_{ij} - \frac{\sum_{l=1}^K \tau_{lj} G_{lj} w_l / M_l}{\sum_{l=1}^K G_{lj} w_l / M_l} \right) \right] \quad [5]$$

where

$$G_{ij} = \exp(-\alpha_{ij} \tau_{ij}) \quad [6]$$

$$\tau_{ij} = \frac{A_{ij}}{T} + B_{ij} \quad [7]$$

$$\alpha_{ij} = \alpha_{ji} \quad [8]$$

A_{ij} , B_{ij} and α_{ij} are parameters of the NRTL model, w is the mass fraction, M is the molecular weight and T is the equilibrium temperature. A_{ij} and B_{ij} represent the interaction energy between compounds i and j and α_{ij} is a nonrandomness parameter that derives from the local composition assumption. The parameter estimation was based on the minimization of the following objective function by using the Simplex Nelder Mead method:

$$OF = \sum_k^D \sum_j^M \sum_i^{N-1} \left[\left(w_{ijk}^{EP,exp} - w_{ijk}^{EP,calc} \right)^2 + \left(w_{ijk}^{OP,exp} - w_{ijk}^{OP,calc} \right)^2 \right] \quad [9]$$

where D is the number of data sets (number of studied temperatures for each system), M is the number of tie-lines in each data set, N is the number of components, the superscripts EP and OP refer to ethanol rich phase and oil rich phase respectively, and the superscripts exp and calc refer to the experimental and calculated values of the liquid-phase concentration. A similar procedure as proposed by Reyes-Labarta et al.²² has been followed in the minimization procedure to calculate the equilibrium concentrations. The concentrations that satisfy the isoactivity criterion and the mass balances in each phase are calculated by the Newton-Raphson method by fixing pressure, temperature and composition of one of the components in one phase. In the fitting procedure, the non-randomness parameter of the NRTL model has been fixed to different values ranging from 0.1 to 0.5. The best results were obtained for $\alpha_{ij} = 0.4$ in the system ethanol + fish oil + tert-pentanol and $\alpha_{ij} = 0.2$ in the system ethanol + fish oil + hexane. Table 9 shows the NRTL binary interaction parameters (A_{ij} , B_{ij}) for these results.

Tie lines calculated by using parameters from Table 9 have been plotted in Figures 3-6. Comparison between experimental and calculated compositions of each component in each of the two phases was made through the root mean-square (RMS) deviation, given by the following expression²³ and it is also shown in Table 9:

$$\delta w = 100 \sqrt{\frac{\sum_i^M \sum_j^N (w_{ijk}^{EP,exp} - w_{ijk}^{EP,calc})^2 + (w_{ijk}^{OP,exp} - w_{ijk}^{OP,calc})^2}{2MN}} \quad [9]$$

From Figures 3-6 can be observed that the NRTL model was able to describe the phase behavior for the ethanol + fish oil system with the two organic solvents, tert-pentanol and hexane, with an average deviation of 0.73 % and 0.51 %, respectively.

4. Conclusions

Experimental liquid-liquid equilibrium data, binodal curve and tie lines, for systems containing fish oil (mixture of tuna and sardine oil), ethanol and tert-pentanol or hexane have been determined. Equilibrium data have been obtained at two different temperatures 303.2 and 323.2 K. As expected, by increasing temperature the biphasic region decreases in the temperature range covered in this work (303.2 to 323.2 K). The miscibility region is slightly larger when using hexane as solvent in the ethanolysis system.

The reliability of the tie-lines was checked by applying the Othmer-Tobias correlation. NRTL activity coefficient model was able to describe the liquid-liquid equilibrium for the two studied systems showing a root mean square deviation lower than 1 %.

Acknowledgment

To the Spanish Government through MINECO (CTQ2012-39131-C02-01) and CDTI (Ref. IDI-20111225) for financial support. To AFAMSA S.L. for kindly supplying the fish oil used in this work. SLB acknowledges support the Secretary of Education Public of Mexico for the fellowship through PROMEP program.

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Table 1. Density of the pure components.^a

Component	$\rho_{\text{exp.}}/\text{kg}\cdot\text{m}^{-3}$		$\rho_{\text{literature}}/\text{kg}\cdot\text{m}^{-3}$		
	303.2 K	323.2 K	298.15 K	303.15 K	323.15 K
Ethanol	782.52	765.73	785.06 ¹¹	781.8 ¹²	765.20 ¹³
Hexane	651.10	632.10	654.80 ¹¹	650.18 ¹¹	--
Tert-pentanol	801.33	783.30	805.00 ¹¹	--	--
Fish oil	917.50	906.53	--	--	--

^a Standard uncertainty u is $u(\rho) = 0.05$.

Table 2. Fatty acid composition of the fish oil used in this work and of the two phases (oil and ethanol rich phases) of a tie line at 303.2 K for the system ethanol (1) + fish oil (2) + hexane (3).^a

Fatty acid		fish oil, %	Tie line	
			OP, %	EP, %
Myristic	C14:0	3.3	3.4	3.8
Palmitic	C16:0	19.5	20.2	20.4
Palmitoleic	C16:1n-7	5.4	5.5	5.9
Stearic	C18:0	6.0	6.2	5.8
Oleic	C18:1n-9	18.0	18.5	18.3
Vaccenic	C18:1n-7	3.0	2.9	2.9
Linoleic cis (LA)	C18:2n-6	2.4	2.4	2.5
α -Linolenic (ALA)	C18:3n-3	0.7	0.7	nd
Steriadonic	C18:4n-3	0.9	0.9	nd
Eicosenoic	C20:1n-9	2.1	2.1	2.0
Eicosatrienoic	C20:3n-3	2.1	2.1	2.2
Eicosapentaenoic (EPA)	C20:5n-3	7.6	7.4	8.0
Docosapentaenoic (DPA)	C22:5n-3	2.0	1.9	1.9
Docosahexaenoic (DHA)	C22:6n-3	27.0	25.8	26.3

EP: ethanol phase; OP: oil phase

^a Standard uncertainties u are $u(\text{percentage}) = 0.5$.

Table 3. Binodal curves for ethanol (1) + fish oil (2) + tert-pentanol (3) at 303.2 K and 323.2 K. ^a

w_1	w_2	w_3	ρ , kg·m ⁻³
303.2 K			
0.9711	0.0289	0.0000	784.55
0.8850	0.0260	0.0890	791.74
0.7410	0.0570	0.2020	797.29
0.6860	0.0700	0.2440	798.12
0.5990	0.1420	0.2590	805.20
0.5040	0.2410	0.2550	820.17
0.4190	0.3420	0.2390	827.52
0.3700	0.4200	0.2100	838.72
0.3380	0.4770	0.1850	847.90
0.2830	0.5820	0.1350	860.14
0.2410	0.6760	0.0830	873.72
0.2200	0.7230	0.0570	880.51
0.1765	0.8080	0.0155	891.17
0.1753	0.8247	0.0000	895.48
323.2 K			
0.9350	0.0650	0.0000	774.08
0.8390	0.0870	0.0740	776.61
0.7600	0.1100	0.1300	780.78
0.6900	0.1550	0.1550	787.70
0.6420	0.2026	0.1554	794.05
0.6100	0.2353	0.1547	798.65
0.5600	0.2890	0.1510	804.31
0.5150	0.3380	0.1470	810.68
0.4700	0.3948	0.1352	815.05
0.4000	0.4900	0.1100	827.92
0.3650	0.5402	0.0948	832.27
0.3320	0.5930	0.0750	843.07
0.3100	0.6340	0.0560	849.13
0.2650	0.7350	0.0000	886.49

^a Standard uncertainties u are $u(T) = 0.5$ K, $u(x) = 0.0005$, $u(\rho) = 0.05$.

Table 4. Binodal curves for ethanol (1) + fish oil (2) + hexane (3) at 303.2 K and 323.2 K. ^a

w ₁	w ₂	w ₃	ρ, kg·m ⁻³
303.2 K			
0.9711	0.0289	0.0000	784.55
0.8800	0.0536	0.0664	778.50
0.8000	0.0800	0.1200	774.23
0.7116	0.1280	0.1604	774.56
0.6090	0.2132	0.1778	780.00
0.5130	0.2934	0.1936	787.70
0.4037	0.4043	0.1920	800.05
0.3395	0.4829	0.1776	815.43
0.2660	0.6057	0.1283	839.40
0.2181	0.7019	0.0800	864.38
0.1940	0.7705	0.0355	878.10
0.1753	0.8247	0.0000	895.48
323.2 K			
0.9350	0.0650	0.0000	774.08
0.9080	0.0720	0.0200	774.45
0.7978	0.1342	0.0680	769.32
0.6770	0.2230	0.1000	767.17
0.5905	0.3052	0.1043	773.91
0.5540	0.3420	0.1040	775.18
0.5300	0.3660	0.1040	778.23
0.4943	0.4057	0.1000	787.76
0.4020	0.5100	0.0880	816.03
0.3564	0.5782	0.0654	837.32
0.3300	0.6100	0.0600	847.51
0.3072	0.6531	0.0397	864.38
0.2650	0.7350	0.0000	886.49

^a Standard uncertainties u are $u(T) = 0.5$ K, $u(x) = 0.0005$, $u(\rho) = 0.05$.

Table 5. Parameters of Equation 1.

T /K	Parameters		r ²
<i>Ethanol (1) + Fish oil (2) + Tert-pentanol (3)</i>			
303.2	A ₁₂ = -10.8661	B ₁₂ = -28.9237	0.9991
	A ₁₃ = -28.407	B ₁₃ = 108.895	
	A ₂₃ = -3.9883	B ₂₃ = -54.5387	
323.2	A ₁₂ = 50.9625	B ₁₂ = -76.6458	0.9994
	A ₁₃ = -31.5295	B ₁₃ = 4.7147	
	A ₂₃ = -27.8921	B ₂₃ = -897.127	
<i>Ethanol (1) + Fish oil (2) + Hexane (3)</i>			
303.2	A ₁₂ = -28.0217	B ₁₂ = -49.4943	0.9993
	A ₁₃ = 44.3121	B ₁₃ = -28.2368	
	A ₂₃ = -133.633	B ₂₃ = 93.2504	
323.2	A ₁₂ = 54.052	B ₁₂ = -72.4196	0.9990
	A ₁₃ = 52.4717	B ₁₃ = 108.657	
	A ₂₃ = -1551.14	B ₂₃ = 2200.44	

Table 6. Ternary LLE Data for the system ethanol (1) + fish oil (2) + tert-pentanol (3) at 303.2 K and 323.2 K.^a

Overall composition			Ethanol-rich phase			Oil-rich phase		
w ₁	w ₂	w ₃	w ₁	w ₂	w ₃	w ₁	w ₂	w ₃
303.2 K								
0.5024	0.4773	0.0203	0.9189	0.0320	0.0491	0.1669	0.8243	0.0088
0.4966	0.4530	0.0504	0.8700	0.0374	0.0926	0.1779	0.8060	0.0161
0.5037	0.4344	0.0619	0.8400	0.0433	0.1167	0.1909	0.7820	0.0271
0.5032	0.4014	0.0954	0.7900	0.0519	0.1581	0.2074	0.7528	0.0398
0.4988	0.3803	0.1209	0.7590	0.0586	0.1824	0.2181	0.7340	0.0479
0.5025	0.3446	0.1529	0.6898	0.0811	0.2291	0.2622	0.6579	0.0799
323.2 K								
0.5979	0.3764	0.0257	0.8900	0.0790	0.0310	0.2554	0.7281	0.0165
0.5995	0.3652	0.0353	0.8743	0.0857	0.0400	0.2680	0.7110	0.0210
0.5990	0.3558	0.0452	0.8485	0.0955	0.0560	0.2860	0.6842	0.0298
0.6002	0.3399	0.0599	0.8271	0.0999	0.0730	0.2937	0.6680	0.0383
0.6003	0.3152	0.0845	0.7723	0.1200	0.1077	0.3403	0.5947	0.0650

^a Standard uncertainties u are $u(T) = 0.5$ K, $u(x) = 0.0005$.

Table 7. Ternary LLE Data for system ethanol (1) + fish oil (2) + hexane (3) at 303.2 K and 323.2 K.^a

Overall composition			Ethanol-rich phase			Oil-rich phase		
w ₁	w ₂	w ₃	w ₁	w ₂	w ₃	w ₁	w ₂	w ₃
303.2 K								
0.5510	0.4176	0.0314	0.9061	0.0540	0.0399	0.2178	0.7400	0.0422
0.5379	0.4095	0.0526	0.8710	0.0714	0.0576	0.2171	0.7190	0.0639
0.5296	0.4005	0.0699	0.8460	0.0850	0.0690	0.2219	0.6925	0.0856
0.5195	0.3905	0.0900	0.8171	0.0914	0.0915	0.2561	0.6390	0.1049
0.4988	0.3711	0.1301	0.7830	0.0895	0.1275	0.2924	0.5695	0.1381
323.2 K								
0.5700	0.4200	0.0100	0.9201	0.0684	0.0115	0.2660	0.7217	0.0123
0.5596	0.4090	0.0314	0.8732	0.0943	0.0325	0.3025	0.6604	0.0371
0.5470	0.3970	0.0560	0.8067	0.1310	0.0623	0.3263	0.6150	0.0587
0.5326	0.3916	0.0758	0.7291	0.1910	0.0799	0.3528	0.5700	0.0772
0.5299	0.3741	0.0960	0.6526	0.2510	0.0964	0.4230	0.4820	0.0950

^a Standard uncertainties u are $u(T) = 0.5$ K, $u(x) = 0.0005$.

Table 8. Correlation constants for the Tobias-Othmer correlation of the ternary system at 303.2 K and 323.2 K

System	T/K	A	B	r²
Ethanol (1) + Fish oil (2) + Tert-pentanol (3)	303.2	-0.3519	0.5313	0.9291
	323.2	0.5101	0.7470	0.9816
Ethanol (1) + Fish oil (2) + Hexane (3)	303.2	0.6053	0.7707	0.9033
	323.2	0.3242	0.5279	0.9734

Table 9. NRTL parameters for the ethanol (1) + fish oil (2) + tert-pentanol (3) and for the ethanol (1) + fish oil (2) + hexane (3)

Pair	A_{ij}/K	A_{ji}/K	B_{ij}	B_{ji}	α_{ij}	RMS
<i>System: Ethanol (1) + fish oil (2) + tert-pentanol (3)</i>						
12	-271.5	3314.3	8.3	-7.5	0.4	
13	7041.2	1930.3	-7.8	-4.4	0.4	0.73
23	1594.6	149.7	2.1	-5.7	0.4	
<i>System: Ethanol (1) + fish oil (2) + hexane (3)</i>						
12	134.8	1605.6	9.0	-8.5	0.2	
13	5537.7	20525.8	-16.0	832.7	0.2	0.51
23	650.5	3162.0	-0.3	-0.8	0.2	

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Figure 1. Phase diagram of ethanol (1) + fish oil (2) + tert-pentanol (3) (\circ 303.2 K; \blacktriangle 323.2 K)

Figure 2. Phase diagram of ethanol (1) + fish oil (2) + hexane (3) (\circ 303.2 K; \blacktriangle 323.2 K)

Figure 3. Liquid-liquid equilibrium for the system ethanol (1) + fish oil (2) + tert-pentanol (3) at 303.2 K: --- binodal curve; \blacklozenge tie lines; — NRTL.

Figure 4. Liquid-liquid equilibrium for the system ethanol (1)+ fish oil (2) + tert-pentanol (3) at 323.2 K: --- binodal curve; \blacklozenge tie lines; — NRTL.

Figure 5. Liquid-liquid equilibrium for the system ethanol (1) + fish oil (2) + hexane (3) at 303.2 K: --- binodal curve; \blacklozenge tie lines; — NRTL.

Figure 6. Liquid-liquid equilibrium for the system ethanol (1) + fish oil (2) + hexane (3) at 323.2 K: --- binodal curve; \blacklozenge tie lines; — NRTL.

Figure 7. Othmer-Tobias plot for the system ethanol (1) + fish oil (2) + tert-pentanol (3) at 303.2 K (\bullet , $r^2 = 0.9290$) and 323.2 K (\circ , $r^2 = 0.9816$) and for the system ethanol (1) + fish oil (2) + hexane (3) at 303.2 K (\blacksquare , $r^2 = 0.9033$) and 323.2 K (\square , $r^2 = 0.9734$).

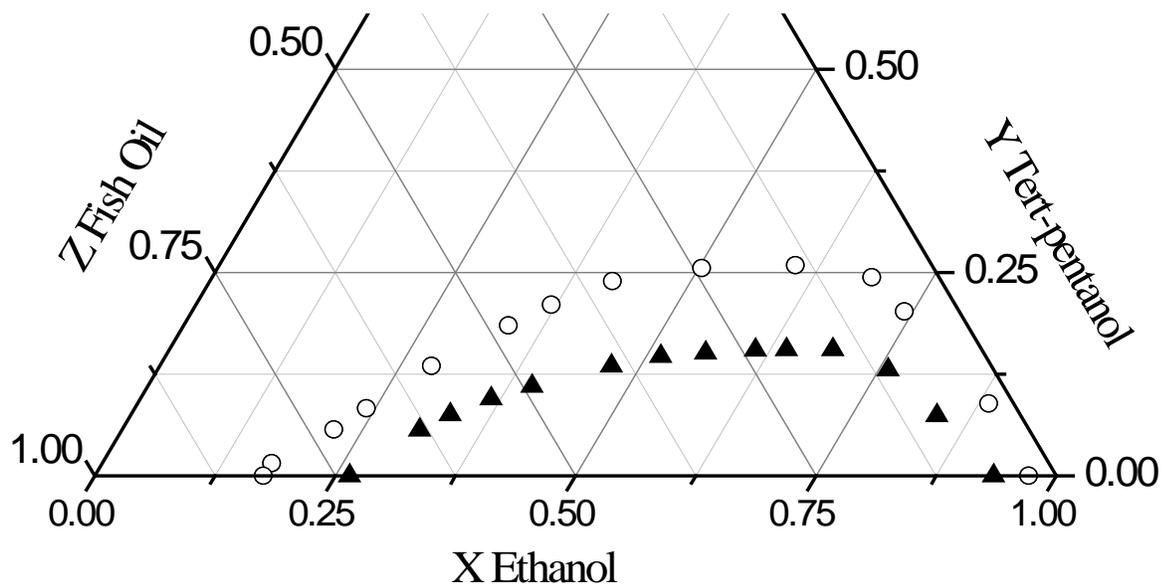


Figure 1. Phase diagram of ethanol (1) + fish oil (2) + tert-pentanol (3) (○ 303.2 K; ▲ 323.2 K).

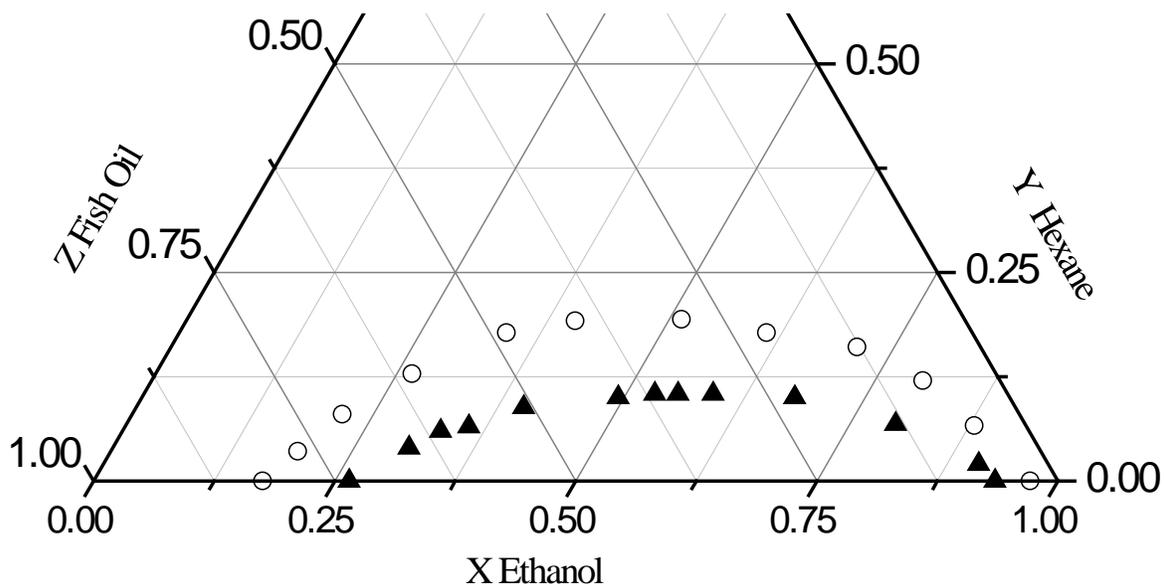


Figure 2. Phase diagram of ethanol (1) + fish oil (2) + hexane (3) (○ 303.2 K; ▲ 323.2 K)

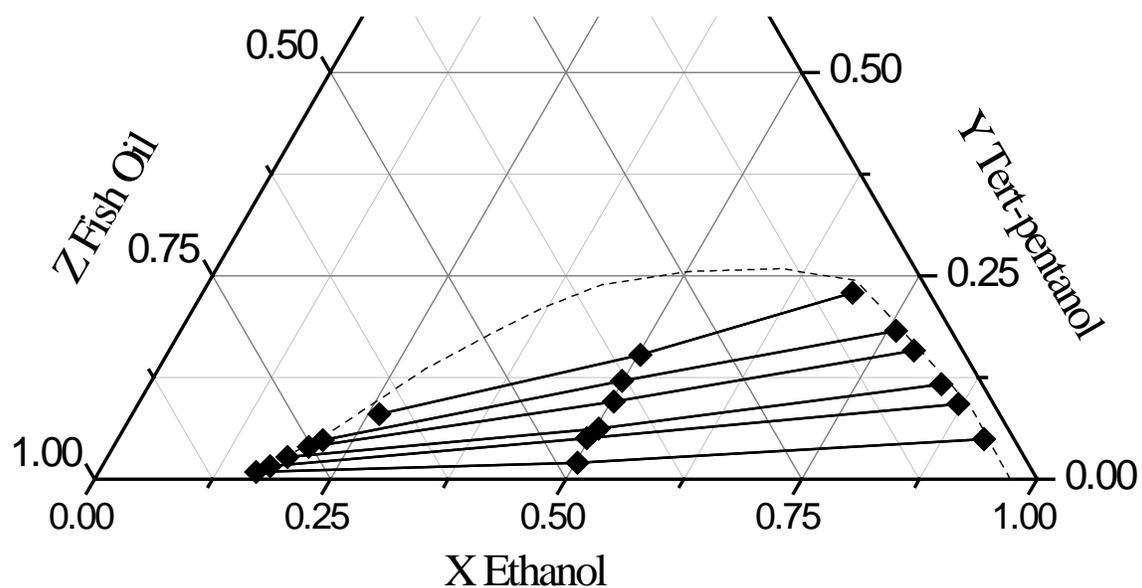


Figure 3. Liquid-liquid equilibrium for the system ethanol (1) + fish oil (2) + tert-pentanol (3) at 303.2 K: --- binodal curve; \blacklozenge tie lines; — NRTL ($\alpha=0.4$).

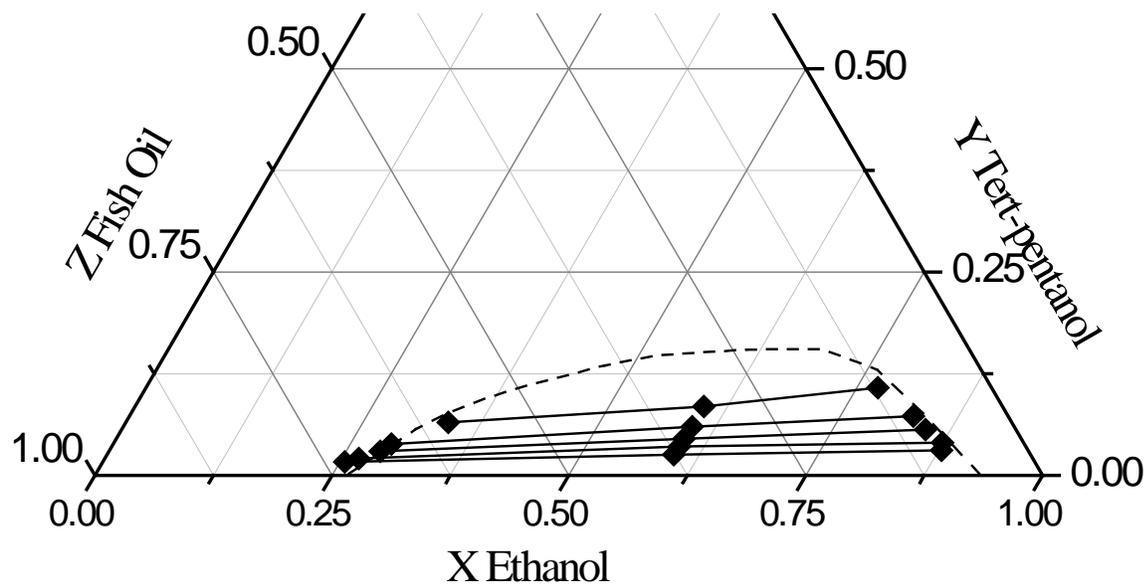


Figure 4. Liquid-liquid equilibrium for the system ethanol (1)+ fish oil (2) + tert-pentanol (3) at 323.2 K: ---binodal curve; \blacklozenge tie lines; — NRTL ($\alpha=0.4$).

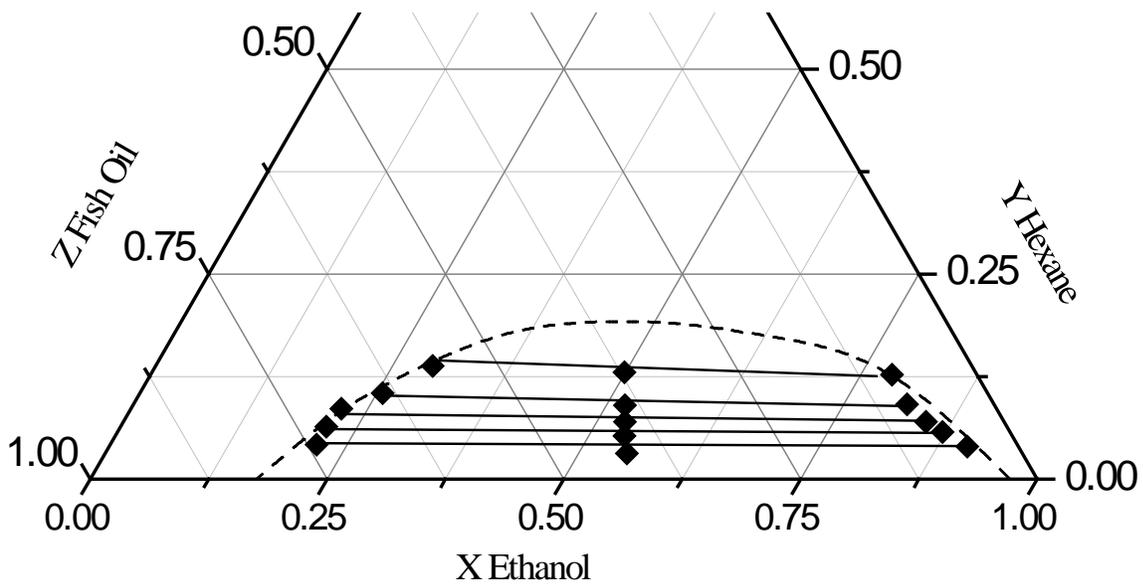


Figure 5. Liquid-liquid equilibrium for the system ethanol (1) + fish oil (2) + hexane (3) at 303.2 K: ---, binodal curve; \blacklozenge tie lines; — NRTL ($\alpha=0.2$).

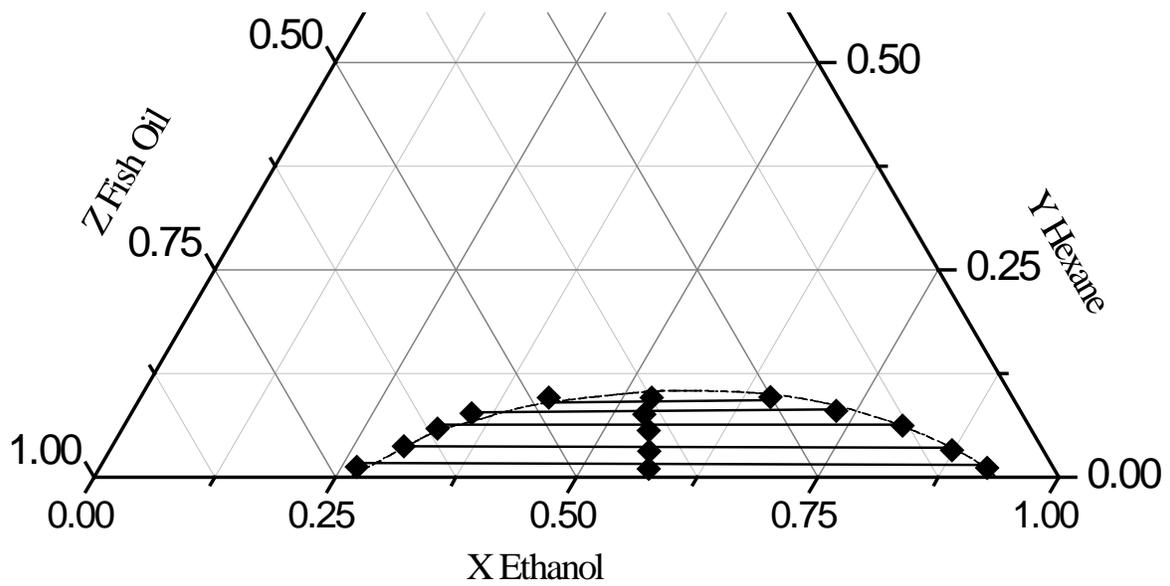


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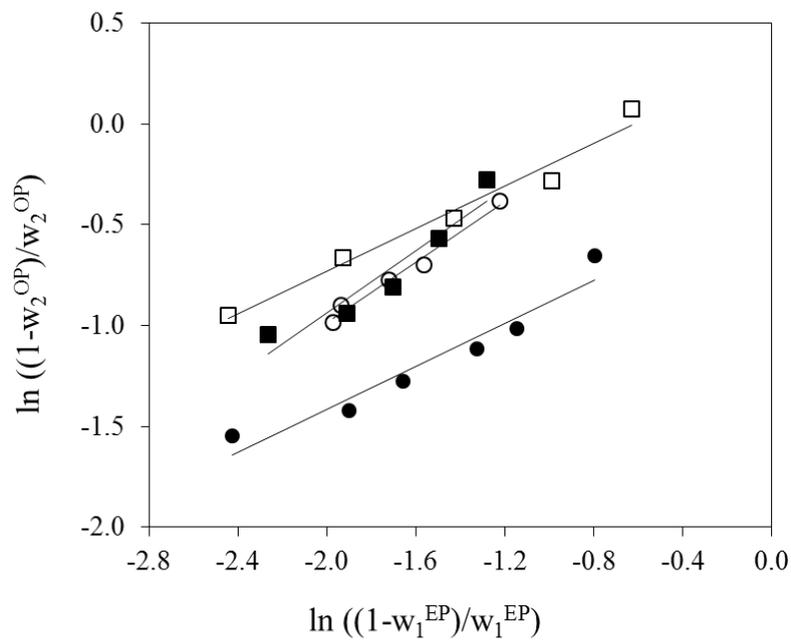


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