Lipase catalyzed interesterification of Amazonian patauá oil and palm stearin for preparation of specific-structured oils

Paula Speranza¹ · Ana Paula Badan Ribeiro² · Gabriela Alves Macedo¹

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Abstract This study showed that enzymatic interesterification of Amazonian oils could be an important tool in order to produce new oils with physicochemical properties that improve the applications of these raw materials. Structured oils of Amazonian patauá oil and palm stearin using two lipases were produced in three different enzymatic systems: first, a crude lipase from the fungus *Rhizopus* sp (a microorganism isolated in our laboratory); second, a commercial lipase; and third, to check any synergistic effect, a mixture of both lipases (*Rhizopus* sp and commercial). The lipase from *Rhizopus* sp was specific in the incorporation of oleic acid at the sn-1,3 positions of the triacylglycerol, resulting in an oil richer in saturated fatty acid in the sn-2 position. This enzyme, produced by solid-state fermentation, even though crude, was fatty acid and positional specific and able to operate at low concentration (2.5 %, w/w). In the second enzyme system, the commercial lipase from *Thermomyces lanuginosus* was not specific in the tested conditions; there was no change in the distribution of saturated and unsaturated fatty acids in the three positions of the triacylglycerol profile, there was only a replacement by the type of fatty acid at the same position. In the third enzyme system, the mixture of both lipases shows no synergic effect. The structured oils retained the concentration of bioactive α - and γ -tocopherol in the three enzyme systems.

Triacylglycerol classes and Thermal behavior tests indicated the formation of more homogeneous triacylglycerols, especially the mono and di-unsaturated.

Keywords Lipase · Amazonian oil · Enzymatic interesterification · Fatty acid · Triacylglycerol · Regiospecificity

Introduction

The interesterification of vegetable oils and fats aims the creation of triacylglycerol species with new and desirable physical, chemical and functional properties (Reshma et al. 2008; Ribeiro et al. 2009). These reactions initially were mainly produced following the conventional chemical process where one of the most commonly used catalysts is sodium methoxide, which 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 structured oils (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 oils, can be produced (Svensson and Adlercreutz 2011). However, as vegetable oils are heterogeneous substrates, containing triacylglycerol formed by very different fatty acids, it's difficult to find a lipase that acts efficiently on all available substrates (Poppe et al. 2015; Issariyakul and Dalai 2014). This fact reflects the extensive research for finding best sources of lipases for optimal reaction rates and conversions for one specific substrate (Poppe et al. 2015; Rosset et al. 2011; Speranza and Macedo 2012).

✉ Paula Speranza
paulasperanza09@gmail.com

Gabriela Alves Macedo
gmacedo@fea.unicamp.br

¹ Department of Food Science, Faculty of Food Engineering,
University of Campinas, 80, Monteiro Lobato St.,
13083-970 Campinas, SP, Brazil

² Department of Food Technology, Faculty of Food Engineering,
University of Campinas, 80, Monteiro Lobato St.,
13083-970 Campinas, SP, Brazil

Among the vegetable oils that can be used in enzymatic interesterification, the Amazon stands out. These oils are rich in bioactive lipophilic compounds that are related to the reduction of various diseases (Lunn 2007; Jiang 2014). The oil obtained from palm *Oenocarpus bataua*, known as pataúá, is rich in unsaturated fatty acids and tocopherol (Montúfar et al. 2010). Use it in enzymatic interesterification reactions with more saturated oils, such as palm stearin, can produce new structured oils with higher commercial interest. Unsaturated fatty acids are beneficial for the prevention of coronary heart disease and reduce platelet aggregation. On the other hand saturated fatty acids undergo less lipid peroxidation than unsaturated fatty acids (Sengupta et al. 2014).

In this study, structured oils of pataúá oil and palm stearin using two lipases were produced and analyzed in three different enzymatic systems: first, a crude lipase from the fungus *Rhizopus* sp (a microorganism isolated in our laboratory); second, a commercial lipase; and third, to check for increasing specificity, a mixture of both lipases (*Rhizopus* sp and commercial). The regiospecificity of lipases, as well as modifying the physicochemical characteristics of structured oils are reported in this paper.

Materials

The crude pataúá oil (melting point 23.9 °C), extracted from the fruit of the palm *Oenocarpus bataua* Mart (Arecaceae), was bought in a local market in the city of Belém, State of Pará, in the Brazilian Amazon. Palm stearin (melting point 43 °C), solid fraction obtained by the fractionation of the palm oil *Elaeis guineensis* Jacq (Arecaceae), was kindly supplied by Agropalma (Pará, Brazil). Crude lipase from *Rhizopus* sp was produced in our laboratory (Macedo et al. 2004 and Speranza and Macedo 2013). Commercial, purified and immobilized lipase (Lipozyme TL-IM) was kindly supplied by Novozymes. All other reagents and solvents were of analytical grade.

Methods

Lipase

Lipase from *Rhizopus* sp was produced according to Macedo et al. (2004) and Speranza and Macedo (2013) in your laboratory. Briefly, the microorganism was grown in solid medium composed of wheat bran (40 % water w/w) for 72 h at 30 °C. Cells were removed by centrifugation and the supernatants treated with ammonium sulphate (80 % saturation). The precipitates were dialyzed against distilled water, freeze-dried and used in powder form as crude lipase preparation. Lipase activities in both enzymes, from *Rhizopus* sp and commercial,

were quantified using olive oil as substrate. One unit of lipase activity (U) is defined as 1 µmol of oleic acid released per minute (Macedo et al. 2004). The analyses were carried out in triplicate and the reported values are the average of three measurements.

Enzymatic interesterification

The enzymatic interesterification between crude pataúá oil and palm stearin was performed in an orbital-shaking water bath at 150 rpm for 24 h at 50 °C under vacuum. The weight ratio of oil to fat was 70:30, with a total weight of 10 g. The most pataúá oil concentration in the reaction, kept the mixture rich in minor compounds. The reactions were performed in three different enzyme systems: commercial lipase (2.5 %, w/w) lipase from *Rhizopus* sp (2.5 and 5.0, w/w) and a mixture of both enzymes (1.25 % and 1.25 % (w/w), totalizing 2.5 %), respectively. Before the reaction, the enzymes were dried in a vacuum oven at 40 °C for 30 min. After completion of the reaction, the structured oils was immediately filtered using a 0.45 µm membrane filter and frozen (Speranza et al. 2015). The non-interesterified blend (physical blend) was also subjected to the same reaction conditions. The free fatty acids and partial acylglycerols of the non-interesterified blend and structured oils after the reaction were removed according to the methodology of Farmani et al. (2006).

Fatty acid composition

Fatty acids methyl esters were prepared according to the Hartman and Lago's method (Hartman and Lago 1973). A Perkin Elmer Clarus 600 gas equipped with a flame ionization detector was used. A capillary chromatographic column (Perkin Elmer-225; 30 m, 0.25 mm id and 0.25 µm film thickness) was used to analyze the fatty acid methyl esters. The split ratio was 1:40, and the injector and detector temperature was 250 °C. The operation conditions of the column were as follows: 100 °C for 5 min, 100 °C - 230 °C (5 °C/min) and 230 °C for 20 min. Helium was used as the carrier gas. 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 analyses were carried out in duplicate and the mean±standard deviation was calculated for each sample.

Regiospecific distribution

Proton-decoupled ¹³C NMR (Nuclear Magnetic Resonance) was used to analyze the positional distribution of classes of fatty acids on the triacylglycerol (TAG) backbone. Lipid samples were dissolved in deuterated chloroform in NMR tubes, and NMR spectra were recorded on a Burkert Advance DPX

spectrometer operating at 300 MHz. The determination of ^{13}C was performed at a frequency of 75.8 MHz, with a 5 mm multinuclear probe operating at 30 °C (Vlahov 1998).

Thermal properties

Thermal analysis of the samples was performed by differential scanning calorimetry according to the AOCS method Cj 1–94 (AOCS 2009). The equipment used was a TA Q2000 thermal analyzer coupled to RCS90 Refrigerated Cooling System (TA Instruments, Waters LLC, New Castle). The data processing software used was V4.7A (TA Instruments, Waters LLC, New Castle). The conditions of analysis were as follows: sample weight, ~10 mg; crystallization curves, 80 °C for 10 min, 80 °C to –40 °C (10 °C/min), and –40 °C for 30 min; and melting curves, –40 °C to 80 °C (5 °C/min). The following parameters were used in evaluating the results: crystallization and melting onset temperatures (T_{oc} and T_{om}), crystallization and melting peak temperatures (T_{pc} and T_{pm}), crystallization and melting enthalpies (ΔH_c and ΔH_m) and crystallization and melting end temperatures (T_{fc} and T_{fm}) (Campos 2005).

Triacylglycerol composition

The fatty acid composition described in item 3.2 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 (Antoniosi Filho et al. 1995). The composition of TAGs present in structured oils 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).

Tocopherol

The determination of the levels of tocopherol was determined according to the AOCS method Ce 8–89 (AOCS 2009). The samples were diluted in hexane at concentration of 0.1 g / ml. The samples were injected into the liquid chromatograph UHPLC. The microparticulate silica column had 250 mm long, 4 mm internal diameter - with each particle measuring approximately 5 μm . The fluorescence detector adjusted with excitation wavelength of 290 nm and an emission wavelength of 330 nm, and the mobile phase HPLC grade was: hexane (99 %) and isopropanol (1 %). The qualitative composition of tocopherol was made by comparison of the retention times of peaks with standard tocopherols. The quantitative composition was performed by normalizing the area under the curves and was expressed in mg / g of oil. All the above tests were carried out in triplicates and the mean $\pm$ standard deviation was calculated. Significant differences between the means were

determined by analysis of variance. The software STATISTICA v.8.0 (StatSoft, Inc., USA) was used for the statistical analyses.

Results and discussion

In this paper, two lipases with different characteristics were tested in interesterification reactions, alone and in combination. The lipase obtained from the fungus *Rhizopus* sp was a crude enzyme, produced by solid-state fermentation, with lipolytic activity of 8.0 U g $^{-1}$. This enzyme has shown positive results in organic syntheses performed in our laboratory (Lopes et al. 2011; Macedo et al. 2004). The commercial lipase, which was purified and immobilized on silica, had a lipase activity of 12.7 U g $^{-1}$. This enzyme is now widely used in enzymatic interesterification reactions (Speranza et al. 2015; Fernandez-Lafuente 2010). The mixture of both enzymes had a lipase activity of 10.3 U g $^{-1}$. These three different enzyme systems displayed different catalytic properties, as described below.

Fatty acid compositions

The results in Table 1 indicate that pataua oil is very rich in oleic acid (74.5 %). Few oils, such as olive oil, exhibit as high concentration of this fatty acid (Nunes et al. 2011). The fatty acid composition also indicates that this oil presents palmitic acid as the major saturated fatty acid (15.7 %). Regarding polyunsaturated linoleic acid, the concentration in this oil does not exceed 5.8 %. Therefore, pataua oil has great potential as a new source of monounsaturated oil. From the biological point of view, evidence from epidemiological studies suggests that oleic acid is linked to a reduced risk of coronary heart disease and reduction of inflammatory responses (Lunn 2007; Pacheco et al. 2008). A few other available few studies also confirm the high concentration of oleic acid in the pataua oil (Montúfar et al. 2010; Rodrigues et al. 2010).

Table 1 Fatty acid composition (%) of pataua oil and palm stearin

Fatty acids	Pataua	Palm stearin	Blend ^a
Myristic acid (C14:0)	0.6 $\pm$ 0.00	6.1 $\pm$ 0.77	2.2
Palmitic acid (C16:0)	15.7 $\pm$ 0.01	46.9 $\pm$ 0.06	25.1
Stearic acid (C18:0)	2.2 $\pm$ 0.00	5.9 $\pm$ 0.12	3.3
Oleic acid (C18:1)	74.5 $\pm$ 0.00	28.1 $\pm$ 0.01	60.6
Linoleic acid (C18:2)	5.8 $\pm$ 0.01	13.0 $\pm$ 0.04	8.0
Arachidic acid (C20:0)	1.3 $\pm$ 0.00	–	0.9
Σ Saturated	19.8	58.9	31.5
Σ Monounsaturated	74.4	28.1	60.5
Σ Polyunsaturated	5.8	13.0	8.0

^a Composed of 70 % of pataua oil and 30 % of palm stearin

Regarding palm stearin, palmitic acid is its main fatty acid (46.0 %), followed by oleic acid (28.1 %) (Table 1). Saturated fatty acids increase the capacity of structured lipid, in addition, providing an important moisture barrier property (O'Brien 2009). Moreover, oils rich in these fatty acids may have a higher antioxidant activity, since, saturated fatty acids undergo less lipid peroxidation (Sengupta et al. 2014).

The choice of patauá oil and palm stearin to enzymatic interesterification was made upon their different compositions: patauá oil is rich in C18:1, whereas palm stearin has a significant content of C16:0. These differences of fatty acid compositions may produce structured oils with a more balanced combination of saturated and unsaturated fatty acids, which may have positive implications of the health and technological points of view.

Regiospecific distribution

The results of regiospecific distribution with the three-enzyme system are presented in Figs. 1 and 2. The enzyme from *Rhizopus* sp, as had never been used in interesterification reactions, was evaluated at concentrations of 2.5 and 5.0 % (w/w). Regarding the reaction catalyzed by the commercial lipase from *Thermomyces lanuginosus* was used just 2.5 % (w/w) in the reaction, since this enzyme is widely used in interesterification reactions (Reshma et al. 2008). In the reaction catalyzed by the mixture of both lipases (*Rhizopus* sp+commercial), it was used 1.25 % (w/w) of each enzyme in the reaction, totaling 2.5 % (w/w), thus enabling the comparison with the other systems that operate in this enzyme concentration. In Fig. 1 is shown the percentages of saturated and unsaturated fatty acids present in the sn-2 position of the glycerol for the non-interesterified blend and structured oils produced with the different enzymes concentrations. While in the Fig. 2, it shows the percentages of saturated and unsaturated fatty acids present in the sn-1 and sn-3 positions of the glycerol. This analysis shows, therefore, the distribution of classes of fatty acids (saturated and unsaturated) in all three TAG positions. It can

Fig. 1 Regiospecific distribution of fatty acids at the sn-2 position of non-interesterified blend and structured oils of patauá oil and palm stearin catalyzed by different enzyme systems

Fig. 2 Regiospecific distribution of fatty acids at the sn-1,3 positions of non-interesterified blend and structured oil of patauá oil and palm stearin catalyzed by different enzyme systems

be seen that the percentages of these fatty acids, in these positions, varies according to the specificity of the enzyme system.

The results obtained with the enzyme from *Rhizopus* sp shown that at both concentrations (2.5 and 5.0 %, w/w), the structured oils formed displayed essentially the same distribution of fatty acids, indicating that lower concentrations of the enzyme were sufficient to saturate the substrate. The oils formed with this enzyme displayed a higher concentration of saturated fatty acids in the sn-2 position of the TAG and unsaturated fatty acids in the sn-1,3 positions of the TAG. These results indicate that the enzyme was specific for unsaturated fatty acids, especially oleic acid, both in the hydrolysis and in the synthesis of fatty acids in the glycerol. During synthesis, the enzyme was also specific for the sn-1,3 position of the TAG. Thus, there was a shift of unsaturated fatty acids of the sn-2 position to the sn-1,3 positions. Therefore, as the position of the fatty acid in TAG influences its absorption, and as saturated and unsaturated fatty acids have various biological functions, such redistribution of the fatty acids ensures the production of a structured oil with new potential applications (Quinlan and Moore 1993; Sengupta et al. 2014). In nature, vegetable oils are generally rich in unsaturated fatty acid in the sn-2 position of the TAG and saturated fatty acids in the sn-1,3 positions of the TAGs (Silva et al. 2010). Methods to produce TAGs rich in palmitic acid at sn-2 position are therefore of great potential industrial interest, as these TAGs are not produced at industrial levels (Jiménez et al. 2010).

In another study, the enzyme from *Rhizopus oryzae* was used in the reaction between palm stearin enriched with palmitic acid and oleic acid (Esteban et al. 2011). The enzyme was specific in the incorporation of oleic acid at the sn-1, 3 positions of the TAG.

Regarding the reaction catalyzed by the commercial lipase from *Thermomyces lanuginosus*, there was no change in the distribution of saturated and unsaturated

Table 2 Melting onset temperature (T_{mo}), melting peak temperature (T_{mp}), melting end temperature (T_{me}) and melting enthalpy (ΔH_m) of the non-interesterified blend and structured oils catalyzed by different enzyme systems

Blend and structured oils	Peak 1				Peak 2				Peak 3			
	T_{mo} (°C)	T_{mp} (°C)	T_{me} (°C)	ΔH_m (J/g)	T_{mo} (°C)	T_{mp} (°C)	T_{me} (°C)	ΔH_m (J/g)	T_{mo} (°C)	T_{mp} (°C)	T_{me} (°C)	ΔH_m (J/g)
Non-interesterified blend	−21.28	−3.66	8.27	63.92	7.69	12.57	41.16	40.73	41.60	44.41	48.03	3.72
2.5 % lipase from <i>Rhizopus</i> sp	−23.90	−0.91	34.79	84.36								
5.0 % lipase from <i>Rhizopus</i> sp	−23.90	−4.67	26.30	84.19								
2.5 % commercial lipase	−20.90	0.85	35.16	79.33								
1.25 % <i>Rhizopus</i> sp+1.25 % comm.	−20.78	0.88	35.91	80.87								

fatty acids in the three positions of the TAG profile, but changes occurred in the physicochemical characteristics of the structured oils (described in the following sections). Most likely what occurred was the replacement of certain saturated fatty acids by other saturated fatty acids at the same position, which is the same as occurred with the unsaturated fatty acids. Although the analysis has not identified what type of fatty acid is present at each position of TAG, due to changes in the physicochemical characteristics of the initial blend, it can be concluded that the enzyme was able to act in the blend and produce new oil.

This enzyme has been used in interesterification reactions using palm stearin and other oils with a lower melting point (Reshma et al. 2008). The enzyme has different specificities depending on enzyme preparations, the reaction conditions and type of substrate (Muralidhar et al. 2002; Fernandez-Lafuente 2010). These conditions may change drastically the enzyme specificity because of structural changes in the enzyme molecule, affecting the size and form of the active site (Muralidhar et al. 2002). For this reason, many results of different groups of researchers appear contradictory (Fernandez-Lafuente 2010).

In the reaction catalyzed by the mixture of both lipases (*Rhizopus* sp+commercial), the same behavior was observed when compared with using *Rhizopus* sp alone. Apparently, the

results indicate that the mixture of enzymes did not display any increase in specificity, and lipase from *Rhizopus* sp at a concentration of 1.25 % was able to catalyze the interesterification reaction alone. In this study, a synergistic effect of the enzymes was not observed.

In other studies, the use of more than one lipase in the reactions has been used as a strategy to improve the properties of the enzymes, increasing specificity and selectivity. Rodrigues and Ayub (2011) evaluated the effect of combining two commercial lipases (TL-IM and RM-IM - Novozymes) on the transesterification and hydrolysis of soybean oil. The results indicated that the mixture of lipases provided synergistic effects in the reaction, and the conversion rate was superior to that obtained with the individual enzymes. Complex substrates such as vegetable oils, because of the variety of fatty acids, have different specificities, and in certain situations, the use of mixtures of lipases may be required for effective transesterification and hydrolysis.

Thermal behavior

The melting and crystallization curves can be subdivided into different regions, reflecting the different types of TAGs present in the mixture before and after interesterification. Interesterification can change the types of TAGs initially present in the blend, thus changing their thermal properties. This

Table 3 Crystallization onset temperature (T_{co}), crystallization peak temperature (T_{cp}), crystallization end temperature (T_{ce}) and crystallization enthalpy (ΔH_c) of the non-interesterified blend and structured oils catalyzed by different enzyme systems

Blend and structured oils	Peak 1				Peak 2			
	T_{co} (°C)	T_{cp} (°C)	T_{ce} (°C)	ΔH_c (J/g)	T_{co} (°C)	T_{cp} (°C)	T_{ce} (°C)	ΔH_c (J/g)
Non-interesterified blend	21.05	19.39	−1.17	11.39	−4.08	−7.50	−29.52	14.32
2.5 % lipase from <i>Rhizopus</i> sp	16.51	10.37	4.33	4.688	0.65	−3.32	−36.82	18.95
5.0 % lipase from <i>Rhizopus</i> sp	13.44	9.87	0.95	6.268	0.12	−2.75	−31.14	13.87
2.5 % commercial lipase	13.81	11.92	5.57	4.084	1.52	−1.39	−25.40	14.11
1.25 % <i>Rhizopus</i> sp+1.25 % comm.	15.43	12.52	1.95	3.913	1.26	−3.41	−24.02	12.01

Table 4 Triacylglycerol classes of pataua oil, palm stearin, non-interesterified blend and structured oils catalyzed by different enzyme systems

TAG ^a	Pataua oil (%)	Palm Stearin (%)	Non-interesterified blend (%)	Structured oils			
				2.5 % <i>Rhizopus</i> (%)	5.0 % <i>Rhizopus</i> (%)	2.5 % Comm. lipase (%)	1.25 % <i>Rhizopus</i> +1.25 % Comm. (%)
S ₃	0.7	20.5	6.5	8.9	9.4	9.9	9.7
S ₂ U	8.7	41.7	18.6	33.1	33.8	38.9	34.5
SU ₂	37.2	29.0	34.8	41.1	40.6	39.3	40.2
U ₃	53.2	8.5	39.9	16.9	16.2	11.9	15.6

^a S₃ - trisaturated; S₂U - disaturated-monounsaturated; SU₂ - monosaturated-diunsaturated; U₃ - triunsaturated

analysis is considered an important tool for characterizing structured oils (Ribeiro et al. 2009).

In this study, the results of thermal melting behavior (Table 2) showed that all structured oils produced using different enzyme systems showed reductions in the number of peaks. The second and third peaks, of more saturated TAGs with higher melting points, disappeared as a result of the elimination of more saturated TAGs in the structured oils. This effect can also be seen in the increase in the value of melting enthalpy of peak 1 (ΔH_m), showing the increased participation of more highly unsaturated TAGs in the new oils produced.

Regarding the thermal behavior in crystallization (Table 3), the same type of modification is observed, as the phenomena of melting and crystallization are thermodynamic reversed in the scan evaluated. In the crystallization of the blends, a reduction in the enthalpy values was observed for the peak 1 (ΔH_c), indicating a lower participation of more highly saturated TAGs in the structured oils.

This analysis indicated that in all structured oils the number of peaks was reduced, indicating a reduction in the classes of TAGs presents in the initial blend and the formation of TAG more unsaturated. The formation of a single endothermic region with more homogeneous TAGs facilitates the use of the

new oils formed, as it prevents separation of oil and fat at a given temperature.

The thermal behavior results confirm the changes in the physicochemical characteristics of the initial blends, indicating that the structured oils obtained with the three different enzyme systems, exhibited characteristics that improve their applicability.

Triacylglycerol composition

Interesterification caused significant alteration in the TAG composition of the blends studied (Table 4). In all structured oils produced with the different enzymes, interesterification produced a significant increase in the percentages of disaturated-monounsaturated (S₂U) TAG content, with a corresponding decrease in triunsaturated (U₃) TAG content.

The structured oils produced with the lipase from *Rhizopus* sp displayed an increase of approximately 80 % in the S₂U TAG content and an approximately 60 % decrease in the concentration of U₃ TAG concentration. The lipase from *Rhizopus* sp, at both concentrations tested, produced blends with very similar TAG classes, indicating again that an increase in enzyme concentration does not change the characteristics of the new oils obtained.

Table 5 Tocopherol content in pataua oil, palm stearin, non-interesterified blend and structured oils

Oils, blend and structured oils	Tocopherol (mg kg ⁻¹)			
	α	β	γ	δ
Pataua	1504.7 ^a ±1.71	ND	258.6 ^a ±1.23	ND
Palm stearin	33.0 ^e ±1.91	ND	5.0 ^e ±0.01	ND
Non-interesterified blend	992.0 ^d ±2.82	ND	161.1 ^b ±1.21	ND
2.5 % lipase from <i>Rhizopus</i> sp	1019.0 ^b ±1.73	ND	158.2 ^c ±1.01	ND
5.0 % lipase from <i>Rhizopus</i> sp	1005.0 ^c ±0.03	ND	159.9 ^c ±1.71	ND
2.5 % commercial lipase	1001.3 ^c ±0.92	ND	152.0 ^d ±0.33	ND
1.25 % <i>Rhizopus</i> sp+1.25 % comm. lipases	1007.4 ^c ±0.97	ND	154.9 ^{cd} ±1.01	ND

^{a-e} The same letters in a column are not significantly different ($P>0.05$)

ND - Not detected

The structured oils produced with the commercial lipase from *Thermomyces lanuginosus* displayed an increase of approximately 110 % in the S₂U TAG content and an approximately 70 % decrease in the concentration of U₃ TAGs. The concentration of monosaturated-diunsaturated (SU₂) and trisaturated (S₃) TAGs slightly increased after the reaction in all oils produced.

The structured oil produced with the mixture of both lipases, as with the regioespecific distribution, presented the same behavior as that observed with the *Rhizopus* sp acting alone.

Therefore, the main TAGs of the non-interesterified blend were of the U₃ class followed SU₂, whereas the main TAGs present in the oils after interesterification were of the SU₂ class, followed by S₂U. The increased level of S₂U after interesterification is quite timely. There are many applications of these TAGs by industry, since they have structuring properties, lubricity, aeration and moisture barrier. These characteristics also favor the production of more stable emulsions, enabling the application of these oils in cosmetic formulations (O'Brien 2009).

Tocopherol

Pataúá oil is naturally rich in α and γ -tocopherols and as the blend is rich in this oil (70 %), it is also rich in these isomers. The isomers β and δ -tocopherols were not detected in pataúá oil and consequently in the blend (Table 5).

The structured oils formed after interesterification with the three different enzyme systems, kept the concentration of α and γ -tocopherol practically constant (Table 5). The oils showed a relatively high concentration of α -tocopherol, even after interesterification with palm stearin, poor in this compound. Although the enzymes have different characteristics (crude and purified) and have catalyzed the production of structured oils with different distribution fatty acid in the glycerol, these features had little influence on tocopherol concentration of the pataúá oil.

Reshma et al. (2008) obtained similar results to those obtained in this study. Lipids with bioactive phytochemicals were produced using interesterified palm stearin and rice bran oil rich in tocopherol and tocotrienol (tocols). The conditions employed for the reaction, showed only a marginal decrease (4–8 %) in the tocols content, with no preferential reduction of any particular isomer. Due to the use of lipase, which allows the reaction occurs under mild conditions, and the use of rice bran oil, which is rich in tocols, the synthesized lipids showed high concentrations of these compounds.

Conclusions

This paper describes, for the first time, the production of Amazonian structured oils using different lipases. These enzymes are capable of catalyzing the reaction with different

specificities and produce structured oils with physicochemical characteristics that best meet the demands of the food and cosmetic industry, without, thereby, losing the minority compounds naturally present in these oils. Thus, the enzymatic interesterification can be an alternative to increase the exploitation of the Amazonian oils.

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