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Article

Chemoenzymatic Synthesis and Anti-Inflammatory Activity of Fatty Acid Amides Prepared from *Bertholletia excelsa* (Brazil Nut) Triglycerides

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This study reports the synthesis of fatty acid amides (FAA), analogous to anandamide, prepared from Brazil nut oil (BNO), using Amano lipase from *Pseudomonas fluorescens* as a catalyst of direct aminolysis, with a good yield (95-68%). The anti-inflammatory effects of BNO and FAA were evaluated by the carrageenan-induced paw edema method. Analysis of the formation of edema showed that these FAA, at all dose used, significantly reduced the development of edema.

Keywords: direct aminolysis, biocatalysis, oleoylethanolamine, palmitoethanolamide, alkylamides

Introduction

Anandamide derivatives are known as *N*-arachidonoyl ethanolamide or fatty acid amides (FAA) and were identified and characterized as neuromodulators of the endogenous endocannabinoid class.[1] At that time, however, little attention was given to these molecules, due to their simplicity[2,3] currently, FAA are one of the most studied endocannanabinoid classes, owing to their vasorelaxant activities, and numerous biological activities, such as analgesic, antimicrobial,[4] antituberculosis,[5] anti-inflammatory,[6-8] larvicidal (against *Toxocara canis*)[9] and anti-carcinogenic activities.[10] FAA are also of considerable interest due to their extensive range of applications in lubricants, surfactants, cosmetics, and detergents.[11,12]

The FAA are frequently synthesized using a fatty acid ester with an alkanolamine, in the presence of a heterogeneous catalyst, such as ZnO,[13] CoO nanoparticle[14] or immobilized enzymes,[15-17] the reaction normally involves two steps, increasing the cost of production and the number of purification steps. One alternative for obtaining fatty amides is via their direct synthesis from triglycerides;[18] however, high temperatures are necessary for this process, in some cases.[19-21] The green chemistry concept has intensified the search for sustainable synthetic methodologies, and the identification and use of enzymatic catalysts have been of particular interest. Advantages of the application of enzymes in chemical synthesis include their high catalytic activity, and high chemo-, regio-, and stereoselectivity.[22,23]

Medicinal interest in products derived from higher plants has increased significantly worldwide. *Bertholletia excelsa*, popularly known as the Brazil nut, is produced by a large tropical forest tree of the Lecythidaceae family, which grows throughout the Amazon Basin of South America;[24] its almond consists of 60-70% fat, 15-20% protein (of good biological quality), liposoluble vitamins (A and E), and minerals (Ca, Fe, Zn, Na, K and Se).[25] Over the last 25 years, anti-inflammatory and antioxidant effects of *Bertholletia excelsa* oil have been identified.[26]

In this study, we report the use of Amano lipase from *Pseudomonas fluorescens* (LPF), as catalyst for the direct

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transformation of triglycerides from Brazil nut oil (BNO) into fatty acid amides (FAA) set. In addition, these FAA demonstrated anti-inflammatory properties, *in vivo*.

Experimental

Reagents and solvents

A sample of Brazil nut oil was purchased from COMAJA® ((Laranjal do Jari, Brazil) and stored at −4 ºC until use. Ethanolamine (99.5%), Amano lipase from *Pseudomonas fluorescens* (20.000 U g^{-1}, CAS No. 9001-62-1) and indomethacin 98.5% (CAS No. 53-86-1) were purchased from Sigma-Aldrich (São Paulo, Brazil). Hexane (98%) was bought from Synth (São Paulo, Brazil) and ethanol (99%) was purchased from Solven (São Paulo, Brazil). Deuterated chloroform was from Cambridge Isotope Laboratories (Andover, USA). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was from Sigma-Aldrich (São Paulo, Brazil) and Dulbecco's modified Eagle's medium (DMEM) was purchased from CULTILAB (Campinas, Brazil).

Transesterification of BNO by enzymatic catalysis (LPF)

The production of fatty acid ethyl ester (FAEE) from BNO by enzymatic catalysis was carried out in 3-mL Erlenmeyer flasks, containing 150 mg (154 µL) BNO, 475 µL ethanol, and 10% of LPF (m/m of the oil). The mixtures were incubated at room temperature (32 °C) on a 130-rpm orbital shaker (Lucadema). After 24 h, the reaction was completed, the reaction mixture was filtered and the organic phase was dried over sodium sulfate. The solvent was then removed under reduced pressure and the product was purified by gel silica column chromatography with a mixture of *n*-hexane:ethyl acetate (9:1) as eluent, and characterized using gas chromatography-mass spectrometry (GC-MS) analysis.[27]

General procedure for the aminolysis reaction of triglycerides from BNO

An amidation reaction was conducted between ethanolamine (3.0 mL; 2.166 g) and BNO (1.0 mL) and varying catalyst concentrations (LPF, 0, 10 or 15% m/m of the oil) with magnetic stirring (300 rpm, 40 ± 2 °C) for 24 or 96 h. After this period, the enzyme was filtered and the mixture washed with hexane (3 × 1.0 mL). The filtrate was evaporated under reduced pressure and purified directly by column chromatography on silica gel using *n*-hexane:ethyl acetate (8:2) as an eluent.

Analyses

GC-MS analysis

GC-MS was performed on a Shimadzu/GC 2010 apparatus coupled to a Shimadzu/AOC-5000 auto-injector and an electron beam impact detector (Shimadzu MS2010 Plus) (70 eV), equipped with a DB-5MS fused silica column (30 m × 0.25 mm × 0.25 mm) (65 kPa). The parameters used were: 1:15 split ratio, helium as the drag gas, 1.0 mL injection volume, injector temperature of 250 ºC, detector temperature of 270 ºC, initial column temperature of 100 ºC for 2 min, heating rate of 6 ºC min^{-1} until 280 ºC for 5 min. The total analysis time was 37 min. Identification of the fatty acid esters was made by comparison of the fragmentation spectrum with those contained in the GC-MS library (MS database, NIST 5.0).[28]

^{13}C nuclear magnetic resonance (NMR) analysis

^{13}C NMR experiments used an Agilent Technologies 400 MHz Premium Shielded spectrometer. All samples (20 µL, approximately) were prepared by dissolving in 600 µL of deuterated chloroform ($CDCl_3$, Cambridge Isotope Laboratories, Andover, USA) and tetramethylsilane (TMS) as the internal standard. The chemical shifts were expressed in ppm.[29]

DPPH antioxidant activity

Aliquots of 0.3 mL of methanolic solutions of each sample (FAA, FAEE and BNO) at concentrations of 250, 125, 62.5, 31.25, 15.62 and 7.81 µg mL^{-1} were added to 2.7 mL of a methanolic solution of 0.1 mmol L^{-1} 2,2-diphenyl-1-picrylhydrazyl (DPPH). After 30 min, absorbance was measured in a UV-Vis spectrophotometer apparatus at a wavelength of 516 nm (in triplicate). A methanolic solution of ascorbic acid was used as a positive control, under the conditions evaluated, and 0.3 mL of each sample concentration and ascorbic acid in 2.7 mL methanol were used as negative controls.[30]

The percentages of remaining DPPH were ($\%DPPH_{rem}$) calculated from the calibration curve and from absorbance values at 30 min for each concentration tested, according to the following equation:

$$DPPH_{rem}\ (\%) = [DPPH]_{t = 30\ min}/[DPPH]_{t = 0\ min} \times 100 \quad (1)$$

$[DPPH]_{t = 30\ min}$ corresponds to the concentration of DPPH after the reaction with the extract at the time of 30 min, $[DPPH]_{t = 0\ min}$ corresponds to the initial concentration of DPPH (40 µg mL^{-1}). The amount of extract required to decrease the initial concentration of DPPH by 50% ($CE_{50\%}$) was determined using linear regression and the SPSS

program,[31] in relation to the concentration of extract or ascorbic acid and the ordered %$DPPH_{rem}$. The antioxidant activities for the concentrations tested were calculated as percentages (%AA) using absorbance values, according to the following equation:

$$AA(\%) = \{[Abs_{control} - (Abs_{sample} - Abs_{blank})] \times 100\}/Abs_{control} \quad (2)$$

$Abs_{control}$ represents the absorbance of the control, Abs_{sample} represents the absorbance of the sample and Abs_{blank} represents the absorbance of the blank.

Kinetic evaluation of DPPH degradation in the presence of extracts and ascorbic acid

Aliquots of 0.3 mL of methanolic solutions of FAA, FAEE or BNO (250 μg mL^{-1}) were added to 2.7 mL of 0.1 mmol L^{-1} DPPH and the absorbance was determined at 1, 5, 10, 20, 30, 40, 50 and 60 min after the start of the reaction. For each concentration, a blank of 0.3 mL of sample in 2.7 mL ascorbic acid in methanol was used. A positive control of 250 μg mL^{-1} was also used under the same conditions.

Animals

Wistar rats from the Multidisciplinary Center for Biological Research in Laboratory Animal Science (CEMIB), University of Campinas, Unicamp, were used for the study. Animals were aged 21 days, were grouped in five animals *per* polyethylene cage, and maintained under controlled temperature conditions (21 ± 2 °C), with free access to food and water *ad libitum*, and light/dark cycles of 12 h. This study was approved by the Ethics Committee of the Federal University of Amapá under the Protocol 01/2017.

Acute toxicity test

The acute toxicity test followed the protocol of literature.[32] Male animals weighing between 300 ± 30 g were separated randomly into four (n = 5) groups: (*i*) vehicle-treated (0.5 mL $animal^{-1}$); (*ii*) treated with 10 mg kg^{-1} indomethacin; (*iii*) FAA20 (treated with 20 mg kg^{-1} FAA); (*iv*) FAA40 (treated with 40 mg kg^{-1} FAA); (*v*) BNO100 (treated with 100 mg kg^{-1} Brazil nut oil (*Bertholletia excelsa*)). Mortality associated with the treatments determined the need for subsequent tests, according to doses determined by OEDC.[32] Behavioral observations, according to toxicological screening, determined changes in motor activity, abdominal contortions, Straub's tail, seizure, defecation, vocal tremor, grasping force, irritability, urination, eyes (watery, exophthalmic), piloerection, posture (ataxia, loss of reflexes), palpebral ptosis, touch response, response to sound stimulus, salivation, tremor, other atypical behaviors and death. For the first 24 h, observations were made at different times (15 min, 1, 2 and 4 h). After 24 h, blood was collected for biochemical evaluation of the animals and followed behavior was monitored until the 14th day.

In vitro cytotoxicity assay

Peritoneal macrophages were obtained from male BALB/c mice (*Mus musculus*). The animals were euthanized in a CO_2 chamber, and 5 mL of Hank's sterile solution was injected into animals' peritoneal cavities for the collection of resident macrophages. Aspirated material was concentrated by centrifugation (1500 rpm, 10 min at 4 ºC). The macrophages were then transferred to 96-well plates and incubated at 37 °C in a 5% CO_2 atmosphere for one hour. Subsequently, the cells were washed with PBS at pH 7.2, to remove non-adherent cells, and then resuspended in DMEM supplemented with 10% fetal bovine serum (FBS).

The cytotoxicity of FAA was assessed by the MTT cell proliferation assay in murine macrophages. The cells (1×10^6 cell mL^{-1}) were plated in 96-well plates and incubated in the presence of different dose of FAA (5, 10, 20, 50, 250 and 500 μg mL^{-1}) for 96 h. MTT dissolved in phosphate-buffered saline (PBS, 0.5 mg mL^{-1}, pH 7.2) was added to each well and incubated for 3 h (37 ºC in a 5% CO_2 atmosphere); the purple colored formazan complex formed was dissolved in 200 μL of dimethyl sulfoxide (DMSO). The absorbance of samples was measured using a spectrophotometer (570 nm) for calculation of percentage cell viability. Dead cells in formaldehyde solution (10%) were used as a negative control. Cell viability is expressed as the CC_{50} value, which is the concentration of the tested compound required to reduce cell viability by 50%, in comparison with the negative control.

Statistical analysis

Results are expressed as means ± standard deviation. For multiple comparisons between the experimental groups, variance analysis (ANOVA) was used, followed by the Tukey's test, employing the Prism software.[33] Results with significance levels of $p < 0.05$ were considered as statistically significant.

Results and Discussion

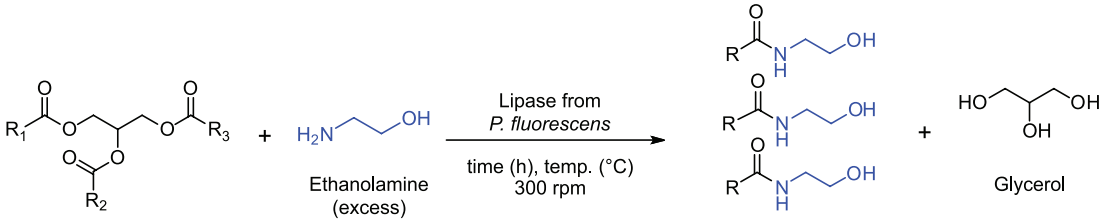
To optimize the production of FAA, initial experiments used Amano lipase from *Pseudomonas fluorescens* (10%) and results are summarized in Table 1. The initial reaction was carried out with 10% lipase using 3 equiv. of ethanolamine as the amine donor. Direct aminolysis, using LPF, provided the desired FAA with a 68% conversion within 24 h (Table 1, entry 1). When the reactions were performed for 96 h, the reaction conversion increased to 86% (Table 1, entry 2). When the amidation reaction of the triglyceride from the *Bertholletia excelsa* oil was performed in the absence of LPF for 96 h with magnetic stirring (300 rpm; 40 °C), the amide product yield was 17%, after purification on a chromatographic column with silica gel (Table 1, entry 3).

The choice of solvent may influence enzymatic activity, sometimes improving selectivity, chemo-selectivity and reaction yield, and other times acting as an inhibitor of enzymatic activity.[34,35] For this reason, the direct amidation reaction of BNO, catalyzed by LPF, was performed in the presence of hexane (1.0 mL) (Table 1, entry 4), with the objective of comparing the yield for fatty amides obtained under solvent-free conditions. The reaction using hexane as solvent, provided a 95% yield at 96 h (Table 1, entry 5). The reaction of direct amidation of BNO, catalyzed by LPF, at 50 ºC for 24 h obtained FAA in a 72% yield (Table 1, entry 6).

However, similar results were observed for solvent-free conditions (yield of 90%), when using 15% lipase (Table 1, entry 4). The objective of using hexane was to act as a solvent, to solubilize the reaction components (oil and amine) and make the reaction medium more homogeneous to facilitate the contact with the catalyst (lipase), consequently increasing the reaction rate. Apolar solvents, such as hexane, can also preserve enzymatic activity, since hydrophobic solvents with higher logP values do not have a tendency to strip the tightly bound water molecules from the enzyme surface, which is essential for the catalytic activity of lipases.[36,37] In general, lipase from *Pseudomonas fluorescens* presented a good catalytic effect when used for amidation in solvent-free reactions.

GC-MS analyses (Table 2 and Figure 1) showed that the lipid composition of the fatty acids present in the BNO studied was predominantly composed of unsaturated fatty acids, such as monounsaturated (C18:1, ʊ-9) oleic acid, with 32% relative abundance, followed by polyunsaturated linoleic acid (C18:2, ʊ-6) (29%) and vaccenic acid (C18:1, ʊ-7) to a lesser extent at 2%. Other fatty acids identified in this BNO sample were palmitic acid (C16:0; 19% relative abundance) followed by stearic acid (17% relative abundance). The profile of the fatty acids identified in the BNO conforms to that described in the literature.[38] The fatty acids present in the triglycerides of BNO were used to obtain their respective amidated fatty compounds, catalyzed by LPF. The FAA structures were identified (GC-MS) and their characteristic ions of fragmentation at *m/z* 116 (base peak), resulting from McLaffery rearrangement and

Table 1. Optimization of the amidation reaction of *Bertholletia excelsa* triglycerides (Brazil nut oil)



entry	time / h	Lipase / %	Temperature / ºC	Yield / %
1[a]	24	10	r.t.	68
2[a]	96	10	r.t.	86
3[a]	96	–	r.t.	17
4[a]	96	15	r.t.	90
5[b]	96	10	r.t.	95[b]
6[a]	24	10	50	72

[a]Reaction: direct solvent-free aminolysis; [b]reaction: direct aminolysis with hexane as solvent. r.t.: room temperature.

γ-cleavage,[1,18] respectively, can be observed in Figure 2b, for the amide compound. In contrast to observations for the ethyl ester structure mass spectra (Figure 2a), which presented the *m/z* 55 fragmentation ion as the base peak and was less abundant than the ion related to the loss of the ethoxide portion (*m/z* 264).

Table 2. Ethyl ester and derivate amide fatty compositions of BNO[a] (based on Figure 1)

Fatty acid[b]	Peak	Fatty acid amide correspondent	Peak	Relative concentration[b] / %
Palmitic (C16:0)	1	*N*-C16:0-ethanolamine	1a	19
Linoleic (C18:2, ʊ-6)	2	*N*-C18:2, ʊ-6-ethanolamine	2a	29
Oleic (C18:1, ʊ-9)	3	*N*-C18:1, ʊ-9-ethanolamine	3a	32
Vaccenic (C18:1, ʊ-7)	4	*N*-C18:1, ʊ-7-ethanolamine	4a	2
Stearic (C18:0)	5	*N*-C18:0-ethanolamine	5a	17
∑Saturated	–			36
∑Monounsaturated	–			34
∑Polyunsaturated	–			29

[a]Percentage of FAEE corresponding fatty acid; [b]MS database (NIST 5.0).

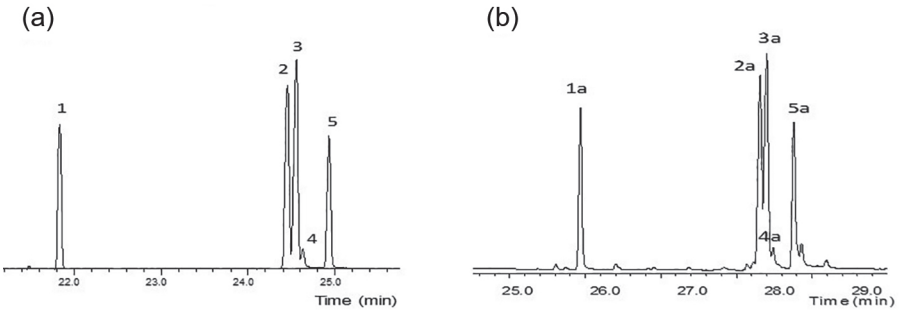


Figure 1. Ethyl ester (a) and derivate amide fatty (b) compositions of BNO, determined by GC-MS analysis.

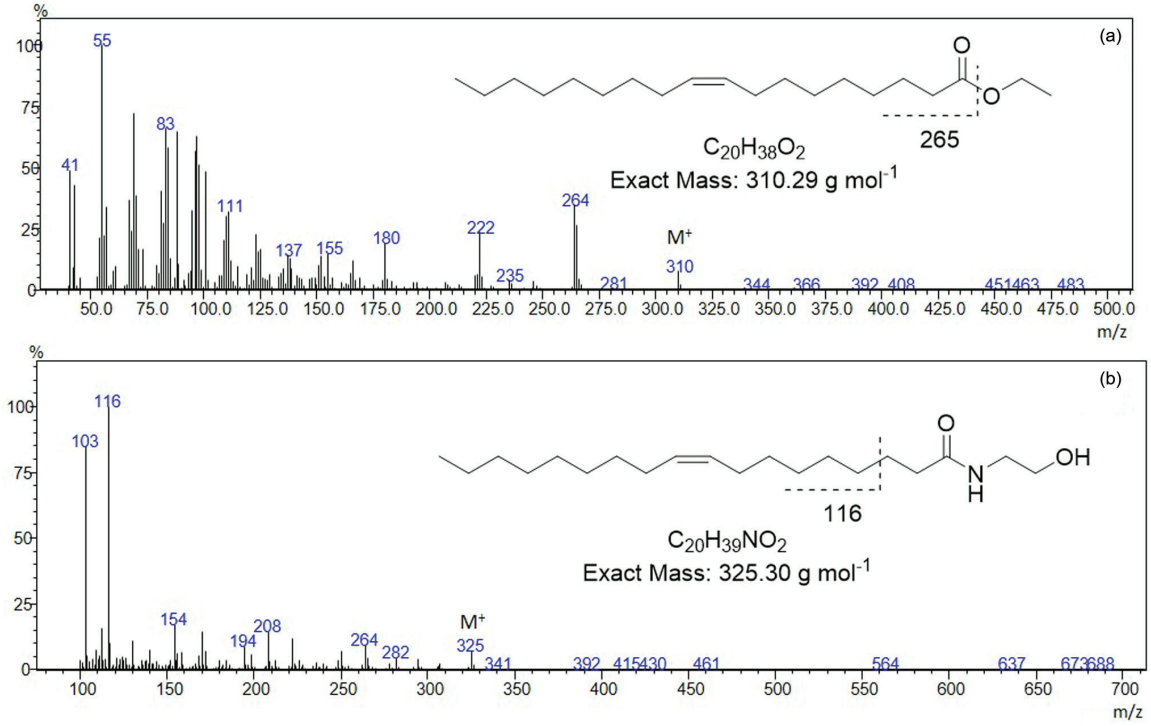


Figure 2. Mass spectra of the ethyl oleate (a) and oleic acid amide (b) compounds.

All the substances in this study (BNO, FAEE and FAA) showed very similar ^{13}C NMR signals (Supplementary Information (SI) section), due to the non-alteration of the alkyl chain present in the starting material (BNO) and its maintenance in the products obtained. The major difference of the ^{13}C NMR spectrum of BNO, compared to those of FAEE and FAA, was the two signals observed at 62.1 and 68.9 ppm, characteristic of glycerol carbons. In contrast, the ^{13}C NMR spectrum of FAEE presented the characteristic signal of the ester mixture $-CH_2$ alkyl carbon; after the transesterification reaction of BNO, this signal appeared at 60.2 ppm. The ^{13}C NMR spectrum of the FAA mixture presented two characteristics $-N-CH_2$ and $-CH_2-OH$ signals at 42.3 and 61.9 ppm, respectively. The absence of glycerol signals also indicated the formation of a new substance from the BNO oil due to enzymatic catalysis (SI section).

The antioxidant activity of the secondary amide product was determined by measuring DPPH radical scavenging, following the protocol of Borges *et al.*,[30] and all the experiments were performed in triplicate.

FAA from BNO was also tested for *in vitro* antioxidant activity against DPPH radicals. Inhibition of DPPH radical formation was not concentration dependent tested and did not increase linearly with increased concentration; however, inhibition was significant as compared to the ascorbate standard (SI section). Fatty isobutylhydroxyamides containing an ethoxy group did not significantly inhibit DPPH radical formation at concentrations of 50 μM.[39] A study by Tanvir *et al.*[40] reported moderate antioxidant activity for long-chained amide derivatives, such as 7-octadecenamide and 9,12-octadecandienamide, against two strains, identified as *Nocardia caishijiensis* (SORS 64b) and *Pseudonocardia carboxydivorans* (AGLS 2), isolated as endophytes from *Sonchus oleraceus* and *Ageratum conyzoides*, respectively. In contrast, our study provides novel findings that FAA from BNO demonstrate antioxidant activity against DPPH radicals. In contrast, the compounds from BNO and FAEE did not inhibit DPPH radical formation (SI section), demonstrating the influence of the amine group (ethanolamine derived) on DPPH activity inhibition.

The anti-inflammatory effects of BNO and FAA (Figure 3) were evaluated using the carrageenan-induced (1%) paw edema method.[41] The anti-inflammatory activities of these natural compounds (FAA and BNO) were evaluated at intervals of 1, 2, 3 and 4 h. During the first hour, significant anti-inflammatory effects ($p < 0.05$) were observed in the groups treated with indomethacin (10 mg kg^{-1}) (positive control) and FAA (40 mg kg^{-1}).

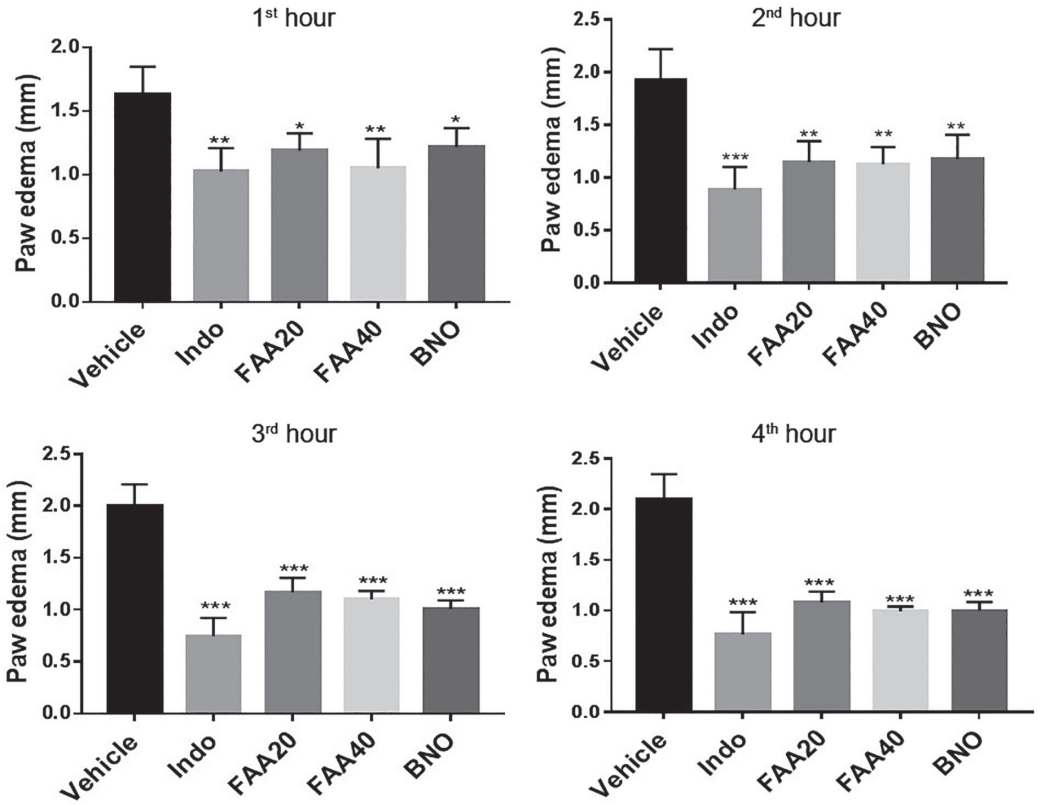


Figure 3. Anti-inflammatory effects of Brazil nut oil (BNO 100 mg kg^{-1}), fatty acid amide (FAA 20 and 40 mg kg^{-1}) and indomethacin (Indo 10 mg kg^{-1}) on paw edema in Wistar rats. Values represents the means ± standard deviation (n = 5 group). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, compared to the vehicle group.

During the time that edema formation was observed, all treatments significantly reduced edema development ($p < 0.001$). At the last time point evaluated (4 h), there was no significant difference between the BNO (100 mg kg^{-1}) and FAA groups (20 and 40 mg kg^{-1}); however, the FAA groups demonstrated greater anti-inflammatory effects, since the concentrations used were lower than those of the BNO groups.

Brazil nut oil has polyunsaturated fatty acid in its chemical constitution, mainly composed of linoleic acid, which has anti-inflammatory proprieties, as demonstrated by different experimental models of inflammation.[41,42] This activity was confirmed herein by in the BNO treatment groups. The mechanisms by which these fatty acids inhibit the production of cytokines are unknown, but may be mediated by the inhibition of the inflammatory cascade at the level of cyclooxygenase (COX) and lipoxygenase (LOX).[43]

The compositions of polyunsaturated fatty acids have been associated with improvements in biochemical parameters, especially lipid, glycyl and inflammatory profiles.[44] In the present study, no significant differences were observed between the biochemical parameters of the treatment groups (Table 3), and treatments demonstrated no toxic effects on the liver and kidneys, considering the normal values for aspartate aminotransferase (AST/GOT), creatinine and urea.[44] However, a significant reduction in triglyceride levels was observed ($p < 0.05$) for the groups that were treatment with FAA (20 and 40 mg kg^{-1}).

Oleoylethanolamine (OEA), FAA unsaturated, majority substance present from Brazil nut oil process, is described that regulates feeding and lipid metabolism.[45] Worth mentioning that, the anorexic effect of OEA seems to be mediated by activation of peroxisome proliferator activated receptor alpha (PPARα) in the intestine, OEA reduces food intake and lowers body-weight gain along with plasma lipid levels.[45]

To investigate the cytotoxicity of FAA triglyceride derivatives from Brazil nut oil on macrophages, we used the MTT assay. The cells were treated with different doses (5, 10, 20, 50, 100, 250, 500 μg mL^{-1}) of the FAA compounds for 72 h. The CC_{50} value determined in the assay for these FAA was 455 μg mL^{-1}, demonstrating reduced cell toxicity (Figure 4). Matysiak *et al*.[46] examined the cytotoxicity of methyl esters of ricinoleic acid and their fatty amide derivatives. Ricinoleic acid amide derivative, prepared with ethanolamine, demonstrated a CC_{50} values of 28.7 μM on lymphocytes.

Evidence indicates that the macrophage lineage is of pivotal importance in tissue homeostasis and the resolution of inflammation.[47] In the present study was observed *in vitro* (Figure 4) that the examined cells, peritoneal macrophages, can be activated in the presence of FAA (20 to 200 μg mL^{-1}), acting as macrophage colony stimulating factor.

Our results demonstrate that FAAs, produced from the triglycerides of Brazil nut oil, may represent an attractive alternative as an anti-inflammatory approach, since they present low cytotoxicity, are easy to administer and have a low production cost, when understood as methodologies already mentioned.[13-15] This group of compounds (FAA) demonstrates broad and overlapping target activities that go far beyond those of the classical cannabinoid receptors.[48]

Conclusions

For first time, FAA were directly synthetized from Brazil nut oil using enzymatic catalysis. This approach resulted in a good yield (67-95%), and represents an easy, economic and efficient method for producing FAA. Many FAA appear to play major roles in the modulation of pain sensitivity and inflammatory processes. Furthermore, FAA set from Brazil nut oil demonstrated anti-inflammatory activity, significantly reducing the development of edema (*in vivo*) and had low cellular toxicity (*in vitro*).

Table 3. Effects of treatment with Brazil nut oil (BNO 100 mg kg^{-1}) and fatty acid amide (FAA 20 mg kg^{-1} and FAA 40 mg kg^{-1}) on biochemical parameters of Wistar rats

Parameter	Vehicle	FAA20	FAA40	BNO
Glucose / (mg dl^{-1})	134.4 ± 12.69	129.7 ± 9.28	124.6 ± 11.30	126.1 ± 9.79
Urea / (mg dl^{-1})	38.57 ± 2.41	44.57 ± 7.65	37.87 ± 6.11	41.21 ± 6.91
Creatinine / (mg dl^{-1})	0.25 ± 0,05	0.22 ± 0.04	0.22 ± 0.01	0.21 ± 0.02
AST / (U dl^{-1})	117.4 ± 2.66	112.2 ± 7.81	117.3 ± 23.09	109.1 ± 15.12
ALT / (U dl^{-1})	39.0 ± 7.6	48.3 ± 10.9	35.2 ± 3.80	43.7 ± 9.2
Triglycerides / (mg dl^{-1})	131.3 ± 22.03	74.1 ± 14.57[a]	87.1 ± 25.74[a]	122.1 ± 11.8
Total cholesterol / (mg dl^{-1})	71.1 ± 7.70	73.63 ± 9.75	66.69 ± 18.87	69.21 ± 12.1

Values represent means ± standard deviation (n = 5 *per* group); [a]$p < 0.05$, compared to vehicle. AST: aspartate aminotransferase; ALT: alanine aminotransferase.

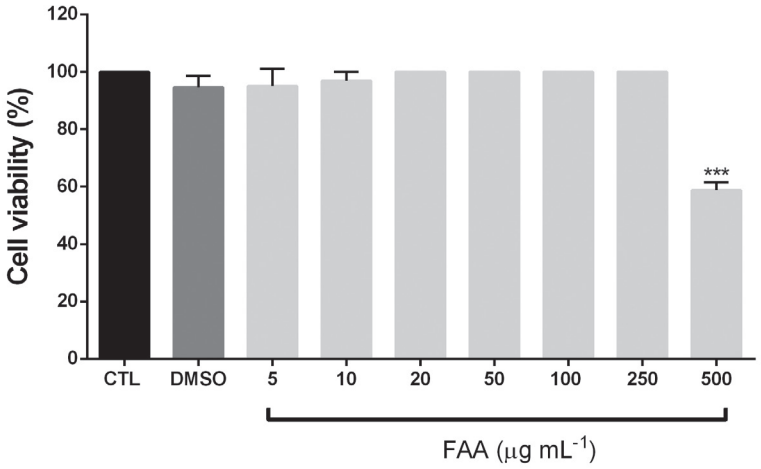


Figure 4. Viability of peritoneal macrophages treated with different concentration of FAA, as determined by the MTT reduction assay, at 72 h after treatment. Analysis of variance (ANOVA), followed by Tukey's test: ***$p < 0.001$. CTL: negative control; DMSO: dimethyl sulfoxide (2%).

Supplementary Information

Supplementary information (^{13}C NMR spectra, and graphic of the antioxidant activity *in vitro* and antioxidant degradation kinetics in DPPH of the compounds BNO, FAEE and FAA) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

The work of synthesis and spectroscopic characterization of FAA was performed by the students P. H. S. Barata and I. R. Sarquis, under the guidance of Prof I. M. Ferreira, participating in all stages of the work and writing the article. A. B. Rodrigues and P. H. S. Barata performed antioxidant activity. H. O. Carvalho, A. S. Barros were responsible of the biological activity *in vivo*, under the guidance of Prof J. C. T. Carvalho. A. J. Galue-Parra and E. O. Silva were responsible of the biological activity *in vitro*.

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