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Stability and Cytotoxicity of a Cosmetic Cream Containing Oil from *Lecythis pisonis* Cambess (Sapucaia) Nuts

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Synopsis

Lecythis pisonis nuts are rich in tocopherols, polyphenols, and fatty acids, and hence, the quality of oil was analyzed for use in the elaboration of skin cream. Nut oil was extracted with hexane in a Soxhlet apparatus. The quality and safety of the oil were tested by using established protocols from Adolf Lutz Institute. Three concentrations, 1%, 5%, and 10%, of oil were incorporated into a formulated cream. The oil formulations were evaluated for their stability, organoleptic characteristics, pH, viscosity, spreadability, challenge test, cellular viability, and their response to microbial contamination, as recommended by the National Health Surveillance Agency (ANVISA). Quality analysis demonstrated the high quality of the obtained oil, with no traces of heavy metals and no toxic effects on ingestion. The cream containing *L. pisonis* nut oil is stable, has an effective preservative system, and is considered safe for use because it presented no toxicity in human cells. A stabilizer is required to maintain the pH in the range suitable for a body cream. Of the formulations tested, the cream containing 5% nut oil was the most stable and had presented the best organoleptic characteristics.

INTRODUCTION

Lecythis pisonis Cambess, Lecythidaceae, is a Brazilian tree also known as "castanha de sapucaia," "sapucainha," with a height of 20–30 m and 90-cm-diameter trunk (1). This species occurs in the Amazon region and rainforest, commonly in Bahia and Espírito Santo (1). The wood is heavy, tough (2), and used in furniture manufacture and civil

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construction (1). The fruits are large (20 cm), with hard shell and a cover that opens when mature, releasing light brown seeds. The fruits form from June to September. The seeds germinate in 40–70 d, are edible, are tasty, and possesses medicinal proprieties (1).

The oleaginous seeds of *L. pisonis* are rich in proteins, lipids, fiber, thiamine, riboflavin, niacin, phosphorus, and potassium (3–5) and reasonable amounts of calcium and magnesium (4,5). Their low sodium content and no cholesterol is beneficial for the cardiovascular system (3,5,6). Therefore, they are considered a functional and nutritional food for humans (6).

In traditional medicine, *L. pisonis* leaves are used to treat pruritus and muscle pain, their analgesic effect in humans might be related to their antinociceptive effects in mice (6).

The antioxidant activity of the ethanolic extract of *L. pisonis* leaves may be associated with high levels of polyphenols and flavonoids (7). Oil from *L. pisonis* showed antioxidant activity that may be related to tocopherols, α and β-tocopherol, and vitamin E (4).

The aim of this work was to develop a skin cream using *L. pisonis* nut oil and evaluate its quality to add value to the oil as a raw material and to the plant as a living resource. This will reduce the need to raze *L. pisonis* trees and promote the maintenance of this species and sustainable development in the Amazon region, creating a potential source of revenue for the region.

MATERIALS AND METHODS

PLANT MATERIAL

Fruits of *L. pisonis* were harvested in Laranja da Terra, Espírito Santo, Brazil, as a part of the 2015 crop acquired by the Instituto Capixaba de Pesquisa e Extensão Rural. The voucher specimen was deposited in the Coleção de Herbário do Jardim Botânico do Rio de Janeiro (JBRJ-Holotype) and Royal Botanic Gardens (K000600113).

After selection, where bruised nuts where discarded, the nuts were divided into four groups and stored at –18°C until analysis. The nuts were peeled and macerated with hexane in a Soxhlet (Unividros, Ribeirão Preto, Brazil) apparatus for 6 h to obtain the oil according to the International Union of Pure and Applied Chemistry method 1.122 (8). After the solvent was removed by evaporation under reduced pressure, the oil was stored in an amber glass bottle under refrigeration (–8°C) until use.

PHYSICAL–CHEMICAL ANALYSIS OF THE OIL OF *L. PISONIS* NUTS

Acidity index of the oil was determined according to the physical–chemical methods for food analysis (9), with modifications. Briefly, 2 g of the sample was dissolved in 25 mL of a 2:1 ether:alcohol mixture and was titrated with a standard solution of 0.01 M potassium hydroxide (KOH) using phenolphthalein as an indicator. The acidity index was calculated and expressed in mg KOH/g oil.

The peroxide index was also determined according to the physical–chemical methods for food analysis (9), with modifications. The oil (5 g) was shaken for solubilization with 30 mL of an (3:2) acetic acid:chloroform mixture. Then, protected from light, 0.5 mL of saturated

potassium iodide solution and 30 ml of water were added. Thereafter, the mixture became yellow and was titrated with a standard solution of 0.1 N sodium thiosulfate until the yellowish color was not perceptible anymore. At this point, on adding 0.5 mL of starch indicator solution, the solution turned blue and titration with a standard solution of 0.1 N sodium thiosulfate was continued until the blue color disappeared. The peroxide index was calculated and expressed as meq KOH/100 g sample.

Also, the lipids and fatty acids in the oil and formulations were determined, where the fat was extracted from the samples according to the Bligh–Dyer method (10). Briefly, 2.5 g of the sample was mixed with a (10:20:8) solution of chloroform, methanol, and milli-Q water. The mixture was shaken and centrifuged at 900 rpm for 30 min, and then 10 mL of chloroform and 10 mL of aqueous solution of 1.5% sodium sulfate were added and shook again for 2 min. Then, the chloroform phase containing the fat was collected and filtered. After the removal of the solvent by evaporation with under-reduced pressure, the total fat content was determined by gravimetry.

The fatty acids extracted from the sample were converted to fatty acid methyl esters (FAMEs) according to Joseph & Ackman (11), using 10% BF_3 in methanol. Briefly, 15 mg of the extracted fat was added to 1.5 mL of 0.5 M sodium hydroxide in methanol in a capped tube. The closed tubes were placed in a boiling water bath for 5 min. After cooling at room temperature, 2 mL of 10% BF_3 in methanol was added, and the tubes were returned to boiling for 30 min. After another cooling step, 1 mL of isooctane was added and shook for 30 s. Finally, 5 mL of saturated sodium chloride solution was added to the tubes and shaken. The isooctane phase (containing FAMEs) was collected. As control, 1 $mg \cdot mL^{-1}$ methyl trioctanoate (C23:0Me) was added to the samples, to allow the correction of FAME quantification due to variability in extraction, analytical instrument, or solvent evaporation.

The resulting FAMEs were injected into a Shimadzu GC-2014 gas chromatograph (GC) with a flame ionization detector (FID) and an HP-INNOWax (Agilent, Santa Clara, CA) capillary column (50 m × 0.20 mm i.d. × 0.20 μm). The chromatographic conditions were as follows. The injector was operated at 250°C in split mode (1:10) for 1.0 min. The nitrogen drag gas flow was 1.25 mL/min, and the detector temperature was 260°C. The oven temperature gradient program was as follows: an initial temperature of 150°C, which was increased at 10°C/min to 260°C, where it was held for 9 minutes. A standard solution of FAMEs (GLC-85, Nu-check) was injected into the GC-FID system under the same conditions as for the samples. All analyses were performed in triplicate.

The fatty acids were quantified according to Visentainer (12) using the FAME areas in the chromatograms; the areas were corrected with the theoretical correction factors (TCFs) and the conversion factors for fatty acids to FAME obtained using internal standards.

To quantify the metals contained in the oil, nuts, arils, shells, and oil were digested with 10% nitric acid (65% purity, Sigma-Aldrich, Darmstadt, Germany) at 150°C using a Marconi digester (model MA 851, Marconi Equipamentos para Laboratório LTDA, Piracicaba, Brazil) and filtered through a 0.22-μm filter (Jet Biofil, Guangzhou, China).

Flame atomic absorption spectrophotometry quantification was performed in triplicate, using an atomic absorption spectrophotometer (iCE 3000, AA05141602 v.1.30, Thermo Scientific, Waltham, MA) and an atomizer with an air/acetylene burner and a hollow cathode lamp (Photron PTY. Ltd., Narre Warren, Australia) as a source of radiation to determine target elements. The calibration curve ranges for each analyte were as follows: Fe (1–15 mg/L), Na (1–15 mg/L), and Pb (10–40 mg/L). The chemicals were analytical

grade, standard solutions (Specsol®, Quimlab Produtos de Química Fina Ltd., Jacareí, Brazil) and acetylene (99.5% purity, Sigma-Aldrich).

COLORIMETRIC CELL VIABILITY TEST FOR THE OIL

Cytotoxicity was evaluated *in vitro* using L929 fibroblasts (ATCC® CRL-6364™, Manassas, VA) and HaCat keratinocytes (BCRJ code: 0341) seeded on 96-well microplates at a cell density of $0.7 \cdot 10^5$ cells/mL in a final volume of 200 µL per well and using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method (13), analyses were carried out in triplicate. The cells were exposed to concentrations of 1,000, 500, 250, 125, 62.5, and 31.2 µg/mL of *L. pisonis* oil. The oil was mixed with dimethyl sulfoxide (DMSO) to have a final concentration of 0.5% of DMSO in the well. The dilutions of the oil/DMSO were made with the cell medium. Cell viability was determinated in relation to the negative control (0.5% DMSO), the limit of nontoxicity of DMSO, considered 100% of surviving cells, and the results are expressed as the mean ± standard deviation.

CREAM OF *L. PISONIS* OIL

The cream base was prepared in a commercial pharmacy (Farmácia Biomédica Manipulação, Vila Velha, Brazil) under supervision by the authors, according to the composition in Table I. The cream was separated in lots for the tests and five different oil formulations were prepared for the assays: F0 (cream base), F1 (1% oil), F2 (5% oil), F3 (10% oil), and control (oil).

EVALUATION OF THE FORMULATION CHARACTERISTICS

A centrifugation test was performed, in triplicate for each formula except for the control group (oil), before and after the stability test according to the National Health Surveillance Agency (ANVISA) (14), with modifications. For each sample, 5 g was transferred to a tube and centrifuged at 3,000 *g* for 30 min and observed if any separation of phases occurs. Also, to evaluate the type of emulsion, five drops of Scarlet Biedrit solution were added to the

Table I
Composition of the Cream Base for *L. pisonis* Oil Cream (3,000 g)

Ingredient	Function	Amount
Cetearyl alcohol/sodium cetearyl sulfate	Self-emulsifying wax	360 g
Isodecyl oleate	Emollient	150 g
Propylene glycol	Solvent	150 g
Phenoxyethanol + parabens	Preservative	15 g
Disodium EDTA	Preservative	4.5 g
Imidazolidinyl urea	Antimicrobial preservative	4.5 g
Demineralized water	Vehicle	2,316 mL
Cupuaçu essence	Essence	—

formulations, followed by homogenization. Thereafter, 100 µL was transferred to a microscopic slide and analyzed on a Leica Galen III microscope (Leica, Wetzlar, Germany).

The pH was evaluated according to ANVISA (14) and measured using a Gehaka digital pH meter (Gehaka, São Paulo, Brazil). The pH of *L. pisonis* oil was measured using pH test strips from MColorpHast® (Merck, Darmstadt, Germany).

The viscosity was evaluated at room temperature (21°C) with a Brookfield viscosimeter using spindle number 4. The applied rotation speeds were 5–11 rpm for the samples tested before the stability evaluation and 8–16 rpm for those tested after the stability evaluation. The results are expressed in centipoise (cP) (14).

The spreadability assay was performed according to Knorst (15), with modifications, for all formulations before and after the stability test. One gram of each sample was placed on the central spot of the acrylic plate, and another plate was placed aligned over the sample. On top, a calibrated weight of 200 g was placed for 2 min. The diameter of the sample spot was recorded in opposing directions, and the median diameter was calculated. A commercial moisture cream from Granado was used as a reference.

For the organoleptic characteristics, all the formulations were visually analyzed for changes in color, odor, and homogeneity throughout the study and after each test (16).

The stability test was performed on all samples in triplicate, according to ANVISA (17). The samples in sterilized glass jars were subjected to different temperature conditions: frozen (−5°C ± 2°C) for 24 h, room temperature (15°–30°C) for 24 h, and heated in an oven (40°C) for 24 h. Three cycles of the stability test were performed. The stability of the samples was evaluated based on the organoleptic properties (16) and physical–chemical characteristics.

MICROBIAL LIMIT TEST

The microbiological safety was assessed on the basis of the 481/99 Brazilian regulation for cosmetic products (18). Samples were randomly selected prior and after the stability test and artificially contaminated with different microorganisms for the challenge test. This test aims to determine the resistance of a product to microbial contamination, thereby reflecting the effectiveness of its preservative system. The formulations were artificially contaminated via inoculation with a suspension of selected microorganisms at 0.5 on the McFarland scale, followed by a survival determination by the plate counting method at 0, 24 h, 48 h, 7 d, 14 d, 21 d, and 28 d. *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* CCCD P003, *Candida albicans* ATCC 10231, and *Aspergillus niger* ATCC 40067 were cultured in Müller–Hinton medium (Kasvi, São José dos Pinhais, Brazil) and then inoculated to 10% of cream for different formulations.

STATISTICAL ANALYSIS

The quantitative variables were expressed as means ± standard deviations, and the data were submitted to Student's *t*-test with a significance level of $p < 0.05$. For the quantification of fatty acids in the oils from different regions, data were analyzed by one-way analysis of variance and Bonferroni post hoc tests. For the quantification of fatty acids in the cream before and after the stability test, a *t*-test was performed. The qualitative variables were expressed in a descriptive manner.

RESULTS AND DISCUSSION

The *L. pisonis* nut oil was transparent and presented a light yellowish green color and a pleasant characteristic oil odor, in agreement with the literature (4,5,19). The average yield of the oil was 35%, which is similar to what is described for other natural oils used in cosmetics, such as tucumã oil (22%) and murumuru oil (34–46.2%) (20), indicating it is a considerable raw material to the development of cosmetics.

Considering the fatty acid contents in *L. pisonis* oils from nuts obtained in different regions of Brazil, they were similar to the one determined in Espírito Santo in which the most abundant fatty acids were oleic and linoleic acids, followed by palmitic acid (Table II). These fatty acids are desirable for moisturizing products used for skin and hair, such as cream, body oil, and emollient or massage purposes (20).

As the physical–chemical characteristics of the nut oil, the acidity index (0.12 mg/g) and peroxide index (6 meq/kg), are in accordance with the ANVISA parameters (maximum of 0.6 mg KOH/g and below 15 meq/kg, respectively) (21), it is indicated that no hydrolysis of free fatty acids occurred during the extraction or oxidation, demonstrating its good quality. Also, comparing with the *L. pisonis* oil from Amazonia, the one from Espírito Santo presented a lower acidity value (0.31 ± 0.01 mg/g) and a higher peroxide value (0.27 ± 0.02 meq/kg) (19). This variation may be due to the difference in the soil composition that interfere in the oil constituents, where the Espírito Santo's oil is more diverse than the one from Amazonia.

The level of lead detected in the nuts, arils, and oil evaluated was below the allowable limit, indicating that the nuts are secure for consumption and use. The content of iron was 2.17 ± 0.47 mg/100 g in nuts and 1.12 ± 0.15 mg/100 g in arils. These values are comparable with those reported for Brazil nuts (2.3 mg/100 g) and coconut (1.8 mg/100 g) (22) and lower than those in *L. pisonis* nuts from the Minas Gerais region (7.0 mg/100 g) (5). For the oil, the lead level was 9.40 μg/g and similar to those found in sunflower, olive, canola, and soy oils (9.14–9.82 μg/g) (23).

The sodium concentrations were 3.27 ± 0.62 mg/100 g in nuts, 6.62 ± 1.06 mg/100 g in arils, and 0.7 μg/g in oil. These values indicate that the tested *L. pisonis* oil is of high

Table II
Fatty Acid Contents in *L. pisonis* Oil Samples from Espírito Santo, Minas Gerais, Amazonia, and Piauí

Fatty acids (%)	Espírito Santo	Minas Gerais (5)	Amazônia (19)	Piauí (4)
Myristic (C14:0)	0.09 ± 0.1	—	—	0.09 ± 0.01
Palmitic (C16:0)	12.20 ± 0.01	11.29	14.49 ± 0.24	14.17 ± 1.17
Palmitoleic (C16:1)	0.41 ± 0.02	0.34	0.21 ± 0.01	0.26 ± 0.08
Margaric (C17:0)	—	—	—	0.08 ± 0.01
Stearic (C18:0)	6.99 ± 0.03	3.77	5.84 ± 0.04	8.09 ± 0.30
Oleic (C18:1 n9)	49.95 ± 0.02	41.37	38.82 ± 0.08	43.17 ± 3.04
Linoleic (C18:2 n6)	29.84 ± 0.01	42.86	39.93 ± 0.11	34.83 ± 4.47
Linolenic (C18:3 n3)	0.2 ± 0.03	0.24	0.24 ± 0.01	0.58 ± 0.06
Arachidic (C20:0)	0.18 ± 0.01	0.13	0.22 ± 0.01	—
(C20:1 n9)	0.07 ± 0.01	—	—	—
Cis-11-eicosenoic (C20:1)	—	—	—	0.07 ± 0.01
Behenic (C22:0)	0.05 ± 0.01	—	Tr[a]	—

[a]Tr = percentage <0.1.

quality, and the levels found in arils are comparable with those found in sesame seeds (3 mg/100 g), raw nuts, and chickpeas (5 mg/100 g) (22).

In addition, the oil from *L. pisonis* could be considered nontoxic and safe for use, being classified as category 5 (24). Biochemical assays showed no significant differences among the groups for liver and kidney damage and that an estimated lethal dose is 5.000 mg/kg.

Regarding acute toxicity, the LD_{50} of *L. pisonis* oil in mice was 2.000 mg/kg, corresponding to an LD_{50} in humans of 405.6 mg/kg. This elevated safe dose could indicate safe application of the oil in products, including topical applications on the face (unpublished results).

The cell survival varied between 98.3 ± 5.2 and 119.3 ± 6.4 for L929 cells and between 113.4 ± 19.9 and 149.5 ± 10.9 for HaCat cells, corresponding to the highest and lowest concentrations of the oil tested, respectively. So, it is possible to infer that the oil is nontoxic because it did not affect the cell growth.

The pharmaceutical form used to incorporate the oil from *L. pisonis* was cream, as it is widely used and accepts well the oil compounds. Analyses of the formulation indicated that the base cream produced is oil-in-water (O/W) emulsion, meaning that oil micelles are dispersed in an aqueous medium. Such emulsions are smooth and easily removed with water (25). Different lipids that enhance the scattering coefficient, such as the green oils from macadamia nuts, wheat germ, and grape seeds, might be used in an O/W emulsion (25). The concentrations of oil used in the formulations were based on studies of other cosmetic formulations with oil using 0.5–10% oil (26,27).

Considering macroscopic aspects, in all samples except F3, no phase separation or changes in texture or odor were observed (Table III). The changes in sample F3 could be attributed to the oil concentration (10%), where despite maintaining the texture, the formulation was less viscous, i.e., when corroborated by the centrifugation test, small droplets were noticed on the surface of F3.

All formulations containing the oil remained stable with no color change during the tests. As excepted, sample F3 presented more pronounced odor after the second cycle of heating and a paler color and rougher texture, different from the other samples, which remained smooth and shiny. The most stable formulations were those with concentrations of 1 and 5% nut oil, in agreement with Christoph et al. (26), where the concentration of melaleuca oil in formulations ranged from 0.5% to 5%.

The control samples remained stable, becoming harder when frozen and regaining normal characteristics after 30 min at room temperature.

The importance of the pH control in a formulation over the entire period relevant to product use is that it should maintain a value between 5.5 and 6.5 to be compatible with the skin pH and remain stable (28), as alteration of pH might decrease the stability of the formulation (16). In the tested formulations, the pH decreased during exposure to heat in the stability test (Table III), indicating that a stability process affects the formulation, but still remains within the acceptable value.

The skin pH is believed to be slightly acidic (4.6–5.8), providing bactericidal and fungicidal protection at the surface (29). The determination and control of skin pH are desirable characteristics of cosmetic products to avoid skin damage and could be controlled by the use of additives, such as antioxidants and vitamin E, to stabilize the oil.

Table III
Physical–Chemical Characteristics of the Formulations

	Phase separation		Texture		Odor		pH		Spreadability		Viscosity		
	BST	AST	BST	AST	BST	AST	BST	AST	BST (cm^2)	AST (cm^2)	Time 0 (cP)	30 days (cP)	Viscosity retrenchment (%)
F0	S	S	S	S	S	S	5.27 ± 0.01	4.77 ± 0.01*	11.15 ± 0.75	13.42 ± 0.38	59,802	38,073*	36.33
F1	S	S	S	S	S	S	5.26 ± 0.02	4.77 ± 0.00*	11.14 ± 0.34	14,41 ± 1.39	60,359	25,830*	57.21
F2	S	S	S	S	S	S	5.21 ± 0.01	4.78 ± 0.01*	11.54 ± 0.46	19,78 ± 1.47	60,272	21,536*	64.26
F3	S	NS	S	S	S+	S+	5.17 ± 0.01	4.78 ± 0.01*	11.74 ± 0.17	18.39 ± 2.48	27,357	18,861*	31.06

BST: before stability test; AST: after stability test. Evaluation of phase separation—S: stable (no phase separation); NS: nonstable (small drops of *L. pisonis* nut oil). Odor evaluation—S: stable (odor of cupuaçu essence); S+: stable with a slight odor of *L. pisonis* nut oil. Texture evaluation—S: stable (smooth and shiny texture); NS: not stable (rough and opaque texture). *$p < 0.05$.

It is possible to infer that after the stability test, the viscosity of the cream decreased, and the same occurred when the oil concentration was increased in the formulation (Table III).

The viscosity of an emulsion could be altered by changing the lipid composition, proportions of the aqueous and oil phases, concentrations of viscosity donors and emulsifiers, and by the presence of polymers (30). Therefore, the viscosity of the oil and of the formulation are interrelated, as the viscosity increases with the ester chain length of fatty acids in the oil and decreases with the level of unsaturation (31).

The changes in viscosity affected the spreadability of most of the samples (Table III). The higher the fatty acid content, the lower the thermal stability of the formulation (32). The samples exhibited similar spreadability before the stability test, and it increased with the oil concentration in the cream. F2 presented the best spreadability.

Table IV presents the fatty acid concentrations in all samples and, notably, the base cream used already contained fatty acids that added to the fatty acids in the formulations.

In addition, the fatty acid composition of the oil did not change during the stability test, as shown in Table IV. Comparing F0 before and after the stability test, it can be observed that the amount of myristic and palmitic acids did not change. Oleic, linoleic, and palmitic acids were the main constituents of the oil and remain the major fatty acids in the creams, expect in F1AST. There were no changes in the quantity of the compounds when comparing the formulations before and after the stability test. The variation in stability may be related to interactions among the cream components and the oil because the oil itself was stable during the test. However, no differences in the cream formulation before and after the stability test were observed.

Although most fatty acids are stable until 300°C, they could change during storage for long periods, being damaged by oxidation or hydrolytic processes (33). That is why stability tests of cosmetics products are important to avoid compromising the health of consumers and ensure a good product.

At time 0 of the challenge test, before the stability test, all samples presented contamination, as expected, and supported growth of *E. coli, S. aureus, P. aeruginosa, C. albicans*, and *A. niger*. After 7 d, there was minimal growth of *S. aureus* and *A. niger.* However, after 28 d, no microbiological growth was detected for all microorganisms tested, showing the efficacy of the preservatives in the base. At time 0 of the experiment performed after the stability test, all samples were positive for contamination. After 28 d, no microbiological growth was detected.

Therefore, it is possible to infer that the preservative system was effective, in compliance with the regulatory standards described in RDC 481/99 (18).

CONCLUSION

So, the developed *L. pisonis* oil cream fulfilled all the requirements of ANVISA, including satisfactory organoleptic and physical–chemical parameters, including the preservative system that is widely used in formulations. The parameters of the 10% oil formulation considerably differed from the other formulations. The viscosity of the cream decreased with an increasing oil concentration, affecting the spreadability. Although no significant changes in the formulation pH could be detected, it is recommended to use a stabilizer to assure a stable pH (5.5–6.5).

Table IV
Relative Concentrations of Fatty Acids in the Cream Formulations before and after the Stability Test

			Formulation							
FAME (%)	OilBST	OilAST	F0BST	F0AST	F1BST	F1AST	F2BST	F2AST	F3BST	F3AST
Myristic (C14:0)	0.09 ± 0.1	0.08 ± 0.04	3.36 ± 0.58	3.55 ± 0.37	1.80 ± 0.32	2.61 ± 0.09	0.39 ± 0.04	0.91 ± 0.01	0.35 ± 0.01	0.70 ± 0.03
Palmitic (C16:0)	12.20 ± 0.01	11.96 ± 0.06	65.89 ± 0.46	46.05 ± 1.01	35.09 ± 0.14	49.34 ± 0.23	16.95 ± 0.11	28.69 ± 0.20	19.28 ± 0.27	23.25 ± 0.31
Palmitoleic (C16:1 n-7)	0.41 ± 0.02	0.31 ± 0.04	—	—	—	—	—	—	—	0.25 ± 0.07
Stearic (C18:0)	6.99 ± 0.03	7.15 ± 0.01	24.47 ± 0.54	18.21 ± 0.67	13.49 ± 0.40	22.93 ± 0.89	8.66 ± 0.03	15.48 ± 0.14	9.95 ± 0.26	12.45 ± 0.11
Oleic (C18:1 n-9)	49.95 ± 0.02	38.24 ± 0.03	2.29 ± 0.17	15.00 ± 0.51	42.27 ± 0.07	9.40 ± 0.90	47.38 ± 0.11	38.84 ± 0.27	48.46 ± 0.67	41.92 ± 0.22
Linoleic (C18:2 n-6)	29.84 ± 0.01	41.67 ± 0.02	2.90 ± 0.27	7.21 ± 0.80	7.36 ± 0.18	4.55 ± 0.44	24.65 ± 0.01	11.78 ± 0.07	31.62 ± 0.43	18.10 ± 0.04
Linolenic (C18:3 n-3)	0.2 ± 0.03	0.20 ± 0.02	—	—	—	—	0.50 ± 0.06	—	—	—
Arachidic (C20:0)	0.18 ± 0.01	0.18 ± 0.03	—	1.75 ± 0.13	—	1.44 ± 0.40	0.16 ± 0.04	0.35 ± 0.01	0.14 ± 0.06	—
C20:1 n-9	0.07 ± 0.01	0.09 ± 0.01	—	—	—	0.90 ± 0.02	0.69 ± 0.06	3.44 ± 0.11	0.91 ± 0.05	2.94 ± 0.26
Behenic (C22:0)	0.05 ± 0.01	0.12 ± 0.01	—	6.73 ± 0.46	—	4.74 ± 1.10	0.62 ± 0.03	—	—	—

BST: before stability test; AST: after stability test.

The oil from *L. pisonis* is a potential material in cosmetic formulations, with good quality, possessing a desirable fatty acid composition for such applications. Now with the most stable concentration being established, it is possible to proceed with the tests in humans to fulfill the parameters to release the *L. pisonis* cream for human use.

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