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Assessment of liquid–liquid phase separation in the composition and oxidation stability of partially hydrolyzed olive oil

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ABSTRACT

Process optimization for producing diacylglycerol-enriched oils has received great attention due the importance of these products for healthier and more balanced diets. This work reports experimental data of liquid-liquid phase equilibrium of hydrolyzed olive oil (which comprises on its composition triacylglycerols, diacylglycerols, monoacylglycerols and free fatty acids) with ethanol and water, and subsequent analysis of oxidative stability of the oil obtained from the liquid phases. Liquid-liquid equilibrium experimental data obtained were modeled using the UNIQUAC thermodynamic model, which was well fitted to experimental data with a root mean square deviation of 1.76 wt%. The results obtained demonstrated the potentiality of liquid-liquid extraction as a suitable procedure for olive oil enrichment in diacylglycerol, after a hydrolysis procedure. It was also observed that liquid-liquid phase separation has a significant effect on the content of important antioxidants present in the olive oil, what affects the oxidative stability of the samples submitted to the liquid phase extraction. From the results obtained in this study, it has been also noticed that the acylglycerols and free fatty acids profile in of the oil samples presented a major rule on their stabilities.

1. Introduction

Despite that it is well known that high fat/oil diets are risk factors for human health (Cheong et al., 2007), low fat/oil diets are usually hard to be tolerated due the loss of palatability associated to fats taste (Kawashima et al., 2008). For that reason, alternatives for conventional fat/oil, which are mainly composed by triacylglycerol (TAG) molecules, have been investigated mainly focusing diacylglycerol (DAG) rich oils (Flickinger and Matsuo, 2003; Kasamatsu et al., 2005; Ming et al., 2007; Voll et al., 2013; Awadallak et al., 2013; Zang et al., 2017). DAG is a natural minor component of several fats and oils, and studies have shown that DAG rich oils were able to reduce post prandial lipid levels and visceral fat accumulation when compared to conventional triacylglycerol oils (Maki et al., 2002). DAG rich oils are well accepted by consumers since, despite the differences in the metabolism between TAG and DAG, the latter is very similar to TAG in terms of appearance and palatability (Kawashima et al., 2008).

DAG rich oil was initially commercialized in 1999, when it was recognized as FOSHU (food for special health use) in Japan (Takase, 2007), being later approved as GRAS (generally recognized as safe) in the United States in 2000. However, DAG rich oil had it sales suspended in 2009 because of its “high levels” of glycidyl fatty acid esters (GEs), which are potential carcinogenic. In the commercial process (Request N° EFSA-Q-2004-089), diacylglycerols were produced by esterification of fatty acids with glycerol or monoacylglycerol in the presence of an immobilized lipase, with a subsequent refining process, which includes distillation (200 °C) and deodorization (230 °C) steps. Since the presence of GEs in oils is related to DAG and monoacylglycerols (MAG) exposure to high temperatures (Cheng et al., 2016), conventional refining process is unfeasible for DAG purification.

DAG-enriching by liquid-liquid extraction can be considered a good alternative to distillation process since it can be performed in mild conditions of temperature and pressure, avoiding energy costs

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and undesirable reactions during the process. Voll et al. (2013) studied the liquid-liquid equilibrium of hydrolyzed palm oil with hydroalcoholic solutions and showed that due the difference in the polarity of DAG and other acylglcyerols presented in oil, a DAG −rich phase can be obtained from the mixture of TAG, DAG, MAG and free fatty acids (FFA). The authors observed that the partition coefficients follow the trend TAG > DAG > FFA > MAG, and the differences between the coefficients of these compounds increase with the water increase in the system. These results are in agreement with reported by Monick and Treybal (1956), who studied the liquid-liquid extraction of MAG, DAG and TAG with aqueous ethanol. The authors concluded that acylglycerols with a higher number of polar hydroxyl groups (and therefore with higher polarity) have higher affinity with the solvent phase. Shiozawa et al. (2015) obtained liquid-liquid equilibrium data for systems containing refined oils, ethanol and small amounts of DAG, MAG and FFA. Those authors observed that MAG and FFA presented higher affinity for the solvent phase while DAG presented for the oil phase, independently of oil source (soybean, cottonseed or rice bran) and the FFA type (oleic or linoleic). This finding indicates that the structural differences between free fatty acids, mono, di and triacylglcyerols (instead of the fatty acid profile) are the primary factor in liquid-liquid phase separation of acylglycerols with polar solvents.

This present work is focused on the experimental and thermodynamic investigation of liquid-liquid equilibrium of TAG, DAG, MAG and FFA obtained from olive oil hydrolysis with ethanol and water. Special focus is given to the effects of liquid-liquid equilibrium and partition phase on the oxidative stability of the oil samples. A better understanding of how the liquid-liquid phase separation procedure affects the antioxidants content of the product and therefore its stability can be useful in establishing suitable conditions for effective enrichment of DAG without compromising its quality and its stability. Extra virgin olive oil was chosen as substrate in this work because it is non-refined oil, with high content of natural antioxidants and monounsaturated fatty acids, which are desirable characteristics for edible oils. Furthermore, olive oil is one of the edible oils with higher content in one particular fatty acid (oleic acid). This makes the assumptions that TAGs, DAGs, MAGs and FFAs can be treated as pseudo-components more reliable for modeling purposes.

2. Materials and methods

2.1. Materials

The oil used in this work was an extra virgin olive oil from Andorinha brand (measured acidity: 1 wt%; saturated fatty acids: 15.8 wt%, unsaturated: 77.5 wt%, polyunsaturated: 6.7 wt%, as declared by the supplier), which was purchased from a local market in Curitiba, Brazil. Commercial enzyme Lipozyme RM IM (activity >30 U/g, where 1 U corresponds to the amount of enzyme which sets free 1 μmol stearic acid per minute at pH 8.0 and 70 °C for tristearin as substrate) used for olive oil hydrolysis was purchased from Sigma-Aldrich. n-Hexane (95%, Panreac) was used for the filtration of the supported enzyme from the hydrolyzed oil. Sodium hydroxide and phenolphthalein (both from Vetec) were used in the acidity determinations with volumetric titration method. Absolute ethanol (99.8%, Sigma-Aldrich) was used for acidity determination and as a solvent in the liquid-liquid phase equilibrium experiments, as well as distillate water. All chemicals were used as received and without further treatment.

2.2. Methods

2.2.1. Experimental procedures

2.2.1.1. Hydrolysis of olive oil. The extra virgin olive oil was submitted to a partial hydrolysis to produce a mixture of triacylglycerols, diacylglycerols, monoacylglycerols and free fatty acids. 400 g of olive oil and 8.4 g of water were added into a magnetically stirred (300 rpm) glass reactor, which was heated by a thermostatic bath to maintain the temperature at 45 °C. After the system reached the desired temperature, 6.5 g of enzyme Lipozyme RM IM was added to start the reaction for 72 h. This procedure was carried out as suggested by Voll et al. (2012) in order to maximize DAG production during hydrolysis. At the end of the reaction, the reactor content was vacuum filtered with the addition of n-hexane to decrease the sample viscosity. The n-hexane was removed from the hydrolyzed oil by rotary evaporation under vacuum and at 85 °C until no condensed was observed.

2.2.1.2. Liquid-liquid equilibrium measurements. Liquid-liquid phase equilibrium measurements involved a systems composed by oil + solvent. In these experiments, the oil could be olive oil, hydrolyzed olive oil, or an oil sample obtained in a previous liquid-liquid phase separation procedure. The solvent could be only ethanol or ethanol and water. Thus, mixtures oil + solvent were initially mechanic stirred on a beaker glass for one hour to promote the intimate contact between the two-liquid phases. The liquid-liquid equilibrium experiments were then carried out in a 500 mL settling funnel which was left inside a controlled temperature oven to maintain the temperature at 45 °C. After 24 h, the liquid phases were collected from the decanting funnel and placed into round bottom flasks. A sample of each phase (around 1 mL) was collected for water quantification by Karl Fisher titration. Ethanol and water were removed from each phase by rotary evaporation under vacuum and at 85 °C until no condensed was observed (usually around 15 min). Volatiles amount (ethanol + water) present in each phase was determined by mass balance. Two samples of dried oil from each phase were therefore collected for the free fatty acid quantification by titration with a standardized solution of NaOH) and acylglycerols quantification by NMR methodology. Finally, 10 g of dried oil from each phase were collected for oxidation stability tests and antioxidants quantification.

2.2.2. Analytical methods

The free fatty acids were quantified according to the AOCS Ca 5a-40 method (AOCS, 1997). Because olive oil contains different free fatty acids in its composition, such molecules were treated as pseudo-components. The free fatty acid molar mass was considered to be equal to 277.875 g mol^{-1} (Lima et al., 2016). This value was estimated as a weighted mean of molar mass of different free fatty acids present in the olive oil used in this work (15.8 wt% of saturated, 77.5 wt% of unsaturated and 6.7 wt% of polyunsaturated free fatty acids, as declared by the supplier). Palmitic acid (256.429 g mol^{-1}) was used to represent the saturated acids, while oleic acid (282.467 g mol^{-1}) and linoleic acid (280 451 g mol^{-1}) were used to represent the unsaturated and polyunsaturated acids, respectively.

The water content on the liquid phases in equilibrium was determined by Karl Fisher titration.

Acylglycerols content was determined by NMR. First, the molar mass of acylglcyerols were determined assuming the triacylglycerol, diacylglycerol and monoacylglycerol molecules are result of the esterification of three, two and one molecules of free