



Direct and Solvent-Free Aminolysis of Triglyceride from *Oenocarpus bataua* (Patawa) Oil Catalyzed by Al_2O_3

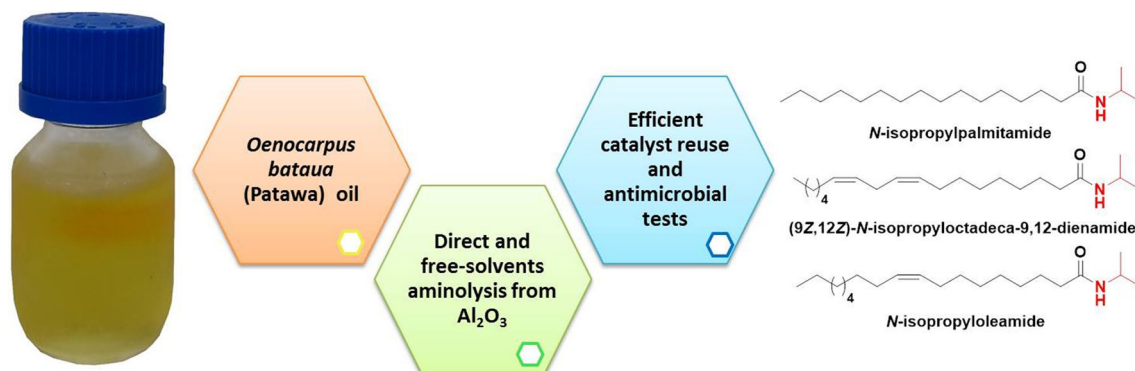
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Abstract

The amidation of fatty acid is an important reaction for synthesis of fatty alkolamines with a variety of pharmacological activities. The objective of this paper was to demonstrate the aminolysis direct and solvent of the triglycerides from the patawa oil by heterogeneous catalysis. The results of the amidation direct free solvents were favorable using 5% Al_2O_3 as catalyst, with a conversion of 94% at 24 h of reaction. After spectroscopic characterization, we decided explore the antimicrobial tests of the fatty amides (FAA) against the *Staphylococcus aureus* strain (ATCC 33591), showing a MIC activity of 1.9612 mg/mL and the fatty ethyl esters (FAEE) a 3.4125 mg/mL MIC. Therefore, the synthesis direct free solvents of FAA by Al_2O_3 from patawa oil was viable and innovative.

Graphical Abstract



Keywords Fatty oil · Amazon oil · Amidation · Heterogeneous catalysis · Aluminum oxide

1 Introduction

The amidation of fatty acid is an important reaction for synthesis of fatty alkanolamines, these compounds have a variety of commercial applications [1], are used as ingredients for detergents, lubricants [2], fungicides [3], corrosion inhibitor, agrochemicals [4], cosmetic [5] and as

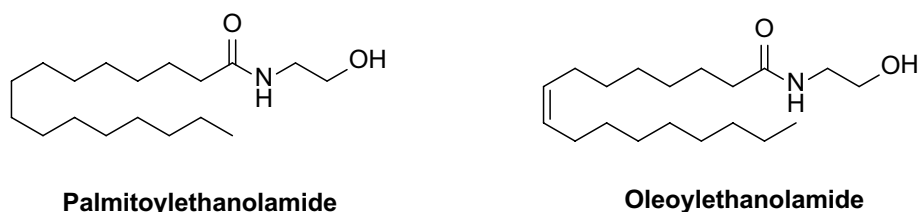
pharmaceutically active compounds, such anti-carcinogenic [6], anti-tuberculosis [7], anti-inflammation [8] and activity against the Alzheimer disease [9].

This class of amides can be naturally found in restricted organism and microorganisms [10]. This amides are considered a neuromodulatory lipid that belongs to a family of signaling molecules collectively termed endocannabinoids [11], which activate receptors of the type CB1 or CB2, as palmitoylethanolamide, and oleoylethanolamide, similar to anandamide (Fig. 1), but characterized by saturated or monounsaturated acyl chains [9].

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Fig. 1 Chemical structures of endocannabinoids



Fatty alkanolamines are typically synthesized by use of fatty acid ester with an alkanolamine in the presence of a metallic catalyst, as such CaO, ZnO, TiO, SiO₂ [12] or biocatalysis, as CAL-B [13], for example normally involves two steps reaction at high temperatures [14] with incomplete product formation, which increases the cost of production and consequently require more steps of purification.

Lopes et al. [10] described the obtaining of fatty amides employing an excess of ethanolamine in a proportion of 1:6 (fatty ester: ethanolamine) at temperatures of 90–130 °C, with a final yield of 88% when sodium methoxide was used as catalyst.

Other studies reports the use of pre-prepared catalysts with high amine ratios, increasing the cost of the process [15] or using alkali metals such as Li or Na supported in crystalline and nanoparticulate CaO at 90 °C [16].

Therefore, the search for new forms of fatty amide synthesis becomes essential for the study of these compounds and the different ways of obtaining products with different pharmacological properties.

The Amazon forest is very rich in oily fruits, which represents a great economic potential for the region. *Oenocarpus bataua* Mart., commonly called patawa, is another Amazonian palm fruit, frequently used by local communities an important source of oil with biological activity [17] traditionally used for medicinal, culinary, and cosmetic purposes, including for hair and skin care [18]. The chemistry composition of oil from seed patawa is being chemically comparable to olive oil, rich in monounsaturated fatty acids, particularly oleic acid (C18:1) [19].

Knowing the need to improve existing chemical methods for the transformation of organic substances, with biological activity, at low cost and free solvents, in this article we showed the use of Al₂O₃ as a heterogeneous catalyst in the reaction direct aminolysis of triglyceride from of the patawa oil and evaluation of the anti-bactericidal activity of the obtained product.

2 Materials and Methods

2.1 Extraction of the Patawa Oil (PO)

The extraction of the oil was made from the pulp of patawa acquired in Saint-Laurent in French Guiana (Western zone

of the country). Hexane was used as the drag solvent by soxhlet extraction for 4 h from 5 to 6 cycles with a mass of 78.0 g of pulp and 300 mL of solvent.

2.2 Chemicals and Reagentes

Isopropylamine (99.5%) was purchased from Sigma-Aldrich, Lipase acrylic resin from *Candida antarctica* (CAL-B ≥ 5000 U/g) expressed in *Aspergillus niger* was purchased from Sigma Aldrich. Hexane (98%) was purchased from Synth (SP, Brazil) and ethanol (99%) was purchased from Solven (SP, Brazil). Deuterated chloroform purchased was from Cambridge Isotope Laboratories. Al₂O₃ (99%) was purchased from VETEC—Sigma Aldrich.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS)

The reactions analyses (fatty ethyl ester = FAEE and fatty amide = FA) were conducted using a gas chromatograph (GCMS-QP 2010) equipped with an auto-sampler injection AOC-20i (Shimadzu). Electron impact detection used as detector (Shimadzu MS2010 Plus), electronic impact of 70 eV and fragments detected from 50 to 550 Da. Separations were performed on a fused silica capillary column (RTX-5MS with i.d. = 0.25 mm, length = 30 m and film thickness = 0.25 μm) in a stream of helium 1.03 mL/min. The sample was solubilized in dichloromethane (2 μg/mL) and 1.0 μL of the solution was subjected to following experimental conditions: injector temperature, 210 °C; detector temperature, 250 °C; carrier gas, Helium; flow rate 3.0 mL/min; split injection with split ratio 1/15. The column temperature was programmed from 90 °C, with an increase of 6 °C/min to 250 °C, ending with a 5 min isothermal at this temperature, the total analysis time was 33.67 min.

2.4 Fourier Transform Infrared Analysis (FTIR)

FTIR spectra were recorded on a Shimadzu IRAffinity spectrometer samples (OP, FAEE and FA) were prepared as thin films on KBr disks. The transmittance was expressed in cm⁻¹ of band between 4000 and 400 cm⁻¹ with resolution of 4 cm⁻¹ and 64 scans.

2.5 Nuclear Magnetic Resonance (NMR)

^{13}C NMR spectra (OP, FAEE and FAA) were recorded on an Agilent Technologies 500/54 Premium Shielded spectrometer. The samples were solubilized in CDCl_3 and chemical shifts expressed in ppm relative to internal standard TMS or deuterated solvents. The chemical shifts were given in ppm.

2.6 Transesterification Reaction of PO by CAL-B

The reaction followed the method described by Ferreira et al. [20] with some modifications. In a vial (3 mL) was added the patawa oil sample (1.0 mL), 10% (10 mg) of the lipase from *C. antarctica* B and 3.0 mL of ethanol on magnetic stirring (300 rpm, $27 \pm 2^\circ\text{C}$) by 24 h. After this period, the enzyme was filtered and washed with acetone (3×1.0 mL). The filtrate was evaporated under reduced pressure and purified by column chromatography on silica gel using hexane:ethyl acetate (9:1) as eluente. The product, fatty acid ethyl esters (FAEE), obtained were analyzed by spectrometric methods.

2.7 General Procedure for Reaction of Aminolysis of Triglycerides from *Oenocarpus bataua* (Patawa) Oil

First, the amidation reaction was conducted between isopropylamine (3.0 mL–2.166 g), PO or FAEE (1.0 mL) and 10% Al_2O_3 , SiO_2 , TiO_2 or CAL-B as catalyst on magnetic stirring (300 rpm, $27 \pm 2^\circ\text{C}$) by 24 h. After this period, the enzyme was filtered and washed with acetone (3×1.0 mL). The filtrate was evaporated under reduced pressure and purified by column chromatography on silica gel using hexane:ethyl acetate (9:1) as eluente. Other experiments were performed varying catalyst concentration and reaction time (Table 1).

Table 1 Variations of direct aminolysis reaction with isopropylamine

Entry	Catalyst	Proportion (%)	Time (h)	Conversion (%) ^a
1	Al_2O_3	10	24	98
2	Al_2O_3	10	12	90
3	Al_2O_3	5	24	94
4	CAL-B	10	24	82
5	SiO_2	10	24	90
6	TiO_2	10	24	92
7	Free catalyst	–	24	Traces

^aConversion by analysis

2.8 Catalyst Reusability on Amylolytic Reaction

At the end of each reaction cycle, the catalyst was filtered and washed with acetone (3×1 mL) for later reuse. The reaction conditions were the same as described above (entry 3, Table 1).

2.9 Preparation of Bacterial Suspension

The reference strain used in the study was *Staphylococcus aureus* ATCC 33591. The microorganism was obtained from the Laboratory of Reference Materials of the National Institute of Health Quality Control (Oswaldo Cruz Foundation—FIOCRUZ, Rio de Janeiro, RJ, Brazil) and reactivated according to the reactivation protocol of the same laboratory. In a test tube (16×160 mm) containing 10 mL of Mueller–Hinton broth the strain *Staphylococcus aureus* ATCC 33591 was inoculated and then incubated at 37°C for 24 h. After incubation, the bacterial suspension was adjusted with saline (0.9% NaCl) to give a turbidity standard of 0.5 McFarland.

2.10 Susceptibility Test—Minimum Inhibitory Concentration (MIC)

The broth microdilution test was performed in triplicate. Sterile 96-well acrylic plate with 500 μL each, arranged in 12 columns of 8 wells. In the first well was added 100 μL of the FAA dissolved in dimethyl sulfoxide (DMSO) at a concentration of 30 $\mu\text{L}/70$ μL , and successive dilutions were performed with 100 μL of solution in the 6 consecutive wells and the latter was used as a discard. Then, 20 μL of the previously prepared bacterial suspension was added in each well and the plate incubated at 37°C for 24 h [21]. After this period, an aqueous solution of 2 mg/mL of tetrazole salt was added and incubated for an additional 4 h to reveal bacterial growth [22]. The MIC was defined as the lowest concentration of the sample capable of inhibiting bacterial growth, detected by the naked eye. The positive control of the experiment was performed with 0.5 Mac Farland bacterial suspension culture medium. Negative controls were the culture medium added with vancomycin and bacterial suspension and culture medium inoculated with bacteria and DMSO, which was the solvent used for the dissolution of the test substance. Statistical comparison units were the sequential dilutions in which MIC was observed. The data were tabulated in the GraphPad Prism 6.01 program (GraphPad for Windows program, San Diego, CA—USA), by which the statistical analysis was derived by the Kruskal–Wallis test and Dunn post-test, adopting a significance level of 95%.

3 Results and Discussion

3.1 Synthesis de Fatty Amides

The patawa oil extracted from the seeds, showed that its main physico-chemical properties are in agreement with the parameters already reported in the literature [23]. Saponification index of 200 mg KOH/g; free acidity of 5.07 mg KOH/g; melting point 33–36 °C and a bulk density 0.9650 g/cm³. Failure to observe the standard in the physico-chemical characteristics may unnerve the reaction process through the formation of unwanted sub products. The solvent free direct aminolysis reaction from the patawa oil was performed with various catalysts at room temperature for 24 h. The catalyst bets for convention of FAA by direct aminolysis free-solvents from OP was the Al₂O₃ (5%) at 27 ± 2 °C for 24 h, with 94% yield (Table 1, entry 3). The yield of the FAA decreased with decreasing at time for 12 h (Table 1, entry 2) and remained unchanged with increasing amount of the catalyst at 5% (Table 1, entry 1). The others Lewis acid as SiO₂, TiO₂ and CAL-B were examined, however the results were inferior to those obtained using Al₂O₃ from OP free solvents.

Based on the principles of Green Chemistry, such as, lower energy expenditure, higher atomic yield and smaller amount of the catalyst used in the reaction [24], we conclude that 5% of the catalyst in 24 h is the optimized condition for this direct amidation reaction from patawa oil.

Some authors described the obtainment of quantitative yields in the reaction of direct amyolysis of cotton oil with diethanolamine in the presence of 3.5% Ca(OH)₂ with Na⁺ ions, 5% Ni/CaO or NaC/CaO (3 wt% Na impregnated in CaO) used as catalyst. However, in all cases, the direct aminolysis reaction was realized at high temperatures (> 100 °C) and the used catalysts were prepared from the calcination process (650 °C) [15, 16, 25, 26].

It is believed that the high catalytic Al₂O₃ activity is due to its specific area in relation to the other catalysts used. Catalytic studies report a larger specific area and a more stable porous face for Al₂O₃ [27, 28] when compared to the catalysts other, as TiO₂ [29] and SiO₂ [30], this aspect directly influences the formation of final products, as in works reported in the literature [31, 32]. The Al₂O₃ stability of Al³⁺ allows a greater selectivity of the active sites besides that the metallic surfaces are restructured when chemisorbed during the reaction process, but with a restructuring only in the superficial region, which characterizes the loss of catalytic activity during the synthesis recycle with the Al₂O₃ [28]. On the other hand, CAL-B, because it is an enzymatic catalyst, has demonstrated the lowest yield due to the lack of the same reaction stability during the reported process, as was the case with metallic

catalysts. The enzyme used is a lipase with a geometric region restricted by the nanostructure [33].

The Fig. 2 below shows the response surface graph, using Statistica 7.0 ® software [34], for the parameters used during the amidation process using isopropylamine for conversion of PO into their respective FAAs. The percentage of catalyst (Al₂O₃) to be used during the reaction process can be calculated by the equation described in the image. It is notorious to say that the reaction time is the main influent in the reaction yield followed by the percentage of the catalyst, which end up influencing linearly in the total percentage of synthesized fatty amides.

Purified products from the FAEE and FAA were analyzed by GC-MS. The Fig. 3 showed the GC chromatogram analysis of fatty acids ethylic ester (FAEE) of the PO next of the transesterification reaction by CAL-B. The fatty acid composition was found to be 18% palmitic (1), 2.5% linoleic (2), 70% oleic (3), 5.1% stearic (4) and 4.4% other not identification (5 and 5a). This composition of fatty acid of the OP was according with Oliveira et al. [19] which described oleic acid (78.88%) as the majority and palmitic (13.7%), stearic (4.76%) and linoleic (2.12%) as the main constituents of patawa oil. The relative percentage of fatty acid esters was calculated from total ion chromatography by integrator area and identified using a MS database (NIST 5.0) and literature. The Table 2 show that after the treatment of PO with isopropylamine all the fatty acids were converted into respective FAA, and major fatty acid amides were *N*-isopropylpalmitamide (1a) and *N*-isopropyleamide (3a).

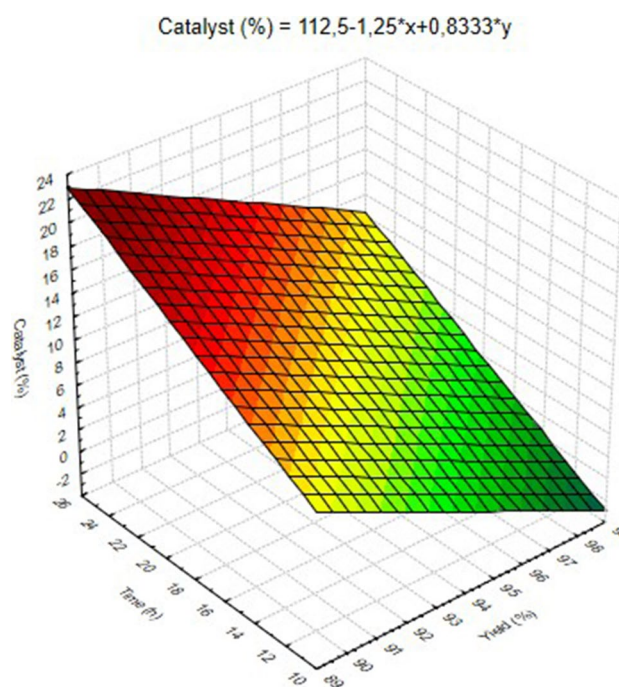
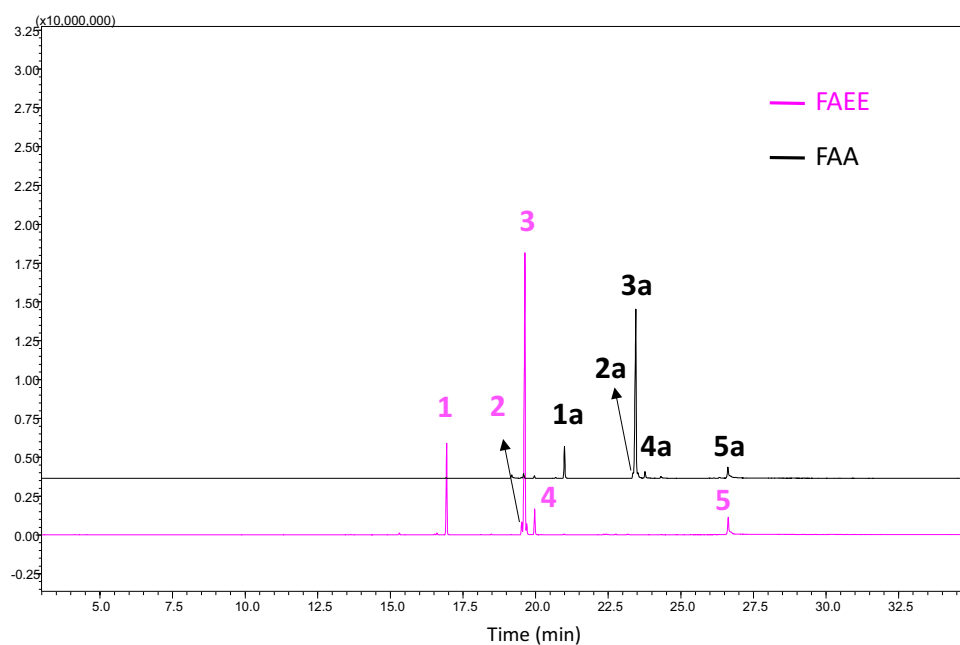


Fig. 2 Response surface graph for getting FAA

Fig. 3 Chromatograms of FAEE and FAA analyzed by GC-MS**Table 2** FAEE and FAA identified in the analysis by GC-MS

FAEE	R. T.* (min)	FAA	R. T.* (min)
<chem>CCCCCCCCCCCCCCCC(=O)OCC</chem> ethyl palmitate (1)	16.93	<chem>CCCCCCCCCCCCCCCC(=O)NC(C)C</chem> <i>N</i> -isopropylpalmitamide (1a)	21.00
<chem>CCCCC=CCCCCCCC(=O)OCC</chem> ethyl linoleate (2)	19.52	<chem>CCCCC=CCCCCCCC(=O)NC(C)C</chem> (9 <i>Z</i> ,12 <i>Z</i>)- <i>N</i> -isopropyloctadeca-9,12-dienamide (2)	23.35
<chem>CCCCC=CCCCCCCC(=O)OCC</chem> ethyl oleate (3)	19.62	<chem>CCCCC=CCCCCCCC(=O)NC(C)C</chem> <i>N</i> -isopropyleamide (3)	23.45
<chem>CCCCCCCCCCCCCCCC(=O)OCC</chem> ethyl stearate (4)	19.96	<chem>CCCCCCCCCCCCCCCC(=O)NC(C)C</chem> <i>N</i> -isopropylstearamide (4a)	23.53

*Retention time (Method described in item 2.3)

The fatty amides from isopropylamine and triglycerides of patawa oil were characterized by MS spectrum with typical peak at m/z 101 and m/z 114, resultants from McLafferty

rearrangement and γ -cleavage, respectively (Fig. 4). For *N*-isopropyleamide ($323.32 \text{ g mol}^{-1}$), in mass spectra the base peak was at m/z 101 with molecular ion at m/z 323 [10].

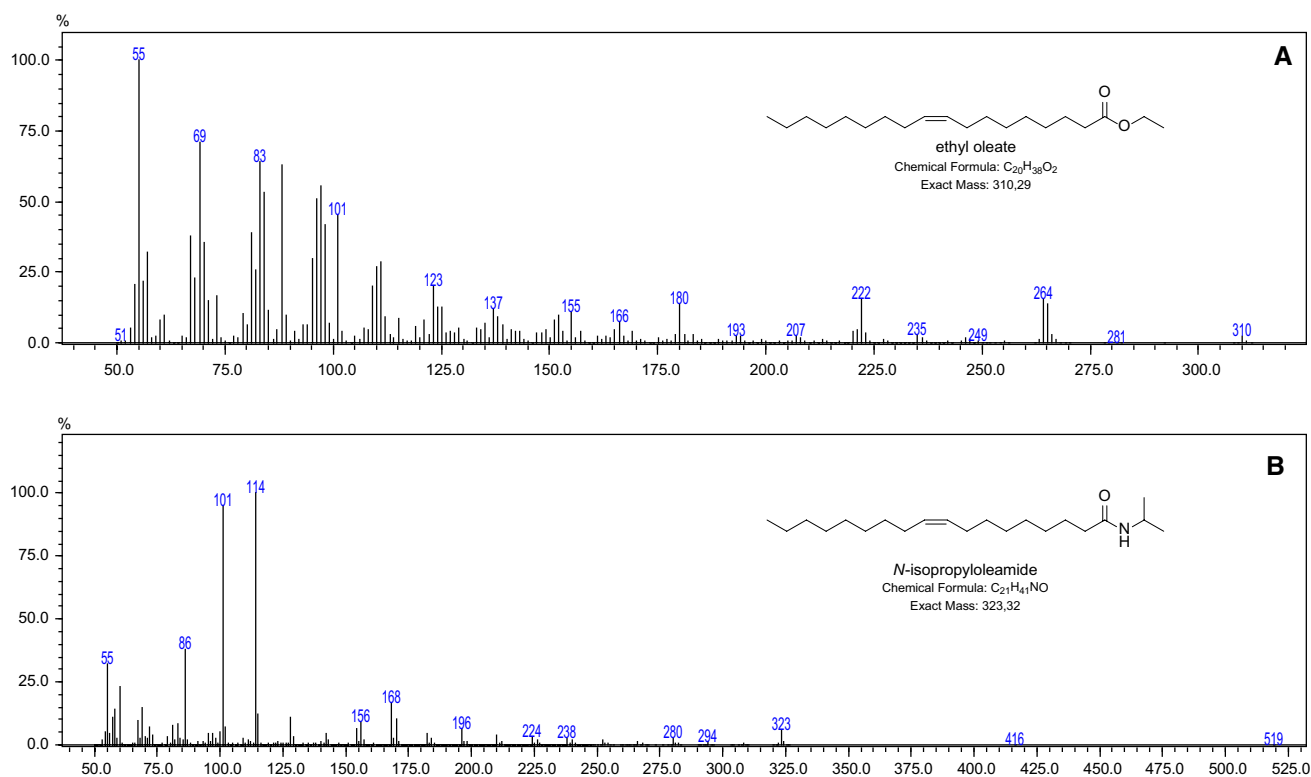


Fig. 4 Mass Spectrum of the FAEE (a) and FAA (b)

The corresponding spectra of the infrared analyzes are shown in Fig. 5. Such spectra show high similarity because they are the saturated and unsaturated alkyl chains derived from the OP triglyceride. The peak in 3005 cm^{-1} corresponds to the stretching of the $-C-H_{(sp^2)}$ and 2924 and 2854 cm^{-1} to the stretching of the ligation $-C-H_{(sp^3)}$, another characteristic peak of triglyceride derived

compounds appeared in 1745 cm^{-1} , related to the stretching of the ligation $-C=O$ of the PO, FAEE and for the FAA. Signals of the amidated compound are listed as the peaks in 3385 and 3304 cm^{-1} which correspond to the stretch $-NH_2$ and the peak in the 1647 and 1658 cm^{-1} correspond to the folding of the ligation $-N-H$, characteristics of the fatty amide synthesized. In the past, the peak for the double band

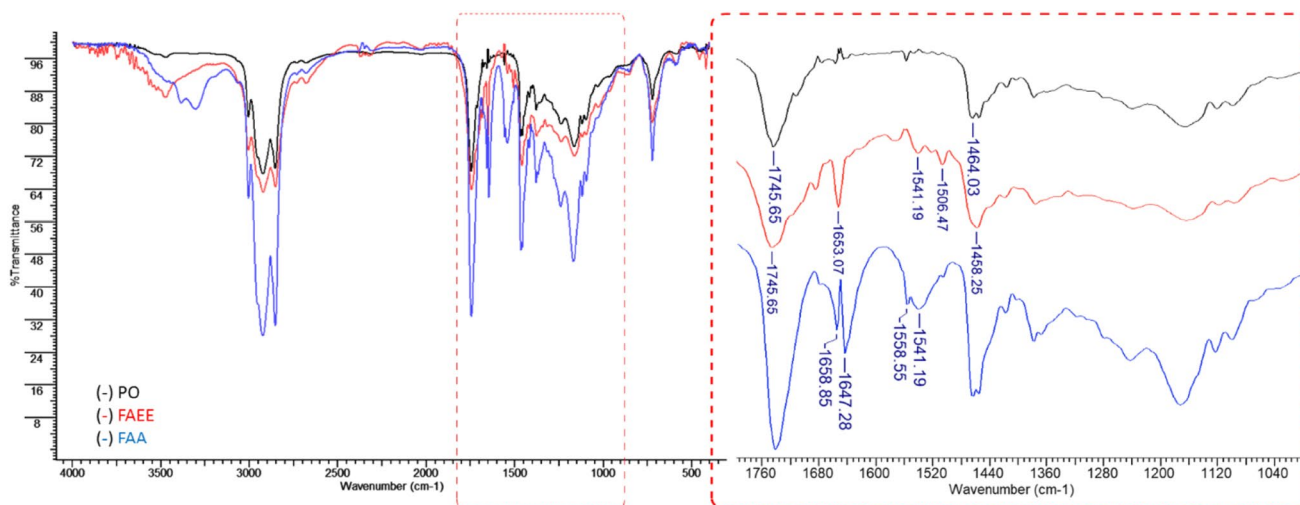
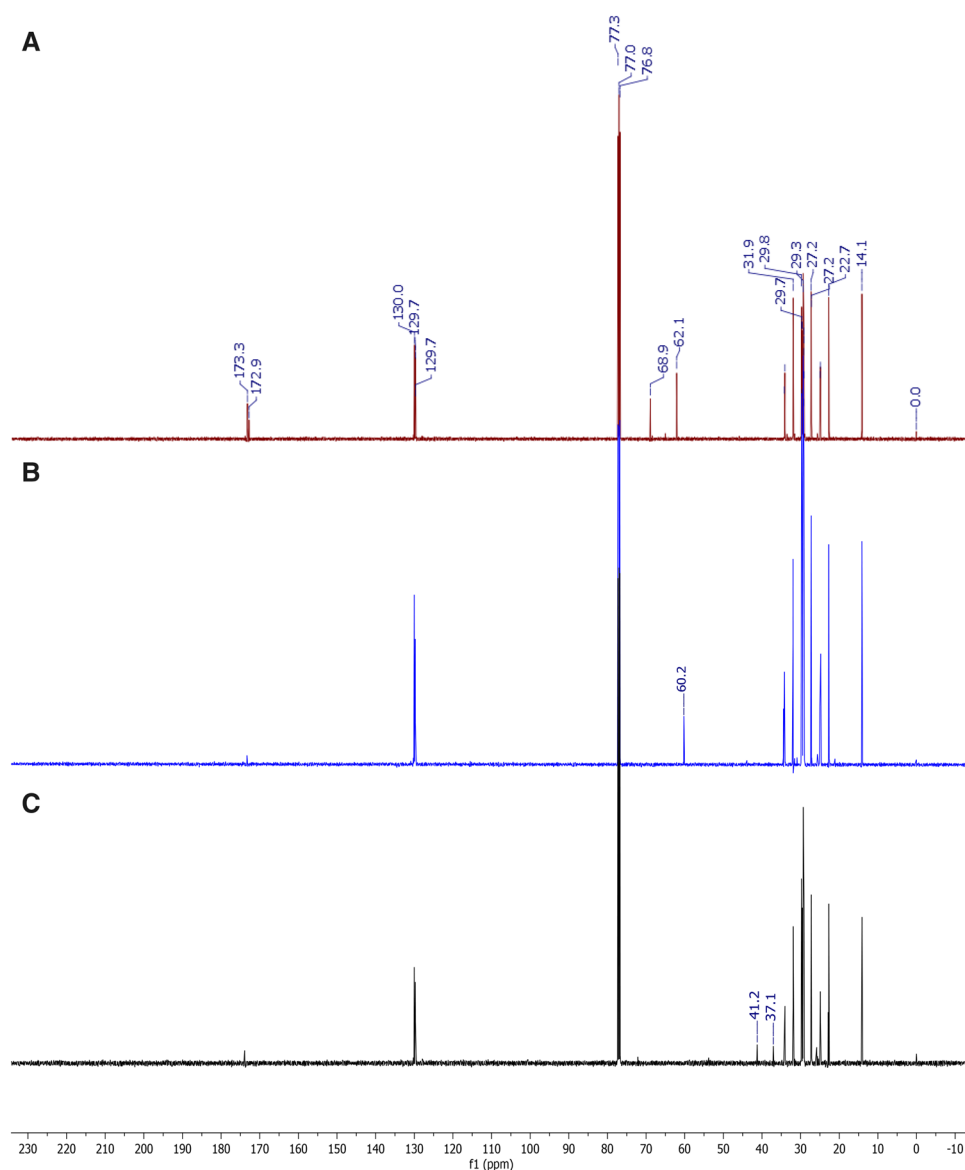


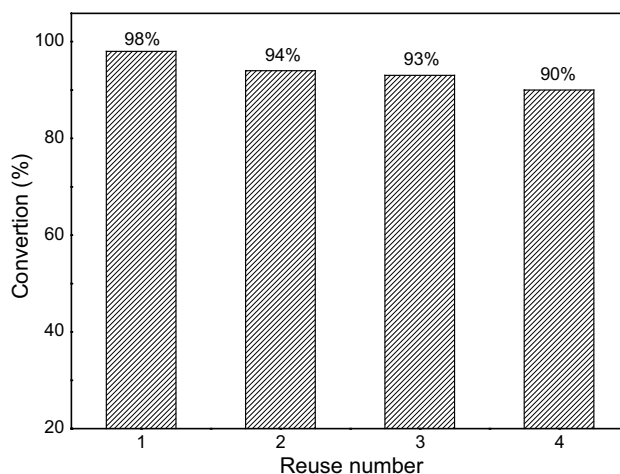
Fig. 5 Analysis IR of PO (-), FAEE (-) and FAA (-)

Fig. 6 ^{13}C NMR spectrum of the PO (a), FAEE (b) and FAA (c)

in 3385 cm^{-1} it refers to the grouping $-\text{NH}$ free in amides and the peak 1558 cm^{-1} referring to $-\text{NH}_2$ corresponding to the symmetrical angular deformation in the plane, confirming the result of the compound.

By analyzing the ^{13}C NMR spectrum corresponding to FAA, it is possible to notice an absence that the characteristic signals of the glycerol peaks in $68.9\text{--}62.1\text{ ppm}$, present in the spectrum corresponding to the PO (Fig. 6a). Other signs that evidence the formation of the FAA appear in 41.2 and 37.1 ppm (Fig. 6c), corresponding to the carbons $-\text{CH}$ and $-\text{CH}_2-$ of the amide (isopropyl).

Figure 7, below, shows the reaction yield of the amidation process using the Al_2O_3 catalyst through four cycles for 24 h by cycle. It is possible to observe the decrease of the yield, this aspect can be characterized by the wear of the active sites present in the pores of the catalyst, consequently, reducing its conversion capacity in function of structural collapse

**Fig. 7** Reuse of the catalyst in the amidation process

and loss of surface area [27]. Once, the selection of the particulate sizes, to about 30 μM , may favor the increase of the catalytic performance, since particles with smaller sizes have a greater specific area of action, because the improvement of the microstructure of the catalyst through the formation of nanoparticles allows a stability of the surface area, with optimized crystalline faces, which avoids the diversity of active sites and, consequently, the low selectivity [28]. However, the catalyst proved to be efficient and favorable for use through the methodology used, observing that there was a decrease of only 8% of the catalytic activity even after use for 4 consecutive cycles.

3.2 Antimicrobial Activity

Table 3, demonstrates the minimum inhibitory concentration (MIC) calculated for the antimicrobial tests against *Staphylococcus aureus* strain ATCC 33591.

The methodology employed in this study was validated by the absence of microbial growth for the control of sterility and positive control (1% azithromycin—1 mg/mL), as well as by the presence of microbial growth control. Knowing that oils are chemicals insoluble in water and chemical complexes [35], the inconsistency of the results obtained was minimized using the broth microdilution technique in order to provide greater contact between the products tested with the bacterial surface. DMSO solvent was also used to allow the dilution of the oils and amides in aqueous medium. The absence of bacterial growth against Azithromycin demonstrates the susceptibility of the samples to a synthetic antimicrobial. Although there was no consensus on the level of inhibition allowed for natural products when compared with standard antibiotics, Aligianis et al. [36] proposed a classification for plant materials based on MIC results, considering as: strong inhibition—MIC up to 500 $\mu\text{g/mL}$; inhibition—MIC between 600 and 1500 $\mu\text{g/mL}$ and as weak inhibition—MIC above 1600 $\mu\text{g/mL}$. Therefore, the MIC results of the three products present medium and weak inhibition for the products synthesized from the patawa oil and do not have significant differences in the bacterial inhibition capacity of *Staphylococcus aureus* ATCC 33591. Kabara et al. [37] showed the relation of lauryl *N,N*-dimethylamide with bacterial inhibition capacity of *Staphylococcus aureus*

in 0.109 $\mu\text{mol/mL}$. For Bakalova et al. [38] fatty amides with alkyl chains up to 10 carbon atoms all showed higher antibacterial activity at pH 9.1 as compared to pH 7.1 for *Escherichia coli*.

4 Conclusion

It was observed that direct aminolysis of the OP with 5% Al_2O_3 and 24 h of reaction showed a high yield of 94%. The chemical analyzes of GC-MS, IR-TF and NMR confirmed the chemical structure of the formed fatty amides, making possible the described process. The decrease in the amount of Al_2O_3 catalyst and the reaction time influenced directly and linearly the amount of product formed, as observed in the response surface graph. The antimicrobial analysis showed that the products synthesized have an activity against *Staphylococcus aureus* strains (ATCC 33591) with a MIC of 1.9612 mg/mL for amides and 3.4125 mg/mL for esters, which when compared to the literature is considerable compared to extracts from other Amazonian oleaginous plants. It is noteworthy that this is the first work that describes the direct synthesis of fatty amides from the solvent-free patawa oil in the Amazon region, without the formation of by-products and with a mild methodology, facilitating the reaction process and replication in new chemical or pharmacological studies of this type of product.

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Table 3 MIC of synthesized products

Product	Concentration (mL/mL) (%)	Concentration (mg/mL)
PO	3.125	0.8484
FAA	6.25	1.9612
FAEE	12.5	3.4125

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