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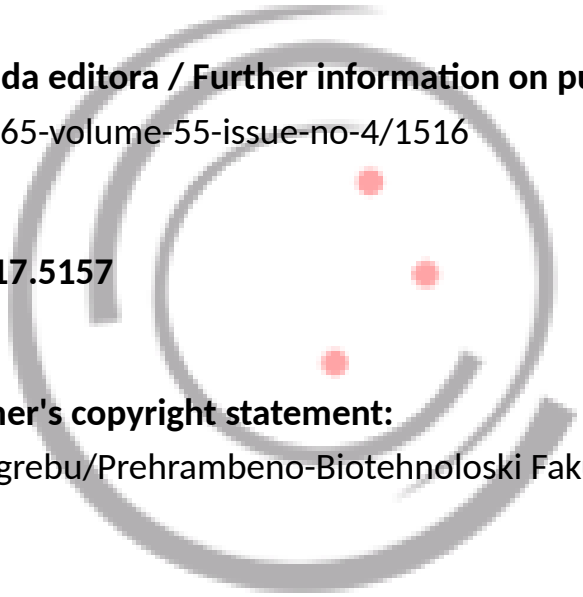

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Antioxidant Potential and Modulatory Effects of Restructured Lipids from the Amazonian Palms on Liver Cells

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Summary

Enzymatic interesterification is used to manipulate oil and fat in order to obtain improved restructured lipids with desired technological properties. However, with raw materials containing significant amounts of bioactive compounds, the influence of this enzymatic process on the bioactivity of the final product is still not clear. Thus, the aim of this study is to evaluate the antioxidant potential and modulatory effects of two raw materials from the Amazonian area, buriti oil and murumuru fat, before and after lipase interesterification, on human hepatoma cells (HepG2). The results indicate that minor bioactive compounds naturally found in the raw materials and their antioxidant capacity are preserved after enzymatic interesterification, and that the restructured lipids modulate HepG2 endogenous antioxidant enzyme.

Key words: enzymatic interesterification, lipases, buriti oil, murumuru fat, HepG2 cells, antioxidant activity

Introduction

Research has demonstrated that products with antioxidant features may reduce the risk of many diseases in which oxidative stress may play a role, especially chronic illnesses such as cancer, cardiovascular and neurodegenerative diseases and inflammation (*1*). Among the compounds with antioxidant potential, exotic raw oils and fats, such as the products from the Amazon rainforest region in Brazil, not well known outside their region of origin, but with a potential for industrial applications, produced according to the tradition of the local population, have shown promising results (*2,3*). Studies have indicated that these types of oil have antioxidant activity due to their fatty acid composition and the presence of minor compounds such as tocols (tocopherols and tocotrienols), carotenoids and phenolic compounds.

Region of Amazon rainforest in Brazil is known to produce raw oils and fats with unique properties. Amazonian palm *Mauritia flexuosa*, known as buriti, produces the oil with high concentration of monounsaturated fatty acids (MUFA) and minor compounds such as tocopherols, carotenoids and polyphenols (*3-5*). In few studies available in the literature, an elevated radical scavenging power of buriti oil was observed (*6*). Also, the concentration of MUFA is similar to the average amount normally present in olive oil, making it an oil with high nutritional quality (*7*). Another, less popular palm found in Amazon region is the *Astrocaryum murumuru*, known as murumuru. This palm produces a type of fat rich in lauric and myristic fatty acids (*8*). There are no studies that evaluate the antioxidant activity of this fat, however, there is evidence of the antioxidant potential of medium chain fatty acids (*9*).

Oil and fat, when subjected to enzymatic interesterification reactions, show a redistribution of the fatty acids present in the glycerol, which may generate new oil with diverse and superior biological properties when compared to the original natural oil. Recent studies have shown that enzymatic interesterification can improve the biological

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characteristics of certain types of oil (*10-13*). Our group previously demonstrated that restructured lipids generated by enzymatic interesterification had superior technological properties compared to the original oil and fat (*8*). The changes in the composition of the original triacylglycerol, compared to a simple oil/fat mixture, modify its physicochemical (*e.g.* crystallization and melting temperatures, structure, lubricity, *etc.*) and nutritional properties, thus increasing the possible applications of these lipids. The use of immobilized commercial lipases in interesterification reactions has been increasing in recent years, presenting positive results in the synthesis of high value-added products such as breast milk fat substitutes and cocoa butter (*14*).

Liver is responsible for a number of important metabolic functions such as processing, storage, distribution of metabolites, hepatic synthesis, and molecular detoxification of the body. Liver cells are widely used in toxicology studies because the liver is the first organ that receives the blood from the passage through the gut, which carries compounds absorbed through the diet (*15*). No studies have evaluated the improvements in the characteristics of restructured oil and its modulatory effects on liver cells.

The aim of this study is to investigate the influence of lipase interesterification (using a commercial lipase and a lipase produced in laboratory by *Rhizopus* sp.) on the antioxidant activity and bioactive properties of buriti and murumuru restructured lipids. Generated oil and fat were characterized by total phenolic compound tocopherol and total carotenoid contents. The *in vitro* antioxidant potential, cytotoxicity and the modulation of endogenous antioxidant enzyme (catalase) in liver HepG2 cells were also determined. This characterization allows the expansion of different applications of exotic types of oil and fat and increase the interest of industrial application of these lipids.

Materials and Methods

Materials

Crude buriti oil and semi-refined murumuru fat, produced through neutralization and bleaching, which are the first two stages of production of refined oils and fats, were donated by Naturais da Amazônia, a cosmetic company located in the Brazilian Amazon (Belém City, Pará State, Brazil). Buriti oil had 4.4 % free fatty acids, 0.02 % moisture, and peroxide value of 4.85 mmol/kg. Murumuru fat had 3.7 % free fatty acids, 0.04 % moisture, and peroxide value of 3.55 mmol/kg. Although the mass fraction of free fatty acids is relatively high, it is an expected value for crude oil and fat, while the peroxide value was within the limits established by Codex Alimentarius standard (max. 5 mmol/kg; *16*).

Rhizopus sp. crude lipase was produced in our laboratory, according to a previously published methodology (*17,18*). Commercial purified immobilized lipase (Lipozyme TL-IM) was generously provided by Novozymes A/S (Bagsværd, Denmark). Human liver hepatocellular carcinoma cell line (HepG2) was donated by the Pharmacological Division of Pluridisciplinary Center for Chemical, Biological, and Agricultural Research (Cpqba-Unicamp, Paulínia, Brazil). All other reagents and solvents were of analytical grade.

Enzymatic activity

The activities of commercial lipase and *Rhizopus* sp. lipase were quantified using olive oil as substrate. One unit of lipase activity (U) was defined as 1 μmol of oleic acid released per minute (*17*).

Enzymatic interesterification of the fat and oil

Three different enzyme preparations were used in the reactions: commercial lipase, lipase produced by *Rhizopus* sp., and a mixture of enzymes. Enzymatic interesterification between buriti oil and murumuru fat was carried out in an orbital shaking water bath (150 rpm, model TE-0532; Tecnal, Piracicaba, Brazil) for 24 h at 40 °C. The ratio of oil to fat was 70:30 (by mass), and total mass was 10 g. Final enzyme mass fraction in all preparations ranged from 2.5 to 10 %. A stream of pure nitrogen was passed through the reaction mixture to prevent fat oxidation. After the reaction, the restructured lipids were filtered through a 0.45-μm membrane filter and frozen. The same reaction conditions were used for the non-interesterified physical blend (*8*).

Determination of bioactive phytochemicals

Total phenolic content

To determine the total phenolic content, Folin-Ciocalteu technique was used (*19*). Phenolic compounds were extracted from the oil with a solution of water/methanol 60:40 (by volume). Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) was added to suitable aliquots of the extracts. After 3 min, a sodium carbonate solution (35 %) was added to the mixture, which was diluted with water to a final volume of 1000 μL. Absorbance was measured after 2 h at a wavelength of 725 nm on a Shimadzu spectrophotometer UV-1800 (Kyoto, Japan). The blank was composed of all the constituents of the reaction except that the phenolic solution was substituted by distilled water. The course of the except was accompanied by a calibration curve with standard gallic acid (Sigma-Aldrich). Total phenolic content was expressed in mg of gallic acid equivalents (GAE) per kg.

Total carotenoid content

Total carotenoid content in oil and fat samples was determined by a spectrophotometric method (*20*) adapted by the Laboratory of Oils and Fats at FEA, Unicamp, Brazil. Samples were diluted in 100 % hexane (Synth, Diadema, SP, Brazil) at a concentration of 0.004 g/mL. The absorbance readings were performed at 453 nm using a Shimadzu spectrophotometer UV-1800. Mass of the tested oil was enough to obtain absorbance values between 0.5 and 0.8. Total carotenoid content was calculated and expressed as μg of β-carotene per g of oil and the specific absorption coefficient of 1 % hexane was $\varepsilon=2592\cdot10^2$ mL/(g·cm).

Tocopherol determination

The mass fractions of tocopherols were determined using the methodology described in AOCS (*21*). Samples,

diluted in hexane at a mass concentration of 0.01 g/mL, were analyzed by UHPLC (Series 200a; PerkinElmer, Waltham, MA, USA), with a LiChrosorb® Si 60 Hibar® HPLC column (250 mm×4 mm, 5 μm i.d.; Sigma-Aldrich) and a fluorescence detector (Series 200a; PerkinElmer). The mobile phase used was hexane (99 %) and isopropanol (1 %) (J.T.Baker, Phillisburg, NJ, USA) and the detection was performed at 290 nm excitation and 330 nm emission. Compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method, and the results were expressed in mg per g of oil samples.

Determination of antioxidant activity

DPPH radical scavenging activity

Radical scavenging activity in the samples was studied by reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich) radicals (*22*). Samples were prepared in ethyl acetate (Synth) at concentrations of 5 to 100 μg/mL. An ethyl acetate solution of DPPH radicals of 0.2 mM was freshly prepared. Then, 50 μL of oil solution and 150 μL of DPPH solution were mixed. The decrease in absorption at 520 nm was measured for 16 min using a NovoStar microplate reader (BMG LABTECH, Ortenberg, Germany). Measurements were performed in triplicate. Radical scavenging activity (in %) was estimated from the differences between the absorbance of DPPH solution with or without control sample and was calculated according to the following equation:

$$\text{Radical scavenging activity}=[(A_{\text{control}}-A_{\text{sample}})/A_{\text{control}}]\cdot 100 \quad /1/$$

ORAC assay

Oxygen radical absorbance capacity (ORAC) assays were performed in a 96-well plate (TPP Techno Plastic Products AG, Trasadingen, Switzerland), using fluorescein (Sigma-Aldrich) as a fluorescent probe (*23*). The measurements were performed in triplicate using a NovoStar Microplate reader (BMG LABTECH) with fluorescence filters at 485 nm excitation and 520 nm emission. The reaction, performed at 37 °C, was started by thermal decomposition of 2,2'-azobis(2-methylpropionamidine) (AAPH; Sigma-Aldrich) in a 75 mM-phosphate buffer (pH=7.4). ORAC values were defined as the difference between the area under the fluorescein decay curve and the blank (net area under curve, AUC). The course of the reaction was accompanied by standard curve with 6-hydroxy-2,5,7,8--tetramethylchroman-2-carboxylic acid (Trolox, Sigma--Aldrich). Regression equations between net AUC and antioxidant concentration were calculated. ORAC-fluorescein values were expressed in μmol of Trolox equivalents (TE) per mg of samples (*23,24*).

Cell culture

Sample preparation

Oil samples were mixed in dimethyl sulfoxide (DMSO)/Tween 80 (9:1) (Sigma-Aldrich) at an initial concentration of 80 mg/mL. Then, Eagle's minimum essential medium (EMEM; Sigma-Aldrich) was added to reach the final oil concentrations from 0.5 to 20 mg/mL. Emulsified systems were used for all tests with mammalian cells and they were processed on ULTRA-TURRAX tube drive workstation (IKA, Staufen, Germany) for 5 min at 4000 rpm.

HepG2 cell maintenance

The cells were cultured on Petri dishes until 80 % confluence using EMEM supplemented with 10 % fetal bovine serum (FBS; Gibco BRL, Thermo Fisher Scientific, Grand Island, NY, USA). Incubation was performed at 37 °C in a humidified atmosphere with 5 % CO_2.

Cytotoxicity assay

Ninety-six well plates (TPP Techno Plastic Products AG) were inoculated with HepG2 cells at a density of $2\cdot10^6$ cell/mL. After incubation for 24 h, the adherent cells were washed with phosphate-buffered saline (PBS; Sigma-Aldrich) and then incubated for 5 h with EMEM (Sigma-Aldrich) containing different concentrations of oil emulsions (0.5-4 mg/mL). Untreated cells were used as positive controls, and the emulsified system without the sample was taken as control. The average result of positive controls was considered as 100 % HepG2 viability. After removing the medium, cells were treated with 10 μL of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (Sigma-Aldrich) and 90 μL of PBS, and the plate was incubated at 37 °C for 3 h (*25*). The formed formazan crystals were dissolved by adding 100 μL of 10 % sodium dodecyl sulfate (Synth) in 0.01 M HCl (Synth) to each well, and the plate was incubated for 18 h. Absorbance measurements (at λ=540 nm) were performed using a NovoStar microplate reader (BMG LABTECH).

HepG2 endogenous catalase activity

HepG2 cells were seeded in 60-mm Petri dishes at $8\cdot10^5$ cell/dish. The cells were incubated in EMEM with 10 % FBS, changing the medium every 2 days until they reached 80 % of confluence. They were washed twice with PBS, treated with 3 mL of the samples (0.5 and 1 mg/mL of oil) and the emulsion control (without the sample) and incubated for 5 h. After that, they were once again washed with PBS at 4 °C, scrapped and centrifuged for 10 min at 4 °C and 266×*g* (Sorval Legend XT; Thermo Fisher Scientific, Waltham, MA, USA). Supernatant was discharged, the pellet was re-suspended in 200 μL of PBS at 4 °C and placed in the ultrasonic cold bath (Unique Ultrasonic Cleaner; Indaiatuba, SP, Brazil) for 5 min. The protein content of the cell extract was evaluated by Bradford assay (*26*). The enzymatic activity of the extracts was analyzed using the catalase assay kit CAT100 (Sigma-Aldrich), according to manufacturer's instructions.

Calculations and statistics

Values are expressed as arithmetic means. Statistical significance of the differences between the groups was analyzed using the Tukey's test. Differences were considered significant at $p<0.05$.

Results and Discussion

Characterization of samples after enzymatic interesterification

Commercial lipase, purified and immobilized on silica, showed lipolytic activity of 12.7 U/g. Crude lipase obtained from the fungus *Rhizopus* sp. showed lipolytic activity of 8.0 U/g. The mixture of both enzymes had lipolytic activity of 10.3 U/g. These enzymes, with different activities and specificities, were able to produce new restructured oil and fat from Amazonian palms.

Full chemical characterization of buriti oil, murumuru fat, the non-interesterified blend and the restructured lipids is described in a previous study published by our research group (*8*). Briefly, the major fatty acid in buriti oil was oleic acid (C18:1, 65.6 %), while 49.6 % of lauric (C12:0) and 26.7 % of myristic acid (C14:0) were detected in murumuru fat. The major triacylglycerol (TAG) classes in the buriti oil were triunsaturated (UUU, 50.0 %) followed by diunsaturated-monosaturated (UUS, 39.3 %). In murumuru fat, the major TAG classes were trisaturated (SSS, 69.7 %) and monounsaturated-disaturated (USS, 25.6 %). In the non-interesterified blend, all TAG classes were present in large mass fractions, predominantly UUU (35.3 %) and UUS (28.8 %). After interesterification, all the restructured lipids produced with the three enzymatic preparations showed reductions in SSS and UUU TAG classes and increases in USS and UUS, with predominance of UUS TAG and low amounts of SSS TAG.

Effect of enzymatic interesterification on bioactive phytochemicals

Tocopherols

Among the natural antioxidants, the most widely used in edible vegetable oil are the tocopherols. Antioxidant activity of these compounds is given by their ability to provide hydrogen of the phenolic group to oil free radicals, interrupting the chain of lipid peroxidation (*27*). In this study, α- and γ-tocopherols were the major tocopherols detected in buriti oil (Table 1). The β- and δ-tocopherol fractions are also present in this oil, although in lower mass fractions. Contrarily, tocopherols were not detected in murumuru fat sample. The non-interesterified blend, mostly produced with buriti oil, is also rich in α- and γ-tocopherols, with low concentrations of β- and δ-tocopherol.

Regardless of the used enzymes, the restructured lipids had similar mass fraction of α-tocopherol as the non-interesterified blend. The γ-tocopherol fraction was significantly increased and the δ-tocopherol fraction was reduced after the interesterification. Commercial lipase, when used alone, caused a more expressive decrease in β-tocopherol fraction. These results may indicate the occurrence of modifications of tocopherol isomers in the restructured lipids, revealing or reducing the antioxidant effect of the molecules. However, further studies need to be conducted to confirm these results.

Overall, the enzymatic interesterification had little effect on the mass fraction of tocopherols. The restructured lipids had significant amounts of α- and γ-tocopherol fractions. The α-tocopherol is considered a potent lipid-soluble antioxidant, playing a role in the *in vivo* mechanisms of action. The importance of this function is to maintain the integrity of long-chain polyunsaturated fatty acids present in the cell membranes (*28*). The γ-tocopherol is recognized as the most active in the removal of reactive nitrogen species with anti-inflammatory activity and possible action in inhibiting carcinogenesis mechanism (*29*).

Total carotenoids

Carotenoids are considered natural antioxidants, like tocopherols. When present in the oil, these compounds act to protect the lipid peroxidation, by scavenging free radicals. In addition to this protection, these natural antioxidants are also known to play a role in chronic disease prevention. Among different forms of carotenes, the β-carotene is the most important to the human body, being an important precursor of vitamin A (*30,31*).

Table 1 shows that buriti oil is a source of total carotenoids determined as β-carotene (781.6 μg/g). Other studies with buriti oil also confirm that it is an important source

Table 1. Bioactive phytochemicals in buriti oil, murumuru fat, non-interesterified blend and restructured lipids

Sample	*w*(tocopherols)/(mg/g)				*w*(total carotenoids)/(μg/g)	*w*(total phenolics as GAE)/(mg/kg)
	α	β	γ	δ		
Buriti oil	(112.5±3.9)[a]	(7.1±0.0)[a]	(107.4±3.4)[a]	(9.4±0.5)[a]	(781.6±67.3)[a]	(107.0±1.2)[a]
Murumuru fat	n.d.	n.d.	n.d.	n.d.	(21.8±1.6)[b]	(16.2±0.7)[b]
Non-interesterified blend	(74.6±0.7)[b]	(1.9±0.2)[b]	(50.1±0.3)[b]	(8.3±0.2)[a]	(643.1±61.8)[a]	(80.7±1.4)[c]
w(restructured lipids)/%						
2.5 (commercial)	(76.0±4.1)[b]	(0.3±0.4)[c]	(69.1±3.9)[c]	(3.5±0.1)[b]	(688.6±91.7)[a]	(85.3 ±3.2)[c]
2.5 (*Rhizopus* sp.)	(69.2±3.0)[b]	n.d.	(63.3±2.7)[c]	(2.8±0.4)[b]	(666.3±26.7)[a]	(70.3±0.7)[c]
1.5 (commercial) + 1.5 (*Rhizopus* sp.)	(73.2±0.3)[b]	(1.9±0.1)[b]	(68.1±0.0)[c]	(3.9±0.8)[b]	(668.0±24.7)[a]	(80.7±2.2)[c]

Values are expressed as mean±standard deviation (S.D.). Mean values with the same letter in a column did not differ significantly ($p \leq 0.05$). n.d.=not detected, GAE=gallic acid equivalents

of β-carotene (*6,7,31*). Murumuru fat had lower mass fraction of total carotenoids than buriti oil and interesterified samples. The effect of interesterification of buriti oil and murumuru fat on carotenoid content is presented in Table 1. Enzymatic interesterification did not significantly alter the final mass fraction of carotenoids. All produced restructured lipids kept similarly high content of carotenoids from the buriti oil. This result is important because one of the most interesting and appealing characteristic of the buriti oil is its high mass fraction of carotenes, and the studied process maintained this attribute in the new lipid samples. Other studies using Lipozyme TLIM treatment in different types of oil showed similar results, reducing the carotenoid content after interesterification (*32,33*). This type of enzymatic interesterification normally occurs under mild conditions, maximizing the retention of minor compounds, contrarily to chemical interesterification (*7,34*).

Total polyphenols

Phenolic compounds are considered the main antioxidant metabolites in plants. They have the ability to donate hydrogen or electrons to form stable radicals (*35*). In the present study, the enzymatic interesterification produced restructured lipids in which the antioxidant phenolic compounds have been preserved. Buriti oil showed to be a valuable source of phenolic compounds (Table 1). Although the mass fraction of total phenols in buriti is lower than in olive oil (170-210 mg/kg), it is larger than in other vegetable oils (*36*). The non-interesterified blend and the restructured lipid samples had the same mass fraction of total phenolics ($p<0.05$), indicating that the interesterification had no effect on the concentration of these compounds. Total phenolics were also determined in the study of Nagaraju and Belur (*37*), where coconut and olive oil were treated with the commercial lipase IM-60 (Novo Nordisk, Copenhagen, Denmark), showing no significant differences between blends and restructured lipids (approx. 115 mg GAE/kg).

Antioxidant activity

The DPPH assay has been widely used to determine the antioxidant capacity of vegetable oil. It is a qualitative study that can separate samples based on their antioxidant capacity (*30*). The ORAC assay measures the ability of an antioxidant to scavenge free radicals by hydrogen atom donation. It serves as a model for antioxidant analyses of food and in physiological systems (*24*). The method was adapted using an emulsion (80 mg per mL of sample) in DMSO/Tween 80 (9:1 by volume) and further diluted in phosphate buffer. Fig. 1 and Table 2 show the DPPH and ORAC values, respectively, of the tested samples.

The DPPH assay results show that the radical scavenging capacity is related to the concentration of the tested oil. The lower concentrations (5 and 10 mg/mL) had scavenging activity between 30 and 40 % in the analyzed samples. However, the restructured lipids produced with the mixture of both enzymes showed lower antioxidant effect. When the concentration of the samples was increased to 50 and 100 mg/mL, it was possible to distinguish significantly the radical scavenging activity of each different sample. At these concentrations, the pure buriti oil and the restructured lipids produced with the enzyme from *Rhizopus* sp. had greater antioxidant potential. These restructured lipids were the ones with the largest reduction in the triunsaturated TAG class content and were more susceptible to oxidation. The large reactivity of the carbon double bonds in unsaturated fatty acids makes these substances primary targets for free radical reactions (*38*). The literature does not usually indicate that enzymatic interesterification significantly affects the antioxidant capacity of the oil. Depending on the used enzyme, enzymatic interesterification can be a great tool to assist in the development of oil with greater technological and antioxidant potential. The different enzymatic preparations applied for the interesterification had no significant influence on

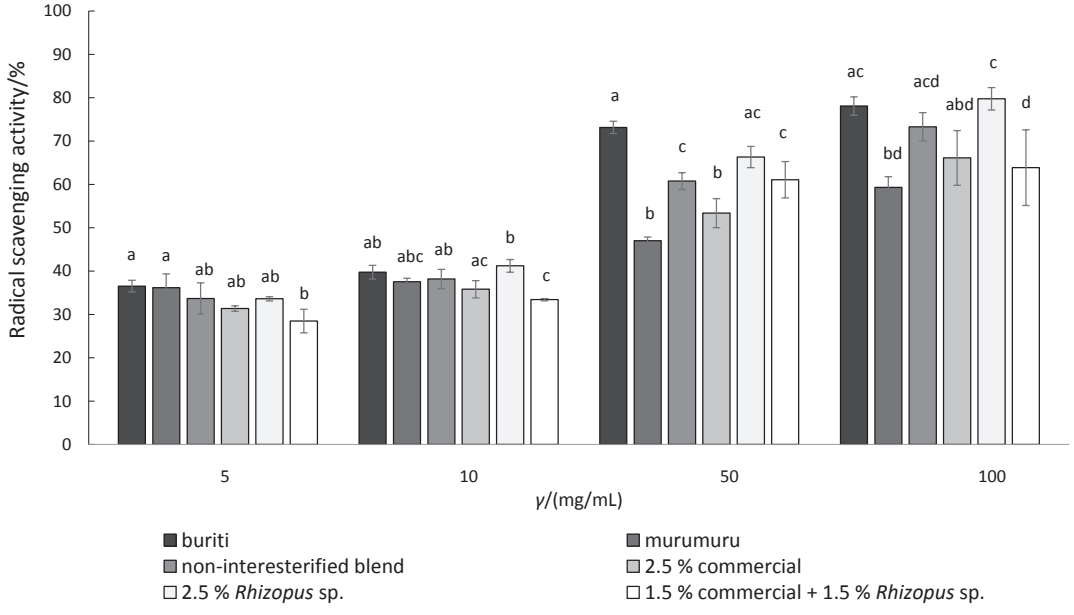

Fig. 1. Radical scavenging activity (DPPH assay) of oil and fat from Amazonian palms. Error bars represent standard deviations. Values with same letters, at the same concentration, are not significantly different ($p<0.05$)

Table 2. Oxygen radical absorbance capacity (ORAC) values and linearity ranges for different concentrations of buriti oil, murumuru fat, non-interesterified blend and restructured lipids

Sample	ORAC as TE	Linearity range	Slope	Intercept	R^2
	μmol/mg	γ/(mg/mL)			
Buriti oil	(95.3±11.9)[a]	4-20	75.7	178.9	0.99
Murumuru fat	(61.2±15.8)[b]	4-20	34.6	241.6	0.98
Non-interesterified blend	(77.4±4.3)[ab]	4-20	71.6	53.8	0.99
w(restructured lipids)/%					
2.5 (commercial)	(104.4±19.2)[a]	4-20	69.6	320.9	0.97
2.5 (*Rhizopus* sp.)	(97.5±13.4)[a]	4-20	74.8	217.4	0.98
1.5 (commercial) + 1.5 (*Rhizopus* sp.)	(77.7±26.6)[ab]	4-20	35.6	377.8	0.93

All results are expressed as mean value±S.D. Mean values with the same letter in a column did not differ significantly ($p \leq 0.05$). TE=Trolox equivalents

the minor antioxidant compounds of the oil; however, they showed great differences in the TAG profiles of the new types of oil, and on the antioxidant potential of these samples against DPPH radical. Further studies should be performed to identify the formation or stabilization of the molecules that confer antioxidant activity in the lipids formed during the enzymatic interesterification.

The ORAC results for antioxidant capacity of the samples corroborate the above mentioned observations and hypothesis. In Table 2, we can observe that the highest ORAC antioxidant capacity was found in the buriti oil and restructured lipids treated with 2.5 % of lipases (with no significant difference among them). The non-interesterified blend showed lower antioxidant potential than the pure buriti oil (probably due to the dilution of the antioxidant compounds by the addition of murumuru fat) and also lower than restructured lipids. This result also indicates that enzymatic interesterification significantly increased the antioxidant capacity of the oil, independently of the content of minor compounds.

HepG2 cells viability

The results of HepG2 cell viability after exposure to different concentrations of emulsified samples are presented in Table 3. The data reveal a dose-dependent relationship, as lower concentrations of the samples (0.5 and 1.0 mg/mL) maintained cell viability around 100 %, and a significant decrease in cell viability was observed in treatments with 1-2 mg/mL of oil emulsions. The decrease in the cell viability was maintained in all samples tested up to the highest concentration (4 mg/mL). The reduction of cell viability was probably caused by increases in the amount of emulsifiers (DMSO and Tween 80) in the system. These compounds showed to be toxic at elevated concentrations to colon cells (*39*).

Based on the results of cytotoxicity, the lower concentrations (0.5 and 1 mg/mL) were chosen for cellular assays. These concentrations maintained cell viability approx. 100 %, showing no citotoxicity of oil samples or emulsifying agents.

Modulatory effects of the oil on HepG2 catalase

The activity of the endogenous antioxidant enzyme catalase in HepG2 cells was analyzed after cell treatment with oil emulsions for 5 h (Fig. 2). The ability to modulate the endogenous antioxidant response by inducing antioxidant enzymes, such as catalase, is one of the possible mechanisms by which the non-enzymatic compounds exert their beneficial effects.

The catalase activity of the cells did not show a dose--response relationship after treatments with different concentrations of oil emulsions. After buriti oil, murumuru fat, and non-interesterified blend treatments at 0.5 mg/mL, HepG2 cells showed higher catalase activity. Contrarily, after treatments with restructured lipids, we can observe a significant modulation of catalase activity, compared to buriti oil, murumuru fat and non-interesterified blend. The biggest difference was observed at 1 mg/mL of oil emulsions between restructured lipids and the non-interesterified blend. A few researches studied the relationship between vegetable oils and the activation of antioxidant enzymes. Oliveiras-López *et al.* (*40*) analyzed the effects of the consumption of extra virgin olive oil in healthy adults, detecting a significant increase in the catalase activity. The authors attribute this increased enzyme activity to minor polar compounds and tocopherols contained in virgin olive oil sample.

Studies of the catalase activity on liver cells show its importance in the prevention of cytotoxicity and cell death induced by oxidative stress through peroxide conversion into hydrogen and oxygen into water (*41,42*). The oil treatments may have stimulated β-oxidation reactions that increase catalase activity to compensate for the generation of peroxides (*42*). Researchers believe that the increased basal catalase activity may be related to a protective effect, an enzymatic activation to generate faster metabolism, and excretion of oxidizing agents (*42,43*). However, when the rate of increase of catalase activity outweighs the basal activity value, it can culminate in cell death and is indicative of pathological conditions or the depletion of other components in the endogenous antioxidant system

Table 3. Viability of human hepatoma cells (HepG2) treated with buriti oil, murumuru fat, non-interesterified blend, restructured lipids and emulsion

Sample	γ/(mg/mL)			
	0.5	1.0	2.0	4.0
	Viability(HepG2)/%			
Buriti oil	$(100.9\pm0.1)^{abc}$	$(103.7\pm0.6)^{abc}$	$(79.5\pm1.9)^{ad}$	$(58.8\pm5.6)^{ac}$
Murumuru fat	$(108.7\pm1.1)^{a}$	$(114.3\pm8.1)^{ac}$	$(67.8\pm1.2)^{abd}$	$(33.5\pm0.7)^{be}$
Non-interesterified blend	$(102.3\pm1.4)^{ab}$	$(99.2\pm2.6)^{abc}$	$(62.2\pm2.5)^{bd}$	$(38.1\pm5.5)^{bc}$
w(restructured lipids)/%				
2.5 (commercial)	$(99.2\pm3.1)^{bc}$	$(101.4\pm1.3)^{abc}$	$(62.8\pm9.2)^{bd}$	$(46.9\pm2.8)^{c}$
2.5 (*Rhizopus* sp.)	$(102.6\pm0.5)^{ab}$	$(93.8\pm3.5)^{bc}$	$(38.2\pm2.0)^{c}$	$(8.6\pm0.1)^{df}$
1.5 (commercial) + 1.5 (*Rhizopus* sp.)	$(100.4\pm3.4)^{abc}$	$(116.1\pm6.6)^{a}$	$(67.5\pm1.2)^{d}$	$(21.7\pm0.1)^{ef}$
Emulsion	$(93.6\pm2.8)^{c}$	$(98.0\pm0.9)^{c}$	$(46.7\pm1.2)^{c}$	$(15.5\pm0.6)^{f}$

All results are expressed as mean value±S.D. Mean values with the same letter in a column did not differ significantly ($p\leq0.05$)

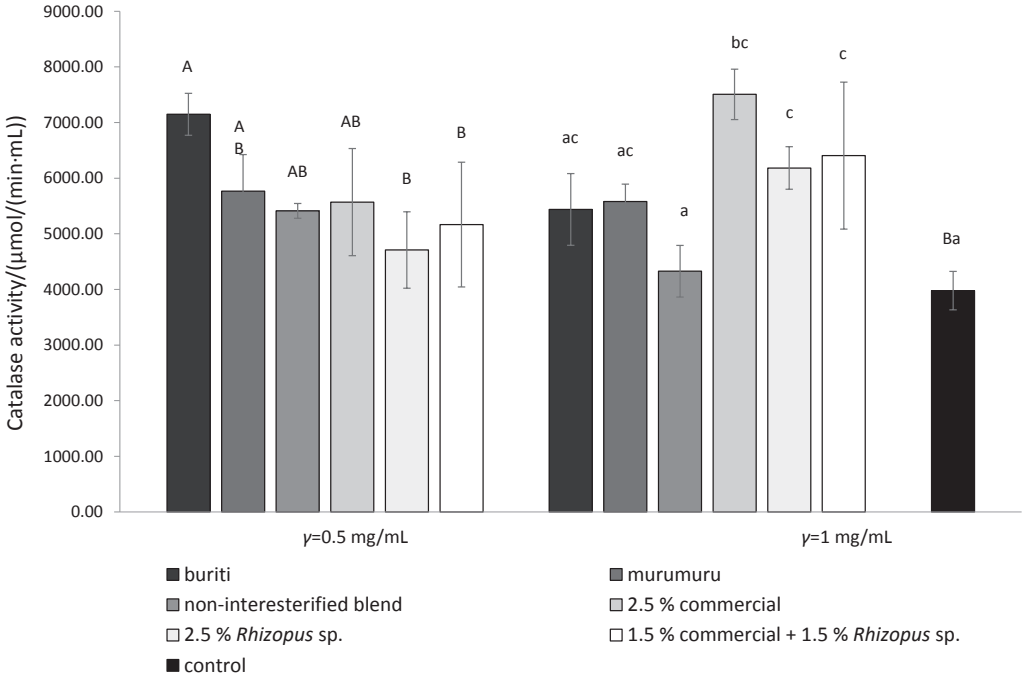

Fig. 2. Catalase activity of human hepatoma cells (HepG2) after treatment with oil and fat from Amazonian palms. Error bars represent standard deviations. Values with same letters, at the same concentration, are not significantly different ($p<0.05$)

(*44*). Such exacerbated response was not observed in this study, showing a beneficial modulatory effect of the oil on the antioxidant enzyme.

Conclusions

The enzymatic process developed for the interesterification of specific types of oil may represent an important and efficient way to restructure lipids with desirable technological properties. These oils and fats show a redistribution of the fatty acids present in the glycerol, which may generate new types of oil, but preserving the presence of biologically active minor compounds and antioxidant capacity, with diverse biological and nutritional properties Results indicate that the enzymatic reactions may have generated or stabilized molecules with antioxidant properties and modulatory effects on the endogenous antioxidant enzyme catalase in liver cells.

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