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Application of lipases to regiospecific interesterification of exotic oils from an Amazonian area

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ABSTRACT

Enzymatic interesterification may favor the development of lipid fractions from Amazonian oils with greater application potential. In this study, the Amazonian buriti oil and murumuru fat were subjected to enzymatic interesterification using two lipases in three different enzyme systems: one with a commercial lipase from *Thermomyces lanuginosa*, a second with the lipase produced by *Rhizopus* sp., and a third with a mixture of both lipases. The three enzyme systems were able to catalyze the reaction, but the enzymes showed different specificities. The commercial lipase was specific for unsaturated fatty acids, whereas the *Rhizopus* sp. lipase was specific for both unsaturated fatty acids and the positions *sn* -1 and *sn* -3 of the fatty acid on the triacylglycerol. The mixture of both lipases showed no synergistic effect: the results were intermediate between the two enzymes applied alone. Interesterification reduced the levels of trisaturated and triunsaturated triacylglycerols and increased the levels of diunsaturated-monosaturated and monounsaturated-disaturated triacylglycerols. The thermal melting behavior indicated the formation of a single endothermic region with more homogeneous triacylglycerols. The content of the bioactive β-carotene was preserved after the interesterification reaction with all three-enzyme systems. The interesterified lipids obtained, because of the characteristics of the oils, may be applied to the formulation of cosmetics and pharmaceuticals.

1. Introduction

There has been increasing interest in manipulating of fatty acids in the triacylglycerol to produce oils with new properties and better performance for different applications. Among the available techniques, interesterification is one. In this technique, a catalyst is used to exchange of fatty acids attached to the glycerol backbone of a fat (Cowan, 2011). With the ability to combine the beneficial characteristics of component fatty acids into one triacylglycerol molecule, interesterification enhance the role fats and oils play in food, nutrition, and health applications (Osborn and Akoh, 2002). These reactions may be catalyzed by chemical or enzymatic processes. The chemical process is efficient in terms of reaction team and yields (Poppe et al., 2015). However, lipase-catalyzed interesterification has many advantages compared to the corresponding chemical process (Svensson and Adlercreutz, 2011). Fewer and simpler process steps are required, fewer by-products are created, less water is needed, and no aggressive chemicals are required to obtain the interesterified lipids (Holm and Cowan, 2008). Furthermore, these enzymes may be specific for the position or type of fatty acid in the glycerol, which allows the variety mixtures of lipids, can be produced (Svensson and Adlercreutz, 2011). In this context, the research on enzyme catalysis for the synthesis of new oils, in special using lipases, is in continuous development to overcome setbacks of the chemical catalysis (Poppe et al., 2015; Hsu et al., 2004).

The oil from *Mauritia flexuosa* Mart. (*Arecaceae*) pulp, know as buriti, has a high concentration of monounsaturated fatty acids (74%) (MUFA), higher than that of olive oil (71%), which is known to have high-quality nutritional oil (Silva et al., 2010, 2009). The unsaponificable fraction of buriti oil has high concentrations of tocopherol (1517 mg kg^{-1}) and carotenoids (1003 mg kg^{-1}), which have functional importance such as antioxidant activity (Silva et al., 2009). The fat from *Astrocaryum murumuru* Mart. (Arecaceae) seed, known as murumuru, is rich in the medium chain fatty acids, lauric (51.6%) and myristic (25.8%) (Mambrim and Barrera-Arellano, 1997; Saraiva et al., 2009). Recent recommendations indicate that the introduction of medium chain fatty acids in the triacylglycerol can improve the health and nutritional quality of

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the produced oil (Sengupta et al., 2014). These fatty acids have shown anti-inflammatory and antimicrobial activity and exhibits good digestibility (Carlson et al., 2015; Che Man and Marina, 2006; Parfene et al., 2013).

Accordingly, the aim of this study was to produce of interesterified lipids enzymatically by introduction of medium chain fatty acids, from murumuru, into buriti oil, and evaluate the pattern of action of enzymes in the reaction. The interesterified lipids were produced using two lipases in three different enzyme systems: one with a commercial lipase from *Thermomyces lanuginosa* (Lipozyme TL-IM), purified and immobilized, widely used in interesterification reactions; a second with a crude lipase from the fungus *Rhizopus* sp. produced by solid-state fermentation with an agro-industrial byproduct; and the third with a mixture of both lipases (commercial and *Rhizopus* sp.).

2. Materials

Crude buriti oil and murumuru fat were bought in a local market in the city of Belém, State of Pará, in the Brazilian Amazon. Crude lipase from *Rhizopus* sp. was produced in wheat bran in our laboratory (Macedo et al., 2004; Speranza and Macedo, 2013). Commercial, purified and immobilized lipase from *T. lanuginosa* (Lipozyme TL-IM) was kindly supplied by Novozymes. All other reagents and solvents were of analytical grade.

3. Methods

3.1. Analyses of the starting materials

The moisture content of the oil and the fat were determined by Karl Fischer titration (Mettler Toledo, model DL31 - Brazil), according to the AOCS method Ca 2e-84. The free fatty acids content was determined according the AOCS method Ca 5a-40 and expressed as% oleic acid for the buriti oil and as% lauric acid for the murumuru fat. The peroxide value was determined according to the AOCS method Cd 8b-90 (AOCS, 2009). Fatty acids methyl esters were prepared according to the Hartman and Lago's (1973) method. A gas chromatograph PerkinElmer Clarus 600 equipped with a flame ionization detector was used. A capillary chromatographic column (PerkinElmer-225; 30 m, 0.25 mm id and 0.25 μm film thickness) was used to analyze the fatty acid methyl esters. The qualitative composition was determined by comparison of the retention times of the peaks with those of the respective standards of fatty acids. The methyl ester profile was quantified based on relative peak areas (Basso et al., 2012). The iodine and saponification values were determined according to the AOCS methods Cd 1c-85 and Cd 3a-94 (AOCS, 2009).

3.2. Lipases

The lipase activities in both enzymes were quantified using olive oil as substrate. The liberated free fatty acid was titrated against 0.05 M of NaOH using phenolphthalein as indicator. One unit of lipase activity (U) is defined as 1 μmol of oleic acid released per minute (Macedo et al., 2004). The protein concentrations were determined according to the method of Bradford using bovine serum albumin as a standard (Bradford, 1976).

3.3. Enzymatic interesterification

The enzymatic interesterification between buriti oil and murumuru fat was performed in an orbital-shaking water bath at 150 rpm for 24 h at 40 °C under vacuum. The weight ratio of oil to fat was 70:30, with a total weight of 10 g. The reactions were performed in three different enzyme systems: commercial lipase, lipase from *Rhizopus* sp. and a mixture of both enzymes. In all systems, the final enzyme concentration ranged between 2.5 and 10% (w/w). Before the reaction, the enzymes were dried in a vacuum oven at 40 °C for 30 min. After completion of the reaction, the interesterified lipid was immediately filtered using a 0.45 μm membrane filter and frozen. The non-interesterified blend (physical blend) was also subjected to the same reaction conditions (Speranza et al., 2015a). After the reaction, the diacylglycerols (DAG), monoacylglycerols (MAG) and free fatty acids (FFA) were removed according to the method of Farmani et al. (2006). These compounds were determined by high performance size exclusion chromatography (HPSEC) and identified by comparing with the elution times of standards (Conto et al., 2011; Guedes et al., 2014).

3.4. Regiospecific distribution

The quantitative high-resolution ^{13}C-nuclear magnetic resonance (NMR) spectroscopic method (^{13}CNMR) was used for the regioespecific analysis of the non-interesterified blend and structured lipids. In this analysis the resonance of ^{13}C were resolved on the basis of the degree of chain unsaturation and their attachment position on the glycerol backbone (Vlahov, 1998; Li Ken Jie and Mustafa, 1997). The samples were analyzed using a Burker Advanced DPX 300 NMR spectrometer (Silberstreifen, Rheinstetten, Germany). The value for ^{13}C were determined at a frequency of 75.8 MHz, with a 5 mm multinuclear probe operating at 30 °C, according to the method described by Vlahov (1998).

3.5. Triacylglycerol composition

The fatty acid composition was used to predict the groups of TAGs in the non-interesterified sample with PrÓleos software, which uses a mathematical algorithm that describes the distribution of fatty acids in TAG molecules (Anon., 2015http://lames.quimica.ufg.br/p/4035-material-didatico, accessed in: September 10, 2015; Antoniosi Filho et al., 1995). For the prediction, the average values of fatty acids with more than 1% of the total composition were used, and TAGs at predicted levels below 0.5% of the total were excluded. The composition of TAGs present in interesterified lipids was analyzed according to the 1,3-random, 2-random theory (non-random redistribution), and 1,2,3-random theory (random redistribution), based on the analysis of regiospecific distribution described in item 3.4 (D'Agostini and Gioielli, 2002; Guedes et al., 2014). The equations used are described below:

1,3-random, 2-random theory:

$$\%SSS = \frac{(\%S_1) \times (\%S_2) \times (\%S_3)}{10^3} \quad (1)$$

$$\%SUS = \frac{(\%S_1) \times (\%U_2) \times (\%S_3)}{10^3} \quad (2)$$

$$\%SSU = \frac{2 \times (\%S_1) \times (\%S_2) \times (\%U_3)}{10^3} \quad (3)$$

$$\%USU = \frac{(\%U_1) \times (\%S_2) \times (\%U_3)}{10^3} \quad (4)$$

$$\%UUS = \frac{2 \times (\%U_1) \times (\%U_2) \times (\%S_3)}{10^3} \quad (5)$$

$$\%UUU = \frac{(\%U_1) \times (\%U_2) \times (\%U_3)}{10^3} \quad (6)$$

1,2,3-random theory:

$$\%SSS = \frac{(\%S) \times (\%S) \times (\%S)}{10^3} \quad (1)$$