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# Liquid-liquid equilibrium of the system glycerolized olive oil + ethanol + glycerol for diacylglycerol enrichment

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ABSTRACT

Edible oils enriched with diacylglycerols are promising choices for healthy cooking oils, and for that reason production and separation processes regarding these molecules have received great attention. This work reports experimental results and thermodynamic modelling of liquid-liquid equilibrium of systems containing glycerolized olive oil (source of triacylglycerols, diacylglycerols, monoacylglycerols and free fatty acids), glycerol and ethanol. The LLE experimental data were used to estimate UNIQUAC binary interaction parameters. The mathematical model calculated the liquid phase's compositions by minimizing the Gibbs free energy of the system, using a combination of stochastic and deterministic optimization methods in order to avoid local minima. The model was well fitted to the experimental data, with a root mean square deviation of 1.43 wt% between experimental and calculated data. The results indicate the feasibility of diacylglycerol separation from other triacylglycerols and free fatty acids through liquid-liquid phase separation using glycerol and ethanol as solvents.

## 1. Introduction

Despite the fact that lipids have important roles in the body as source of energy, essential fatty acids and fat-soluble vitamins, high fat diets are also associated to obesity and cardiovascular diseases [1]. On the other hand, low fat diets are usually not well tolerated by consumers due their lack in palatability [2]. In this sense, there is a growing interest in investigating functional oils that can be used as a replacement of conventional oils, without compromising palatability, and at the same time avoid health problems related high fat diets.

Oils and fats are basically composed by triacylglycerols (TAG), but they also present diacylglycerols (DAG), monoacylglycerols (MAG) and free fatty acids (FFA) as minor components. Studies have shown that the consumption of an enriched in DAG oil in place of conventional rich TAG oils resulted of reduction post prandial lipid levels and visceral fat accumulation of consumers [3]. For this reason, in addition to its similarity to conventional oils in terms of appearance and palatability [2], DAG rich oils have attracted the attention of researchers [4–6].

DAG can be produced directly from TAG by partial hydrolysis [1,7], glycerolysis [8] or even thought a complete hydrolysis of TAG followed by esterification of free fatty acids with glycerol. In all cases, besides the DAG formed, TAG, MAG and FFA will be present in the reaction product, which can be further separated from DAG by distillation at high temperatures and low pressures [4,9]. However, it is know that the exposure of DAG and MAG to high temperatures is related to the formation of Glycidyl fatty acids esters (GEs) [10] e 3-monochloropropane-1,2-diol fatty acid esters (3-MCPDEs) [11]. Although there is no evidence that GEs and 3-MCPDEs are toxic for humans or animals, their presence in food products is undesirable due the toxicity of their hydrolysates [10].

Voll et al. [12] studied the liquid-liquid phase separation of TAG, DAG, MAG and FFA from hydrolyzed palm oil with ethanol and water. The experiments were performed at a relatively low temperature (50 °C) and showed the potential of liquid-liquid extraction as a suitable method for enriching edible oil with DAG avoiding the use of high temperatures.

It is worth mentioning that GEs and 3-MCPDEs are formed during deodorization process in the production of conventional refined vegetables oils. For that reason, extra virgin olive oil (EVOO), which is unrefined oil, is likely a proper choice of TAG source for production of rich DAG oil free of contaminants. This work was than focused on the experimental and thermodynamic investigation of the (liquid + liquid) equilibrium of TAG, DAG, MAG and FFA (obtained after glycerolysis of EVOO) with ethanol and glycerol. Such results can be useful for design and optimization

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of a liquid-liquid extraction process aiming the DAG enrichment in olive oil.

## 2. Materials and methods

### 2.1. Materials

Commercial extra virgin olive oil from Andorinha brand was purchased at a local market in the city of Curitiba (Paraná, Brazil) and used as the substrate of glycerolysis reaction. Commercial enzyme Lipozyme RM IM (Sigma-Aldrich) was used as catalyst for the glycerolysis reaction. All other materials used for reactions, analytical methods and LLE measurements are presented in Table 1.

**Table 1**
Sample table.

| Chemical name | CAS number | Source | Mass fraction purity* |
|---|---|---|---|
| Glycerol | 56-81-5 | Biotec | 0.995 |
| Ethanol | 64-17-5 | Neon | 0.998 |
| Hexane | 110-54-3 | Neon | 0.950 |
| Heptane | 142-82-5 | Sigma | >0.99 |
| Acetic acid | 64-19-7 | Neon | 0.998 |
| Ammonium acetate | 631-61-8 | Neon | >0.97 |
| Acetylacetone | 123-54-6 | Neon | 0.995 |
| Sodium periodate | 7790-28-5 | Vetec | 0.998 |
| Monoolein | 111-03-5 | Sigma | >0.99 |
| Diolein | 2465-32-9 | Sigma | >0.99 |
| Triolein | 122-32-7 | Sigma | >0.99 |
| Tricaprin | 621-71-6 | Sigma | >0.99 |
| Oleic acid | 112-80-1 | Sigma-Aldrich | >0.99 |
| Lauric acid | 143-07-7 | Sigma-Aldrich | >0.99 |
| MSTFA (*N*-methyl-*N*-trimethysiltrifluoroacetamide) | 24589-78-4 | Sigma-Aldrich | >0.985 |
| Helium | 7440-59-7 | White Martins | 0.999990 |

* All reagents were used as received without purification.

### 2.2. Methods

#### 2.2.1. Olive oil glycerolysis

The glycerolysis reaction of olive oil for producing acylglycerols was performed in a 500 mL glass reactor, on witch 250 g of oil, 26.45 g of glycerol and 2.76 g of immobilized enzyme were added. The reaction medium was maintained at 45 °C and mechanically stirred at 340 rpm for 48 h. Hexane was added in the reaction product for decreasing its viscosity and this mixture was filtered for separating the immobilized enzyme from the liquid. Glycerol was separated from the hexane rich lipid phase, which was further rotary evaporated to remove hexane and obtaining a reaction product composed by TAG, DAG, MAG and FFA. This product was maintained in an oven for 30 min at 120 °C in order to make sure that no active enzyme would be present in the glycerolized oil avoiding undesirable reactions during liquid-liquid equilibrium experiments.

#### 2.2.2. Analytical methods

Glycerol was quantified by the spectrophotometric method proposed by Bondioli and Bella [13]. Acylglycerols and free fatty acids were quantified by Gas Chromatography in a GC-2010 Plus (Shimadzu) equipped with an autosampler, a split/splitless injector at 1:10 split ratio, a flame ionization detector (FID) and a Select biodiesel (Agilent) capillary column (15 m × 0.32 mm × 0.10 μm). The injection and detector temperatures were 380 °C and 400 °C, respectively. The oven temperature was programmed to increase from 50 °C to 180 °C (15 °C.min$^{-1}$), followed by a rate of 7 °C.min$^{-1}$ up to 230 °C, and 10 °C.min$^{-1}$ up to 380 °C, remaining for 6 min, resulting in a total running time of 36.81 min. Helium was used as carrier gas in a flow rate of 29.2 cm$^3$.min$^{-1}$. The procedure used to prepare samples, standards and quantify TAG, DAG, MAG and FFA was based on EN 14105 of the European Committee for Standardization.

#### 2.2.3. LLE measurements

Liquid-liquid equilibrium (LLE) experiments were conducted in a jacket-glass cell of 100 cm$^3$ at 35 °C in an ultra-thermostatic bath where the equilibrium temperature was controlled. Specific amounts of glycerolized olive oil, ethanol and glycerol were added in the cell and vigorously magnetically stirred for 1 h in order to promote contact between the components. Although it was clear that after the first hours of rest the two phases and the interface were already well-defined, samples were only collected from each phase after 8 h. Three samples were collected from each phase and then stored under refrigeration until analysis. The first sample was weighed and dried in an oven (120 °C until constant mass) so the mass of ethanol in that samples could be calculated. The second sample was used for glycerol quantification. The last sample was used for quantifying TAG, DAG, MAG and FFA by GC.

The LLE experiments were carried out at atmospheric pressure ($p$ = 91.2 kPa with standard uncertainty $u(p)$ = 0.3 kPa (Curitiba City, State of Parana, Brazil). The two liquid phases, considered to be in equilibrium, were sampled and then analyzed by the methodology described above. The uncertainties of the mole fractions, temperature and pressure measurements were estimated using the method of standard uncertainties (Type B) as suggested by Taylor and Kuyatt [14] and are reported in tables with the experimental data.

#### 2.2.4. LLE modelling

The experimental data obtained in this work were used to estimate the UNIQUAC parameters [15]. The values of $r_i$ and $q_i$ were calculated accordingly to our previous work [12].

The UNIQUAC binary interaction parameters were estimated through the minimization of the sum of the square differences between experimental and calculated data:

$$of = \sum_{p}^{NP}\sum_{n}^{NT}\sum_{i}^{NC}\left(w_{in}^{p,exp} - w_{in}^{p,calc}\right)^2 \tag{1}$$

where $w_{i.n}^{p,exp}$ and $w_{i.n}^{p,calc}$ are the experimental and calculated mass fraction values of the $i$ component at the $n$ tie line in the $p$ phase, respectively. The values of $w_{i.n}^{p,calc}$ were calculated by Gibbs free energy minimization of systems with known overall compositions. Since it is considered that no reaction happens together with the phase separation, there is no need for taking into account the terms with chemical potential values of each component when writing the Gibbs free energy to be minimized. Therefore, Eq. (2) was minimized for mass fractions calculated [12]:

$$G^* = \sum_{i=1}^{NC}\sum_{p=1}^{NP} n_i^p \cdot \left(\ln(n_i^p) - \ln(nt^p)\right) + \sum_{i=1}^{NC}\sum_{p=1}^{NP} n_i^p \cdot \ln(\gamma_i^p) \tag{2}$$

where $n_i^p$ is the number of moles of each component $i$ on phase $p$, $nt^p$ is the total number of moles of phase $p$, and $\gamma_i^p$ is activity coefficient of $i$ in phase $p$. For each tie line, the values of $w_{i.n}^{p,calc}$ (necessary for Eq. (1)) are directly calculated from $n_i^p$, $nt^p$ and molar masses information. The free fatty acid molar mass was considered to be equal to 277.875 g mol$^{-1}$, since we used the same oil used in the work of Lima et al. [16]. The molar mass of acylglcyerols were determined assuming the triacylglycerol, diacylglycerol and