Liquid liquid equilibria for systems glycerol + sardine oil + tert-alcohols

Ángela García Solaesa, Silvia Liliana Bucio, María Teresa Sanz, Sagrario Beltrán, Sara Rebolleda*

Department of Biotechnology and Food Science (Chemical Engineering Section), University of Burgos, 09001 Burgos. Spain

Abstract

Monoacylglycerols (MAGs) can be produced by lipase-catalyzed glycerolysis of oils and fats at atmospheric pressure and low temperature. The use of organic solvents as reaction media helps to create a homogeneous reaction system between the immiscible reactants glycerol and oil. In this work liquid-liquid equilibrium at two different temperatures (303.2 and 323.2 K) and at atmospheric pressure has been determined for two solvent-systems in the glycerolysis of fish oil (sardine oil): glycerol + sardine oil + tert-butanol and glycerol + sardine oil + tert-pentanol. From the experimental solubility (binodal) curves and tie-lines, it could be observed that the system mutual solubility does not significantly increase by increasing temperature from 303.2 to 323.2 K. The Othmer-Tobias correlation was used to

* Corresponding author. Tel.: +34 947 258810. Fax: ++34947258831. E-mail address tersanz@ubu.es

analyze the consistency of the tie-line data. The experimental liquid-liquid data were correlated satisfactorily by using the NRTL model for the activity coefficient calculation.

Keywords: Liquid-liquid equilibria; Fish oil; glycerolysis; tertiary alcohols.

1. Introduction

Fish oil is one of the main sources of omega 3 polyunsaturated fatty acids (n-3 PUFA), specially eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA). These compounds have been reported to have beneficial effects on cardiovascular diseases, reduction of blood pressure and plasma triglyceride levels, and control of overactive immune functions [1]. Glycerolysis of fats and oils is often carried out to concentrate these PUFA in their natural monoacylglycerides form (2-MAG). The enzymatic catalysis in non-aqueous media using lipases is a usual method for synthesizing structured lipids. Lipase-catalyzed glycerolysis of oils using 1,3-specific lipases has been shown as an interesting alternative to the chemical methods due to the mild reaction conditions for reactions involving the highly unstable n-3 polyunsaturated fatty acids [2]. For this bioconversion it is necessary to introduce a solvent in the reaction system to improve the solubility of the reactants, oil and glycerol. Since the work of Zaks and Klibanov [3], organic solvents have been employed extensively in enzymatic reactions. Among the different solvents considered in the literature for glycerolysis systems, alcohols with more than five carbons are one of the best options since they contain a polar –OH group and a nonpolar carbon chain. Since alcohols are competitors to glycerol, tertiary alcohols are considered because of its tertiary structure that makes them to have a strong steric hindrance for the enzymatic reaction [4]. According to Damstrup et al. [5] the relative low log P values of tert-butanol and tert-pentanol indicate

both hydrophilic and hydrophobic characteristics, with predominant hydrophilic characteristics. This fact makes them suitable solvents for both, oil and glycerol.

Knowledge of the phase behavior for systems containing fish oil, glycerol and the solvent added as reaction media is important for a correct design of the glycerolysis process since this can influence the reaction pathway as well as the further purification steps [6].

Composition of the oil from individual fish species varies, depending on its diet, time of the year and location in the same way as do the oils from vegetable sources [7]. Fish oil is a multicomponent mixture. In the refined process, polar lipids, mainly phospholipids, free fatty acids, and other minor compounds are removed. A neutral lipid analysis for the sardine oil used in this work shows that nearly 99.5 % of the sardine oil are triacylglycerols (TAG). In spite of difference in TAG composition among sardine oil species, determination of phase equilibrium data on natural mixtures is important to estimate the proper process conditions. In literature, phase equilibrium studies concerning vegetable oils are more abundant; however phase equilibrium studies involving mammals or fish oil are scarce.

This work presents liquid-liquid equilibrium data for two ternary systems in the glycerolysis of sardine oil: glycerol + sardine oil + tert-butanol and glycerol + sardine oil + tert-pentanol at 303.2 K and 323.2 K. Binodal curves were obtained by the cloud-point method. Tie-lines have been directly determined by using a high temperature chromatograph capillary column (HT-GC). The results were compared with indirect measurements of tie lines through density measurement of the two phases. The Othmer Tobias equation was applied to confirm the reliability of experimentally measured tie line data. The experimental data were correlated by the nonrandom two-liquid (NRTL) activity coefficient model, using the simplex minimization method with a weight composition-based objective function.

2. Experimental section

2.1. Materials

Glycerol was purchased from Sigma Aldrich with a purity of > 99.5% and a water content of 0.04%. Tert-butanol and tert-pentanol were purchased from Merck with a purity of ≥99% and a water content of 0.298 ± 0.033 % and 0.065 ± 0.023 % respectively. Refined sardine oil was kindly provided by Industrias Afines S.L.

Densities of the compounds were measured by using an Anton Paar DMA 5000 and are presented in Table 1 together with some values found in the literature [8, 9].

2.2. Apparatus and procedure

Binodal curves

The binodal curve of the two ternary systems studied in this work was determined at 303.2 ± 0.5 K and 323.2 ± 0.5 K and atmospheric pressure by turbidimetric analysis using the titration method. Different binary mixtures of sardine oil + tertiary alcohol and glycerol + tertiary alcohol have been prepared at various concentrations by using an analytical balance (Sartorius Basic, accurate ± 0.0001 g). These binary mixtures were titrated with the third component (glycerol or sardine oil) by using a syringe needle until a change from transparent to turbid was observed by using a turbidimeter (Eutech Instruments TN-100). The uncertainty of the drop has been estimated to be ± 0.005 g for glycerol and ± 0.0018 g for sardine oil. The liquid mixtures have been vigorously agitated by a magnetic stirrer. Experimental points with a high content in glycerol, due to its high viscosity, were stirrer for more than 15 minutes to assurance a sufficient mixture of the compounds at the operating temperature. 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 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 can be obtained. Each experimental point was replicated at least twice.

Tie lines determination

Experiments were carried out in equilibrium cells of 20 cm^3. A mixture of sardine oil, glycerol and tertiary alcohol, at a given composition, was prepared directly inside the equilibrium cell by weighing known quantities of each component in an analytical balance (Sartorius Basic, accurate ± 0.0001 g). The equilibrium temperature was controlled by a thermostatic bath (± 0.5 K). The ternary mixture was then vigorously stirred for at least 3 h to allow contact between the two liquid phases. After that, the mixture was allowed to stand for at least 24 h at constant temperature to ensure equilibrium was reached and two transparent liquid phases with a defined interface could be clearly observed. The upper phase was the oil-rich phase and the lower phase was the glycerol-rich phase. Samples of both phases were collected and composition was determined by HT-GC and density measurements. Tie line experiments were replicated twice.

2.3. Analytical methods

The sardine oil used in this work was analyzed by gas chromatography to determine the fatty acid profile by the AOAC method [10]. 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 chromatographic method can be found elsewhere [11]. Table 2 shows the fatty acid composition of the sardine oil. Bandarra et al. [12] analyzed the seasonal change in lipid composition of sardine oil in terms of fatty acid profile. Although difference can be found among the individual fatty acids, a similar fatty acid profile as the reported in this work (Table 2) is obtained when comparing the total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (SFA = 27 ± 1; MUFA = 23 ± 2 and PUFA = 43 ± 3; [12]). Based on the fatty acid profile obtained in this work, a molecular weight for sardine oil of 879 $g \cdot mol^{-1}$ has been estimated.

The free fatty acid (FFA) content of the sardine oil has been determined according to AOCS Official Method Ca 5a-40 [13]. An automatic titrator Methrom, model Titrando 905 was used. The FFA content for the refined sardine oil was 0.2 ± 0.1 % expressed as percentage of oleic acid. Due to the low free fatty acid content, the studied systems have been considered as a pseudoternary mixture as it will be explained in section 3.2.

The composition of the tie lines has been determined by using High-Temperature Gas Chromatography (HT-GC). 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 Methylpolisiloxane (65HT) as a stationary phase and Agilent Technologies 7683B Series automatic injector was used. The initial oven temperature was 120 ºC for 2 min, and was

then raised to 340 ºC at a rate of 15.0 ºCmin^{-1}. Then it was raised again to 365 ºC at a rate of 1.5 ºCmin^{-1} and held isothermally for 4 min. The injector temperature was kept at 380 ºC, while the detector temperature was 400 ºC. Helium (1 mLmin^{-1} column constant flow) was used as carrier gas. Split injection mode was used with a ratio of 1:40.

Tertiary alcohols and oil have been successfully quantified; however quantification of glycerol was not very reliable due to the bad resolution of the glycerol peak by using this kind of columns. In the last years HT-GC has been proved to be an affective technique to characterize TAGs from different vegetable sources. However, HT-GC could thermally degrade triacylglycerol species that contain polyunsaturated fatty acids, as in fish oils [14]. Nevertheless, in this work, the objective is the quantification of sardine oil in terms of total amount of oil and it is not expected to characterize the different TAGs of sardine oil. To quantify total amount of sardine oil a convenient calibration has been performed, as well as for the other components of the mixture. Although degradation of some TAGs species could have been taken place during the HT-GC analysis, this fact would be convenient corrected by using the calibration curve. To show the reliability of HT-GC to quantify sardine oil, composition of the three components in the tie line was also determined by density measurements. According to Maduro and Aznar [15], a density calibration curve was obtained from the cloud point determination as a function of the composition of the three components, although the composition of the third component can be obtained by a simple mass balance; therefore, for tie line measurements, density and only one composition, in this work tertiary alcohols composition, must be known to determine the composition of the other components through a density expression of the three components of the mixture and by material balance.

3. Results and discussion

3.1 Experimental data

The binodal curve and the density data at 303.2 K and 323.2 K for two systems: glycerol + sardine oil + tert-butanol and for glycerol + sardine oil + tert-pentanol are presented in Tables 3 and 4, respectively. In this work, 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 [16], has been used. The expression is:

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

The coefficients of the empirical Eq. 1 were determined by using the Marquardt algorithm. Table 5 lists the values of the adjustable parameters for each system at the two studied temperatures.

Figures 1 and 2 show the binodal curves for the systems studied in this work. The large two-phase region shows high immiscibility between glycerol and sardine oil even in the presence of the tertiary alcohols. The information provided by the binodal curve is necessary to optimize the amount of solvent used to create a homogeneous system containing the reactants, glycerol and sardine oil, taking into account the corresponding reactant molar ratio of the glycerolysis system. It can be observed that, in the temperature range covered in this work (303.2–323.2 K), the effect of temperature is not significant on decreasing the biphasic region. This behaviour has been also observed for ternary mixtures involving biodiesel and glycerol together with different alcohols [17-19]. This means that the reaction temperature of the glycerolysis system can be determined in terms of kinetic

parameters rather than in order to increase the mutual solubility of reactants. Additionally, it can be observed that at a given temperature, the miscibility region is bigger for tert-pentanol than for tert-butanol (see Figures 1 and 2).

The tie line data for the system glycerol + sardine oil + tert-butanol (or tert-pentanol) are presented in Tables 6 and 7 respectively. Composition of the tie lines was determined by HT-GC as well as by density calibration using the tertiary alcohols as key components. By using the parameters from Table 5, composition of sardine oil and glycerol could be also determined. Composition of sardine oil and glycerol calculated by density calibration and by HT-GC were similar. Deviations between both methods are lower than 2 % for the major compound in the equilibrium phases. However relative deviations found for the minor compound in the equilibrium phases are noticeably higher. From the shape of the binodal curves for both systems it can be observed that the amount of oil and glycerol in the glycerol and oil phases respectively is very small. This fact can be clearly observed in the composition of oil in the glycerol-rich phase, since for most experimental tie lines, oil content in the glycerol-rich phase is lower than 1 %. Therefore, relative deviations between the two analytical methods for oil and glycerol composition in the glycerol-rich phase and in the oil-rich phase, respectively, are higher. Relative deviation can reach values up to 50 %, or even higher, specially for oil content in the glycerol-rich phase. Values reported in Tables 6 and 7 correspond to the values obtained by HT-CG analysis.

Tie lines have been plotted in Figures 3-4 and 5-6 for the systems glycerol + sardine oil + tert-butanol and glycerol + sardine oil + tert-pentanol respectively. Tie lines show that for the tert-butanol system, the glycerol phase is richer in tert-butanol than the oil phase. However for the ternary system with tert-pentanol, the oil phase is richer in the tertiary

alcohol, although in this case the slope of the tie line is lower than in the system with tert-butanol. In the literature, it has been also shown a different thermodynamic behaviour of tert-butanol in relation to other 2-methyl-2-alcohols [20]. González et al. [20], in the extension of DISQUAC equation to different mixtures with tert-alcohols, concluded that the same quasichemical coefficients characteristic of each tert-alcohols series could be used, excluding tert-butanol. The different behaviour observed for tert-butanol and tert-pentanol could be related with the corresponding values of the dielectric constant for both tert-acohols ($\varepsilon_{\text{tert-butanol, 25ºC}}$ = 12.47; $\varepsilon_{\text{tert-pentanol, 25ºC}}$ = 5.78) [8]. Dielectric constant of a compound is an index of its polarity. Based on the different values of the dielectric constant for the two tert-alcohols studied in this work, it seems reasonable that tie lines for the tert-butanol system present positive slope since glycerol present a high value of the dielectric constant ($\varepsilon_{\text{glycerol, 25ºC}}$ = 42.5), specially when comparing this value with the dielectric constant of fish oil ($\varepsilon_{\text{fish oil, 25ºC}}$ = 2.76) [21]. The differences observed in the log P for tert-butanol and tert-pentanol ($\log P_{\text{tert-butanol}}$ = 0.35 and $\log P_{\text{tert-pentanol}}$ = 0.89) could also support the different slope observed in the tie lines [5].

The reliability of the tie-line data were tested by Othmer–Tobias equation [22]:

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

where w_2^{OP} is the mass fraction of sardine oil in the oil-rich phase and w_1^{GP} is the mass fraction of glycerol in the glycerol-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.

3.2 Data correlation

In the correlation of the liquid-liquid equilibrium data, the sardine oil has been treated as a single compound. This assumption implies that the different triacylglycerols present in the sardine oil behave in a similar way in the liquid-liquid system. Table 1 shows, along with the fatty acid composition of the sardine oil, the fatty acid composition of the corresponding oil-rich phase and glycerol-rich phase for a tie line obtained at 303.2 K in the system glycerol (1) + sardine oil (2) + tert-butanol (3). Fatty acid distribution in the oil-rich phase is similar to the fatty acid profile of sardine oil. Relative deviations range between 1.0 % for oleic acid and 5.6 % for palmitoleic acid. However, deviations found in the fatty acid profile of the glycerol-rich phase are higher due to low content of oil in the glycerol rich phase. Quantification of fatty acids depends on the detection limit of the detector in the GC. In fact, minority fatty acids in sardine oil, such as α-linolenic or eicosatrienoic acids, could not be properly detected in the glycerol-rich phase (see Table 6 and 7). Anyway, it can be assumed a similar distribution of the fatty acids in both phases. Similar results were obtained for the other tie lines studied in this work. The same approach has been used in the literature when dealing with different types of oil [23]. This result supports the previous assumption of considering the sardine oil as a single compound.

Experimental tie line data were correlated to the NRTL model. 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 is usual in most of the literature dealing with systems including different kinds of vegetable oils [6, 23, 24]. This way, the isoactivity criterion of phase equilibrium can be expressed in mass fraction units as follows:

$$\left(\gamma_i^w w_i\right)^{GP} = \left(\gamma_i^w w_i\right)^{OP} \qquad [3]$$

The mass fraction-scale activity coefficient γ_i^w must be related to the NRTL activity coefficient γ_i by the following equation [24-26]:

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

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.

Rodrigues et al and Gonçalves et al. [23, 27] showed 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

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

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

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

A_{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. The NRTL model has three parameters for each binary mixture: A_{ij} and A_{ji} represent the interaction energy between compounds i and j and α_{ij} is a nonrandomness parameter derived 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:

$$OF = \sum_{k}^{D} \sum_{j}^{M} \sum_{i}^{N-1} \left[\left(w_{ijk}^{GP,exp} - w_{ijk}^{GP,calc} \right)^2 + \left(w_{ijk}^{OP,exp} - w_{ijk}^{OP,calc} \right)^2 \right] \quad [9]$$

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 GP and OP refer to glycerol-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.

In the minimization procedure to calculate the equilibrium concentrations a procedure similar to the proposed by Reyes-Labarta et al. [28] has been followed. 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.

A constant value for the NRTL non-randomness parameter, α_{ij}, has been used in the fitting procedure reducing the number of adjustable parameters to just two for each pair of compounds. α_{ij} was set to different values between 0.1 and 0.5. The best results were achieved for the values presented in Table 8.

Figures 3-6 show the experimental liquid-liquid equilibrium data and the tie lines calculated by using the NRTL model with the parameters listed in Table 8 for the systems studied in this work. As it can be observed, the NRTL model was able to accurately describe the phase behavior. Comparison between experimental and calculated composition of each component in each of the two phases were made through root mean-square (RMS) deviation, given by following expression [27] (see Table 8):

$$\delta w = 100 \sqrt{\frac{\sum_{i}^{M} \sum_{j}^{N} \left(w_{ijk}^{GP,exp} - w_{ijk}^{GP,calc} \right)^2 + \left(w_{ijk}^{OP,exp} - w_{ijk}^{OP,calc} \right)^2}{2MN}} \quad [10]$$

Additionally, in the fitting procedure, α_{ij} was considered as an optimization parameter for each pair of substances (see Table 8). RMS was reduced from 0.58 to 0.48 and from 0.48 to 0.31 for systems with tert-butanol and tert-pentanol, respectively.

4. Conclusions

Liquid-liquid equilibrium for systems containing sardine oil, glycerol and tertiary alcohol as organic solvents has been studied. Specifically, both binodal and tie-lines were determined for the following ternary systems: glycerol + sardine oil + tert-butanol and glycerol + sardine oil + tert-pentanol at two different temperatures, 303.2 K and 323.2 K.

The biphasic region seems not to be significantly reduced by increasing temperate in the range covered in this work (303.2 K – 323.2 K). The miscibility region is bigger for tert-pentanol than for tert-butanol. The experimental data presented in this work are of interest in the correct design of a glycerolysis system to create a homogenous reaction system.

The reliability of the tie-lines was verified 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 squared deviation lower than 1%.

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Table 1. Density of the pure components. [a]

	$\rho_{exp.}$/kg·m^{-3}		$\rho_{literature}$/kg·m^{-3}
Component	303.2 K	323.2 K	
Glycerol	1252.11	1240.28	$1255.12^{303.15}$ [8]
Tert-butanol	775.48	754.03	$775.45^{303.15}$ [8]
			$775.85^{303.15}$ [9]
Tert-pentanol	801.33	783.30	$805.0^{298.15}$ [8]
Sardine oil	922.18	908.42	---

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

Table 2. Fatty acid composition of the sardine oil and for the two phases (oil and glycerol phases) of a tie line at temperature T = 303.2 K for the system glycerol (1) + sardine oil (2) + tert-butanol (3).[a]

			Tie line	
Fatty acid		Sardine oil, %	OP, %	GP, %
Myristic	C14:0	7.6	7.7	10.7
Palmitic	C16:0	18.1	19.1	22.1
Palmitoleic	C16:1	8.9	8.9	11.3
Stearic	C18:0	3.6	3.8	2.2
Oleic	C18:1n-9	10.0	10.1	11.7
Vaccenic	C18:1n-7	3.8	3.9	2.2
Linoleic cis (LA)	C18:2n-6	2.5	2.5	nd
α-Linolenic (ALA)	C18:3n-3	1.1	1.1	nd
Steriadonic	C18:4n-3	3.6	3.4	2.9
Eicosatrienoic	C20:3n-3	1.7	1.7	nd
Eicosapentaenoic (EPA)	C20:5n-3	25.9	25.0	26.3
Docosapentaenoic (DPA)	C22:5n-3	2.7	2.7	nd
Docosahexaenoic (DHA)	C22:6n-3	10.6	10.3	10.6

GP: glycerol phase; OP: oil phase

[a] Standard uncertainties *u* are *u*(percentage) = 0.5.

Table 3. Experimental (liquid + liquid) equilibrium weight fractions *w* (binodal curve data) for the system glycerol (1) + sardine oil (2) + tert-butanol (3) at temperature T = 303.2 K and 323.2 K.[a]

w_1	w_2	w_3	ρ/ kg·m^{-3}
	303. 2 K		
0.0016	0.9984	0.0000	923.48
0.0069	0.8648	0.1283	903.29
0.0256	0.7163	0.2581	884.94
0.0365	0.6056	0.3579	870.28
0.0521	0.5144	0.4335	864.06
0.0586	0.4809	0.4605	861.47
0.0717	0.4039	0.5244	854.94
0.1044	0.2851	0.6105	849.67
0.1271	0.1953	0.6776	845.95
0.1649	0.1149	0.7202	851.99
0.2076	0.0689	0.7235	858.72
0.3122	0.0153	0.6725	887.25
0.3330	0.0168	0.6502	903.10
0.4244	0.0039	0.5717	934.64
0.7361	0.0006	0.2633	1092.49
0.9967	0.0000	0.0033	1244.91
	323. 2 K		
0.0043	0.9769	0.0188	906.54
0.0100	0.9048	0.0852	897.25
0.0302	0.6840	0.2858	867.26
0.0378	0.6298	0.3324	862.68
0.0471	0.5743	0.3786	856.39
0.0612	0.5293	0.4095	853.12
0.1037	0.3825	0.5138	843.60
0.1480	0.2437	0.6083	841.47
0.1844	0.1713	0.6443	846.82
0.2542	0.0723	0.6735	855.92
0.3361	0.0285	0.6354	888.24

0.4725	0.0047	0.5228	942.45
0.4995	0.0042	0.4963	954.57
0.8011	0.0019	0.1970	1095.16

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

Table 4. Experimental (liquid + liquid) equilibrium weight fractions *w* (binodal curve data) for the system glycerol (1) + sardine 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		
0.0027	0.9056	0.0917	909.61
0.0025	0.8665	0.1310	905.46
0.0045	0.8247	0.1708	900.43
0.0021	0.7361	0.2618	893.52
0.0159	0.6315	0.3526	885.58
0.0523	0.5175	0.4302	881.25
0.0683	0.4783	0.4534	879.86
0.1105	0.3756	0.5139	881.20
0.1635	0.2539	0.5826	888.48
0.2192	0.1589	0.6219	893.66
0.3038	0.0640	0.6322	914.05
0.4170	0.0199	0.5631	953.34
0.4700	0.0101	0.5199	973.88
0.6544	0.0033	0.3423	1058.99
	323. 2 K		
0.0051	0.8414	0.1535	886.88
0.0066	0.8074	0.1860	883.61
0.0223	0.6274	0.3503	868.91
0.0854	0.4406	0.4740	865.18
0.1561	0.2969	0.5470	869.77
0.1843	0.2490	0.5667	874.89
0.2155	0.2005	0.5840	881.34
0.2817	0.1179	0.6004	893.46
0.3124	0.0885	0.5991	902.49
0.4948	0.0147	0.4905	969.68
0.7151	0.0043	0.2806	1076.68
0.8462	0.0017	0.1521	1143.25

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

Table 5. Parameters of equation 1

System	T /K	Parameters		r^2
Glycerol (1) + sardine oil (2) + tert-butanol (3)	303.2	A_{12}= 562.24	B_{12}= 606.77	0.9997
		A_{13}= -181.51	B_{13}= 8.20	
		A_{23}= -77.64	B_{23}= 70.33	
	323.2	A_{12}= -828.77	B_{12}= -989.29	0.9986
		A_{13}= -168.48	B_{13}= -199.14	
		A_{23}= 19.24	B_{23}= -68.10	
Glycerol (1) + sardine oil (2) + tert-pentanol (3)	303.2	A_{12}= -370.85	B_{12}= 334.08	0.9998
		A_{13}= -163.54	B_{13}= -11.81	
		A_{23}= 57.31	B_{23}= -85.09	
	323.2	A_{12}= -386.53	B_{12}= 37.75	0.9998
		A_{13}= -160.66	B_{13}= -36.12	
		A_{23}= 39.15	B_{23}= -78.65	

Table 6. Experimental (liquid + liquid) equilibrium data for the system glycerol (1) + sardine oil (2) + tert-butanol (3) for weight fractions w at temperature T = 303.2 K and T = 323.2 K.[a]

Overall composition			Glycerol-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				
0.2503	0.2504	0.4993	0.3470	0.0397	0.6133	0.0125	0.7508	0.2367
0.3003	0.2998	0.3999	0.4412	0.0223	0.5365	0.0169	0.7930	0.1901
0.3504	0.3499	0.2997	0.5690	0.0191	0.4119	0.0161	0.8243	0.1596
0.4002	0.3999	0.1999	0.7280	0.0020	0.2700	0.0075	0.8487	0.1438
0.4485	0.4500	0.1015	0.8594	0.0191	0.1215	0.0055	0.8744	0.1201
				323.2 K				
0.2502	0.2504	0.4994	0.3673	0.0238	0.6089	0.0245	0.6994	0.2761
0.2998	0.3003	0.3999	0.4843	0.0095	0.5062	0.0185	0.7323	0.2492
0.3499	0.3501	0.3000	0.6070	0.0076	0.3854	0.0106	0.7539	0.2355
0.3984	0.3963	0.2053	0.7491	0.0066	0.2443	0.0062	0.8299	0.1639
0.4500	0.4501	0.0999	0.8944	0.0004	0.1052	0.0051	0.9044	0.0905

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

Table 7. Experimental (liquid + liquid) equilibrium data for the system glycerol (1) + sardine oil (2) + tert-pentanol (3) for weight fractions w at temperature T = 303.2 K and T = 323.2 K.[a]

Overall composition			Glycerol-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				
0.3506	0.3502	0.2992	0.7451	0.0027	0.2522	0.0303	0.6238	0.3459
0.4002	0.3907	0.2091	0.8470	0.0121	0.1409	0.0108	0.7155	0.2737
0.4199	0.4203	0.1598	0.8776	0.0158	0.1066	0.0123	0.7729	0.2148
0.4492	0.4433	0.1075	0.9182	0.0085	0.0733	0.0074	0.8476	0.1450
				323.2 K				
0.3505	0.3501	0.2994	0.7922	0.0007	0.2071	0.0389	0.6117	0.3494
0.3797	0.3803	0.2400	0.8545	0.0048	0.1407	0.0257	0.6622	0.3121
0.3994	0.4003	0.2003	0.8914	0.0054	0.1032	0.0105	0.7110	0.2785
0.4492	0.4486	0.1022	0.9411	0.0015	0.0574	0.0121	0.8317	0.1562

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

Table 8. NRTL parameters for the systems: glycerol (1) + sardine oil (2) + tert-butanol (3) and for the glycerol (1) +sardine oil (2) + tert-pentanol (3)

Pair	A_{ij}/K	A_{ji}/K	α_{ij}	RMS
System: glycerol (1) + sardine oil (2) + tert-butanol (3)				
12	2781.9	1365.1	0.5	
13	620.6	941.1	0.5	0.58
23	-155.6	1649.8	0.5	
12	5382.9	3825.9	0.309	
13	720.0	1045.3	0.429	0.48
23	614.5	1506.2	0.501	
System: glycerol (1) + sardine oil (2) + tert-pentanol (3)				
12	4219.8	6102.6	0.4	
13	840.1	1010.3	0.4	0.48
23	48.1	1744.1	0.4	
12	5012.5	2523.4	0.362	
13	841.1	964.56	0.408	0.31
23	439.4	1626.4	0.454	

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Figure 1. Phase diagram of the system glycerol + sardine oil +tert-butanol (○ 303.2 K; ▲ 323.2 K)

Figure 2. Phase diagram of the system glycerol + sardine oil +tert-pentanol (○ 303.2 K; ▲ 323.2 K)

Figure 3. Liquid-liquid equilibrium for the system glycerol + sardine oil + tert-butanol at 303.2 K: ---, binodal curve; ♦ tie lines; — NRTL.

Figure 4. Liquid-liquid equilibrium for the system glycerol + sardine oil + tert-butanol at 323.2 K: ---, binodal curve; ♦ tie lines; — NRTL.

Figure 5. Liquid-liquid equilibrium for the system glycerol + sardine oil + tert-pentanol at 303.2 K: ---, binodal curve; ♦ tie lines; — NRTL.

Figure 6. Liquid-liquid equilibrium for the system glycerol + sardine oil + tert-pentanol at 323.2 K: ---, binodal curve; ♦ tie lines; — NRTL.

Figure 7. Othmer-Tobias plot for the system glycerol (1) + sardine oil (2) + tert-butanol (3) at 303.2 K (●, r^2 = 0.9683) and 323.2 K (○, r^2 = 0.9704) and for the system glycerol (1) + sardine oil (2) + tert-pentanol (3) at 303.2 K (■, r^2 = 0.9775) and 323.2 K (□, r^2 = 0.9701).

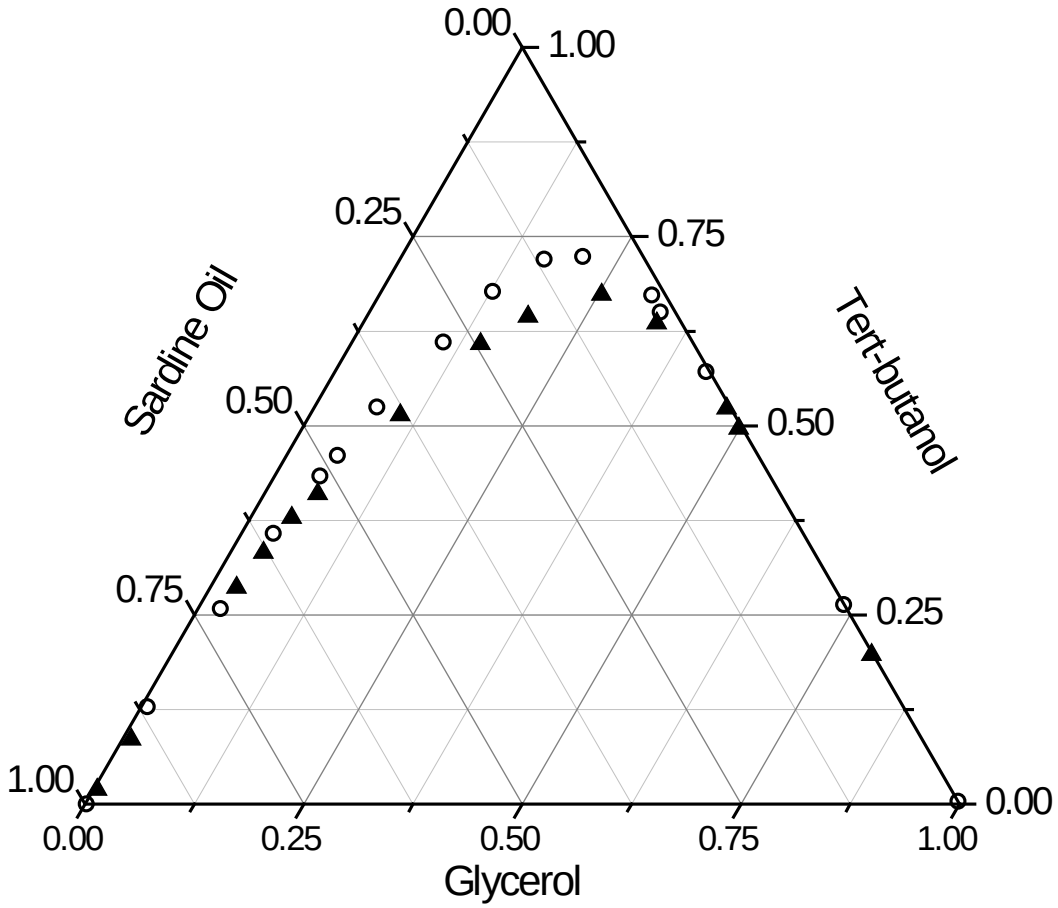


Figure 1. Phase diagram of the system glycerol + sardine oil + tert-butanol (○ 303.2 K; ▲ 323.2 K)

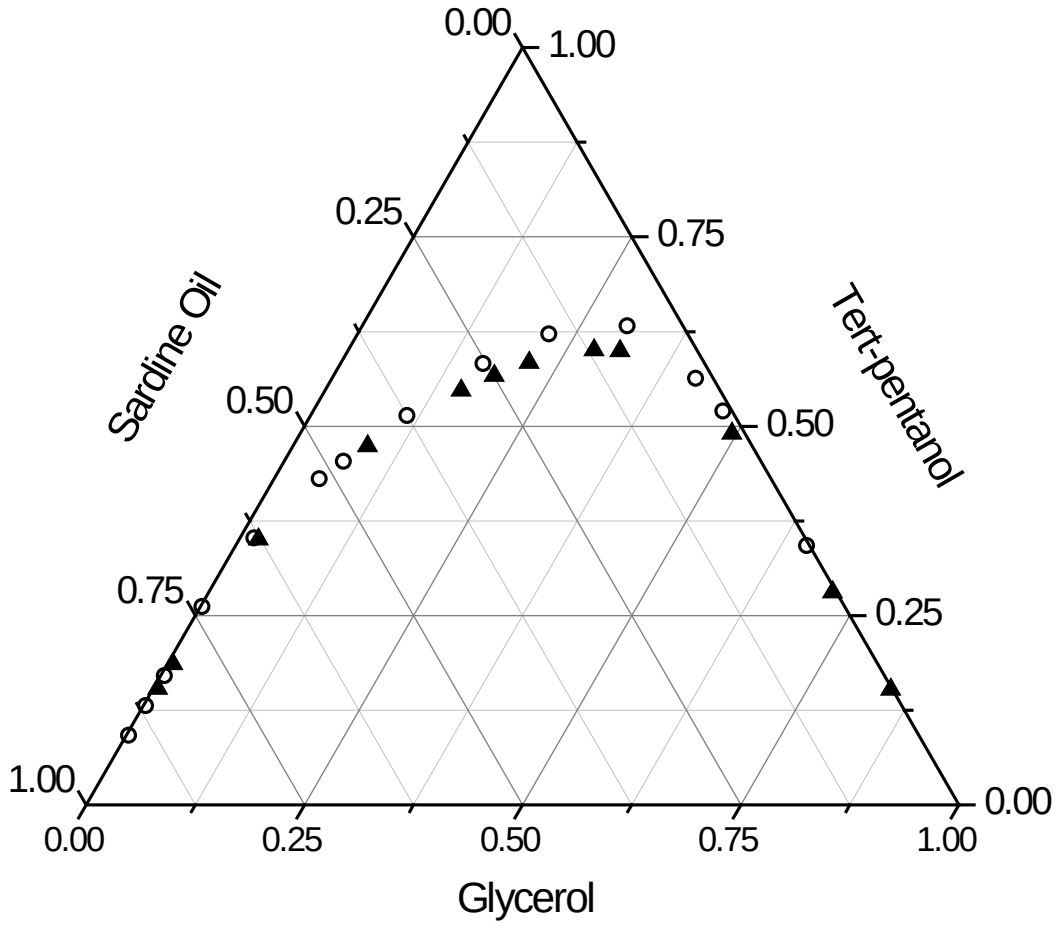


Figure 2. Phase diagram of the system glycerol + sardine oil + tert-pentanol (○ 303.2 K; ▲ 323.2 K)

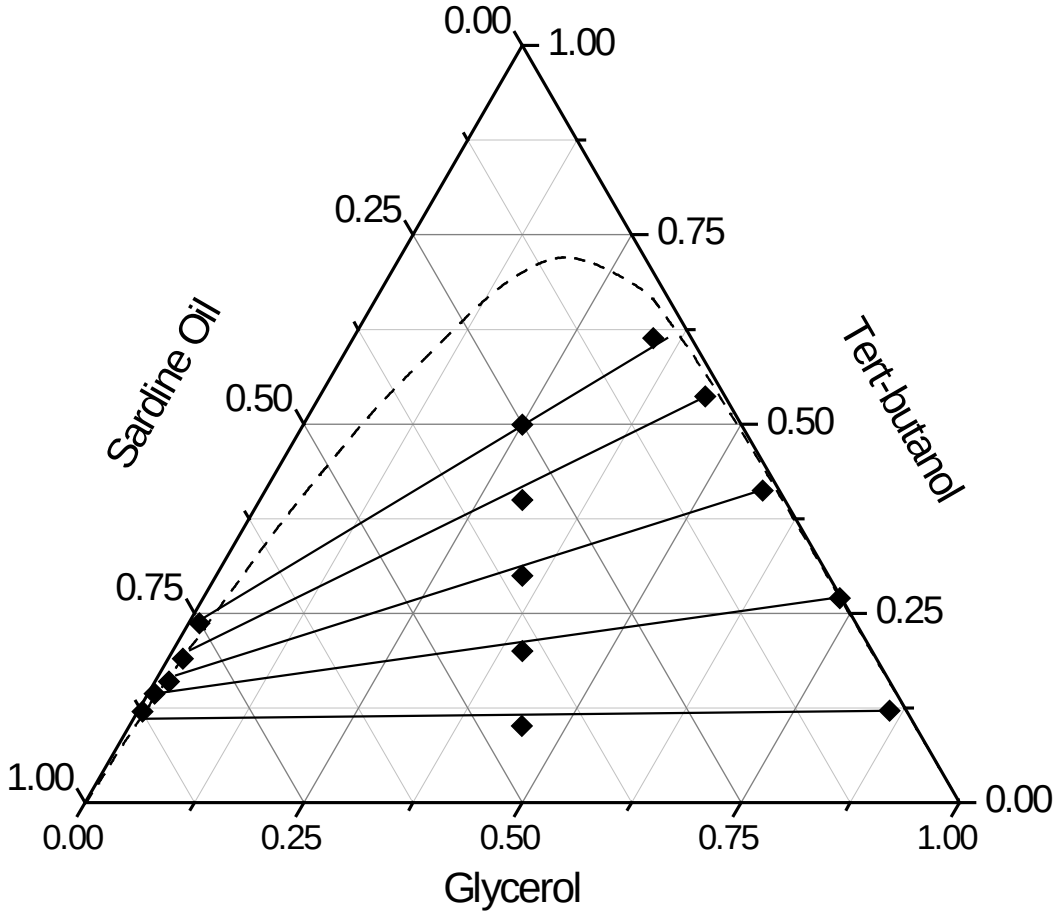
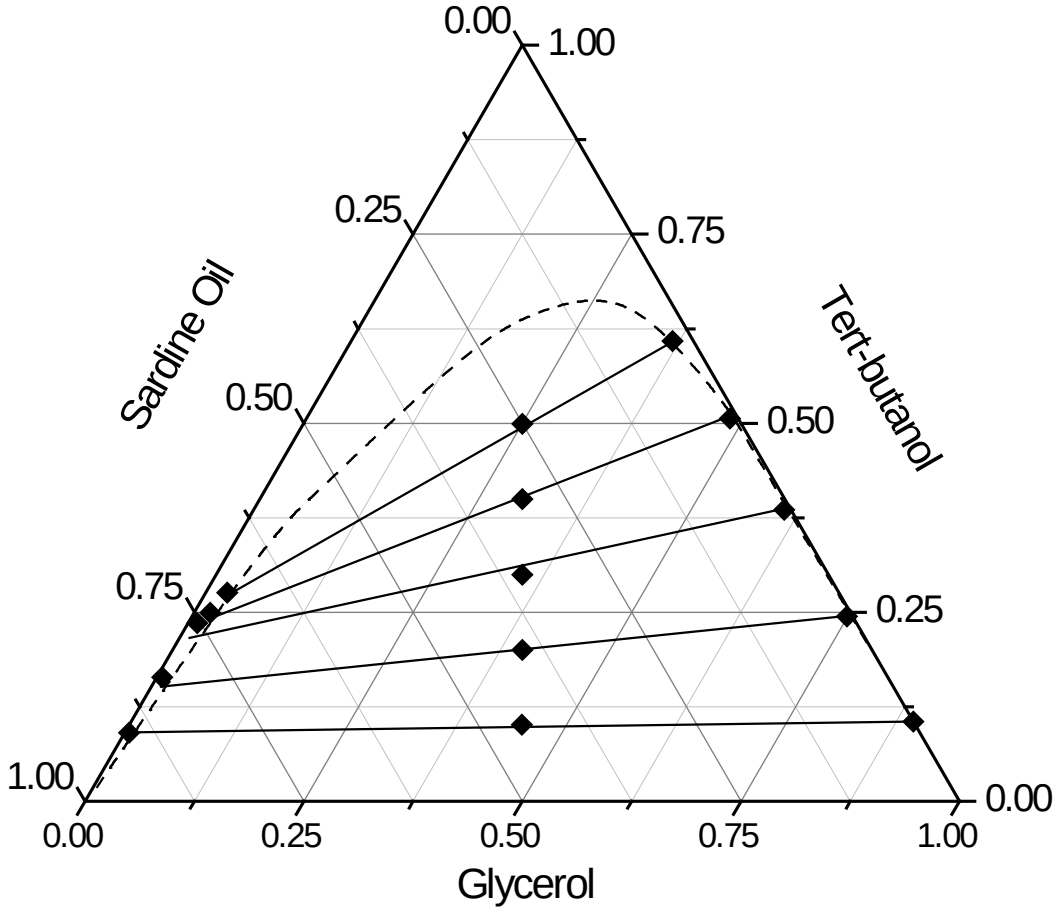


Figure 3. Liquid-liquid equilibrium for the system glycerol + sardine oil + tert-butanol at 303.2 K: ---, binodal curve; ♦ tie lines; — NRTL (α = 0.5).



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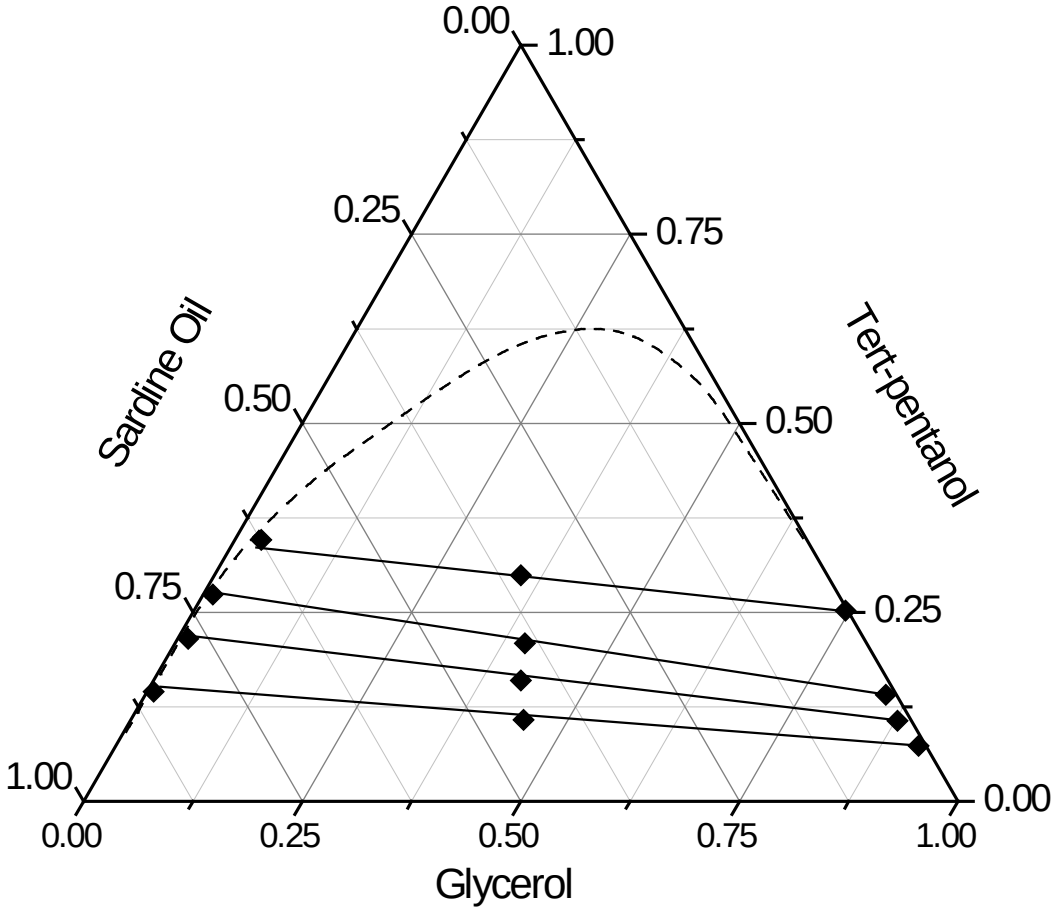


Figure 5. Liquid-liquid equilibrium for the system glycerol + sardine oil + tert-pentanol at 303.2 K: ---, binodal curve; ♦ tie lines; — NRTL (α = 0.4).

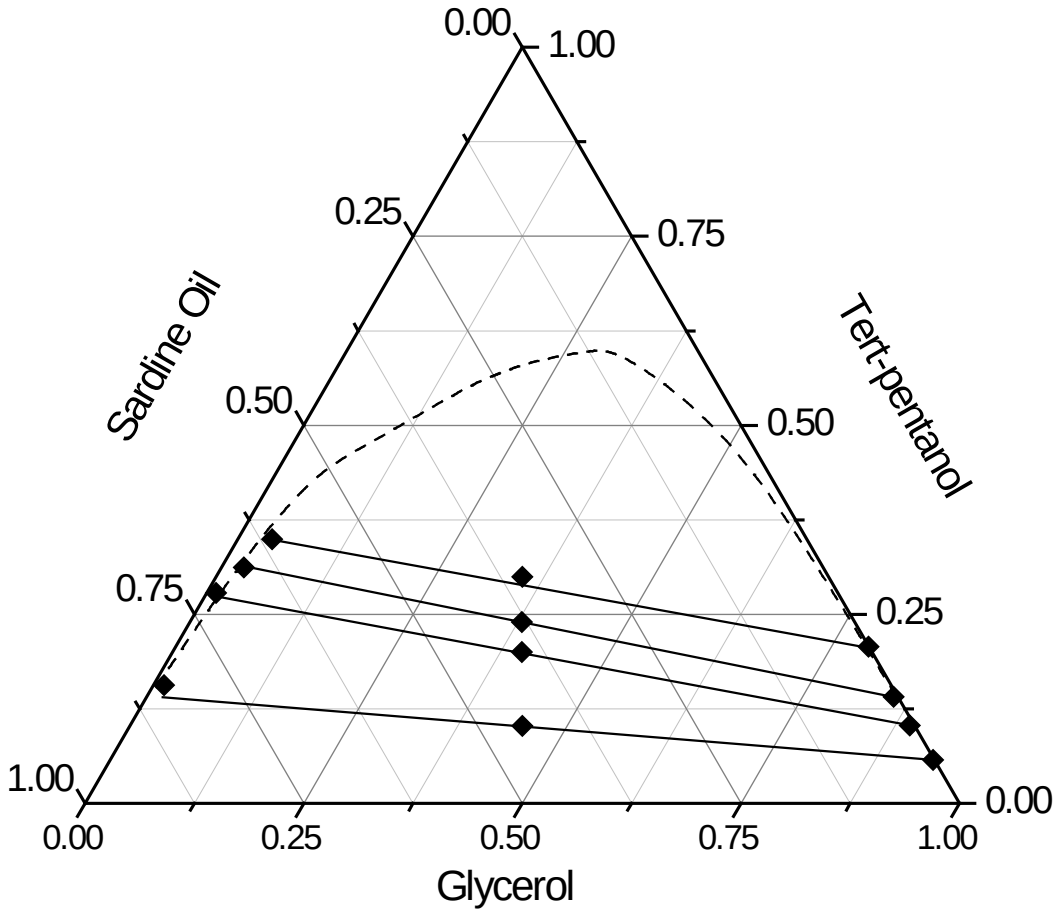
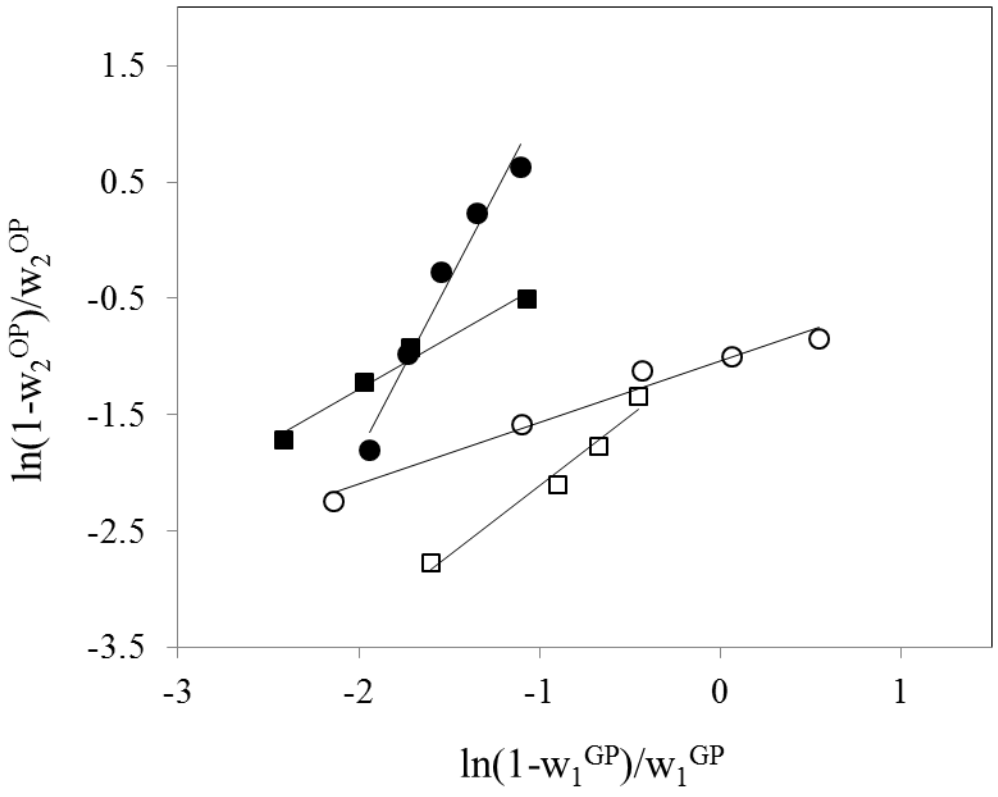


Figure 6. Liquid-liquid equilibrium for the system glycerol + sardine oil + tert-pentanol at 323.2 K: ---, binodal curve; ♦ tie lines; — NRTL (α = 0.4).



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