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REVISTA BRASILEIRA DE FARMÁCIA (RBF)
BRAZILIAN JOURNAL OF PHARMACY (BJP)
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PESQUISA / RESEARCH

Amazon native flora oils: *in vitro* photoprotective activity and major fatty acids constituents

Óleos da flora nativa da Amazônia: atividade fotoprotetora *in vitro* e composição principal de ácidos graxos

Recebido em 28/10/2011

Aceito em /01/2012

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RESUMO

Os óleos de *Astrocaryum vulgare* Mart. (tucumã), *Bertholletia excelsa* Bonpl. (castanha do Brasil), *Carapa guianensis* Aubl. (andiroba), *Caryocar villosum* (Aubl.) Pers. (pequi), *Euterpe oleraceae* M. (açaí), *Mauritia flexuosa* Lf (buriti), *Oenocarpus bataua* Mart. (patauá), e *Pentaclethra macroloba* (Willd.) Kuntze (pracaxi) foram estudados, a fim de verificar a sua atividade fotoprotetora *in vitro*, isoladamente ou em associação com uma emulsão a 10% de TiO_2. A atividade foi avaliada pela determinação do Fator de Proteção Solar, Razão UVA / UVB e comprimento de onda crítico. A sua composição de ácidos graxos foi avaliada por cromatografia gasosa utilizando detector com ionização de chama, a fim de determinar os seus componentes principais (controlo de qualidade). Nenhuma das espécies analisadas alcançou valores de fotoproteção satisfatórios, além disso, não foram úteis como matéria-prima para os protetores solares, embora possam ser utilizadas para outros fins, uma vez que elas podem atuar como emolientes, hidratantes e lubrificantes.

Palavras-chave: Óleos vegetais, Ácidos graxos, Filtros solares, Espectrofotometria de transmitância difusa, Cromatografia gasosa

ABSTRACT

The oils *from Astrocaryum vulgare* Mart. (tucumã), *Bertholletia excelsa* Bonpl. (castanha do Brasil), *Carapa guianensis* Aubl. (andiroba), *Caryocar villosum* (Aubl.) Pers. (pequi), *Euterpe oleraceae* M. (açaí), *Mauritia flexuosa* L.f. (buriti), *Oenocarpus bataua* Mart. (patauá), and *Pentaclethra macroloba* (Willd.) *Kuntze* (pracaxi) were studied in order to verify their in vitro photoprotective activity, either alone or in association with a 10% TiO2 emulsion. The activity was assessed by the determination of the Sunburn Protection Factor, UVA/UVB Ratio and Critical Wavelength. Their fatty acid composition was assessed by gas chromatography – flame ionization detection, in order to determine their major components (quality control purposes). None of the analyzed species achieved satisfactory photoprotection values, therefore not being useful as raw materials for marketable sunscreen products, although they can be used for other purposes since they can act as emollients, moisturizers and lubricants.

Keywords: Plant oils, Fatty acids, Sunscreening agents, Diffuse transmittance spectrophotometry, Gas chromatography

INTRODUCTION

Currently, there is a worldwide trend in the use of natural products in order to offer to consumers safer products that lead to minimal ecological impact (Che, 2009). In this context, the pharmaceutical market and cosmetic science researchers tend to develop products with many components of vegetable origin, rationally exploiting the

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Brazilian biodiversity (Iha *et al.*, 2008), including sunscreens. That is why sunscreens containing natural products have reduced side effects and are less harmful to the environment, compared to synthetic ones (Che, 2009). This is perceived with great interest by the international market, especially when the raw material already has scientific data proving its safety and efficacy, besides the commitment to sustainable development.

The use of sunscreens has been gaining relevance throughout the last few years, especially due to the alarming rise of skin cancer incidence (Kumar *et al.*, 2007). It is estimated that one in every three new cases of cancer is a skin cancer, and this number is expected to increase even more (World Health Organization, 2011). The exposure to ultraviolet radiation (UVR) is considered as the main environmental trigger of this disease (Ministério da Saúde, 2009; Polonini *et al.*, 2011).

The carcinogenic potential of sunlight is due to isolated or synergistic action of UVA and UVB radiations. UVB radiation is more energetic when compared with the UVA one, and it is absorbed directly by a series of cellular components such as nucleic acids, proteins and urocanic acid. Also, it has a mutational effect (Applegate & Frenk, 1995). UVA radiation penetrates easily through the epidermis and acts in the proliferative basal layer and even in blood components of the dermis. It acts indirectly on the cellular components through oxidative mechanisms that form reactive oxygen species (Chandra *et al.*, 2007). These have a relatively short lifespan but are highly reactive, causing cellular damage. In addition, ultraviolet rays also produce inflammation, erythema, burns, immunosuppression, and photoaging (Polonini *et al.*, 2011).

Thus, sunscreens play an important role in preventing skin cancer. They act by blocking or absorbing solar radiation, which is carcinogenic to humans, specifically ultraviolet radiation. It is estimated that at least 10% of all new cases of skin cancer could be avoided if the population made a correct and continuous use of sunscreens (Ministério da Saúde, 2009; Polonini *et al.*, 2011).

In this light, the objective of this study was to evaluate the photoprotective activity of natural sunscreens containing vegetable oils from Brazilian plant species, specifically from Amazon native flora, and so to verify the possibility of their incorporation in the cosmetics market. In addition, the fatty acids composition of the essential oils was determined by gas chromatography, in order to determine the main components of these oils and to correlate our findings with data available from literature. This part is particularly important to situate our work in relation to what is already known about these oils, and thus validate our data. Once it is the first time these species are being studied with photoprotection purpose, and for some species even their chemical composition is not yet known, it is highlighted the uniqueness and the importance of the present work.

MATERIALS AND METHODS

Chemicals

Analytical-grade absolute ethanol, potassium hydroxide, hydrochloric acid, sodium chloride, ethyl acetate, boron trifluoride (Vetec, Brazil) and ultrapure water obtained in an aquaMAX - *Ultra* 370 Series equipment (YoungLin, Korea) were used throughout analysis. Square-shaped (50 x 50 mm) polymethylmethacrylate (PMMA) Helioplate™ HD6 (HelioScreen, France) plates with roughened surface on one side (Sa ≈ 6 µm) were used as the substrate for the determination of SPF by diffuse transmittance spectrophotometry.

Equipments

The *in vitro* photoprotection experiments were conducted in an UV-2000S Ultraviolet Transmittance Analyzer (Labsphere, USA), composed by two photodiode array spectrographs and equipped with an integrating sphere and a xenon flashlamp that emits a continuous spectrum of radiation with no peaks and supplies energy for a 290-450 nm spectral range, with an increment wavelength step of 1 nm, and has a low irradiance such that the photostability of the product is not unduly challenged. Electronic analytical balance AY-220 (Shimadzu, Japan) was used on the compounding of the sunscreening products and positive-displacement manual pipette (Mettler Toledo, USA) was used for the preparation of the PMMA substrates.

The fatty acids analysis was performed in a gas chromatograph HP5890 (HP, USA), with flame ionization detector. The instrument was equipped with a 15m x 0.25mm HP-INNOWax column, injection (1/50 split) of 1 µL, and hydrogen as carrier gas (2 mL min^{-1}). The temperature of both the detector and the injector was maintained at 250 ºC, and for the column it was used a temperature gradient (initial = 80 ºC; then an increase of 7 ºC min^{-1} until 240 ºC). The identification of the peaks was made by comparison with Supelco 37 methylated fatty acids (Supelco, USA), using the same conditions as above.

Plant material

Vegetable oils of *Astrocaryum vulgare* Mart., Arecaceae, popular name: tucumã; *Bertholletia excelsa* Bonpl., Lecythidaceae, popular name: castanha do Brasil; *Carapa guianensis* Aubl., Meliaceae, popular name: andiroba; *Caryocar villosum* (Aubl.) Pers., Caryocaraceae, popular name: pequi; *Euterpe oleraceae* M., Areaceae, popular name: açaí; *Mauritia flexurosa* L.f., Arecaceae, popular name: buriti; *Oenocarpus bataua* Mart., Arecaceae, popular name: patauá; *Pentaclethra macr*oloba (Willd.) Kuntze, Mimosaceae, popular name: pracaxi; were acquired from Amazon Oil Industry (Pará, Brazil).

Each of the oils were incorporated into a Lanette® lotion at a rate of 10% and into a 10% titanium dioxide (TiO_2) Lanette® lotion also in a rate of 10%. These products were used in the photoprotection assays.

***In vitro* photoprotection assay**

The natural sunscreens compounded products were accurately and quickly weighed (to reduce product evaporation and dryness) to satisfy the application rate of 0.75 mg cm^{-2} in each PMMA plate (actual quantity applied: 17.85 mg). They were directly weighed on the plate surface, applied as a large number of small droplets of approximate equal mass, and distributed in an evenly manner on the roughened surface of the plate. Then, the products were spread over the whole surface with a fingertip covered with a vinyl glove and pre-saturated with the product, to prevent eventual losses of the amount weighed. The spreading was achieved in two steps: (i) quick distribution of the product, without pressure (20-30 seconds); and (ii) rubbing it into the rough surface using pressure (20-30 seconds too). For each product, three plates were prepared, which were kept protected from light exposure in a dark chamber at room temperature (≈ 20 ºC) for 15 minutes, in order to facilitate the formation of a standard stabilized sunscreen film.

After this period, the plates containing the product were placed in the light-path of the transmittance analyzer. The transmission of UV radiation through the sample was measured from 290 to 450 nm at 1 nm intervals on 9 different sites of the plates (total measurement area = 2.0 cm^2). The blank was prepared using the HD6 plates covered with a thin film-coating of absolute ethanol, because of its non-fluorescence and UV transparency, as recommended by the European Agency (Colipa, 2011).

When all sample scans were recorded satisfactorily for each plate, the equipment *UV-2000* software used the transmission data to calculate: UVB protection efficacy as SPF; and UVA protection efficacy as UVA/UVB Ratio and Critical Wavelength. The SPF was calculated accordingly to equation 1:

$$SPF = \frac{\int_{290}^{400} E_{(\lambda)} S_{(\lambda)} d_{(\lambda)}}{\int_{290}^{400} E_{(\lambda)} S_{(\lambda)} T_{(\lambda)} d_{(\lambda)}} \quad (1)$$

where E(λ) is the erythema action spectrum, S(λ) is the solar spectral irradiance, and T(λ) is the spectral transmittance of the sample with the integral calculated across the 290-400 nm wavelength limits.

The λc was calculated according to equations 2 and 3:

$$\lambda_c = Min(\lambda') \quad (2)$$

such that λ' satisfies the relationship:

$$\frac{\sum_{\lambda=290}^{\lambda'} A_\lambda}{\sum_{\lambda=290}^{400} A_\lambda} \geq 0{,}9 \quad (3)$$

where A_λ is the absorbance at wavelength λ.

The UVA/UVB Ratio was calculated using the Boots Star Method (2008), without exposing the samples to any source of radiation, according to equation 4:

$$\text{UVA/UVB Ratio} = \frac{\frac{\int_{320}^{400} A_{(\lambda)} d\lambda}{\int_{320}^{400} d\lambda}}{\frac{\int_{290}^{320} A_{(\lambda)} d\lambda}{\int_{290}^{320} d\lambda}} \quad (4)$$

where $A(\lambda)$ is the monochromatic absorbance averaged across the sample plate for each wavelength of the UVA/UVB spectrum.

Fatty acids assay

For the essential oils lipids hydrolysis, 10 mg of the oils were dissolved in 100 µL of a mixture of ethanol and 1 mol L^{-1} potassium hydroxide (95 : 5, v/v), in 2 mL cryogenic tubes. After a 10-seconds vigorous mechanical agitation, the oils were hydrolyzed in a microwave equipment (Panasonic, Japan), using an 80W potency for 5 minutes. Then they were cooled, and the following reagents were added to the tubes, in this order: 400 mL of 20% hydrochloric acid, one spatula tip of sodium chloride, and 600 µL of ethyl acetate. New 10-seconds agitation was made, followed by a 5-minutes rest period, when a 300-µL aliquot from the organic layer was transferred to a microcentrifuge tube and dried in oven, in order to obtain the free fatty acids. The residues obtained were methylated with 14% boron trifluoride in methanol and heated in water bath for 10 minutes, at 80 ºC. The resulted solution was injected in the gas chromatograph.

Statistical analysis

All data were reported as mean, and the SPF values were reported as mean ± standard deviation. The verification of the accordance between the values obtained with the different oils and the reference titanium dioxide product (SPF = 16) was achieved through a one sample *t* test (95% confidence), performed in the Statistical Package for the Social Sciences® (SPSS) v. 14.0.

RESULTS AND DISCUSSION

The SPF is a worldwide known parameter that measures the protection of a sunscreen agent against the UV solar energy. Its values correspond to the amount of UV radiation capable of producing sunburn on sunscreen-applied human skin, compared to the amount of solar energy required to cause the same reaction onto an unprotected skin area (FDA, 2011), therefore this being the most disseminated criterion for labeling the photoprotection efficacy.

Given what this parameter represents, its measurement - on the purpose of registration by official agencies – must be done *in vivo*, on human volunteers (FDA, 2007). However, *in vitro* measurements are currently available for both screening and lot-to-lot quality control studying, once they are faster and less complex. Although over the time the classical UV spectrophotometry has been used on this purpose, today it is known that such method is insufficient on that matter. That is why the currently adopted method is the diffuse transmittance spectrophotometry, which detects the absorbed/transmitted light and also the reflected/scattered one. Thus, its main advantage is the capability of determining the SPF of formulations with high SPF levels containing sunblockers, which act by scattering the UV radiation through both UVA and UVB regions. This protection can be measured through the determination of the UVA/UVB Ratio and the Critical Wavelength, which measure the spectrum of protection of the product.

Using the refered method, the photoprotection potential of eight different raw materials from Amazon flora was assessed. The vegetable species were selected due to their relevance to the Brazilian flora, once all of them are native from Amazon. The photoprotection results of the oils-incorporated base emulsion are shown in Table 1, as well as their absorptive profile can be seen in Figure 1.

As one can see, all sunscreens incorporated with 10% plant oils showed low *in vit*ro SPF, and a product can only label its SPF if it shows a value greater than 2 (Brasil, 2002). Since none of the analyzed species achieved such value, they are not adequate to be used as raw materials for generating on sunscreens an SPF value, although they can be used for other purposes in the formulations, as they can act as emollients, moisturizers and lubricants. The confirmation of the reliability of this technique is given by the 10% TiO_2 control product, which had a SPF determined as 16 ± 2.1, correspondent to the theoretical value (BASF, 2011).

These findings are in agreement with the study of Ferrari *et al.* (2007), which showed that *Carapa guianensis* Aubl. did not possess significant photoprotective activity. On the other hand, none of the species here studied have comparable published data, highlighting the uniqueness of the present work.

The incorporation of these plant materials to a 10% TiO_2 was made in order to verify whether some of them could improve its SPF, by acting synergistically with the inorganic filter. However, the SPF results for these products showed that the photoprotection is similar to the control, i.e., the products without the plant material. This type of study has been already conducted by Ramos *et al.* (1996), which have showed that *Hamamelis virginiana* L., *Rhamnus purshiana* DC and *Cinnamomum zeylanicum* Nees have synergism with synthetic filters. However, there is no data regarding the species here studied.

Table 1. *In vitro* photoprotective activity of oil-free base emulsion containing 10% Amazon oils, with or without 10% titanium dioxide (TiO_2)

	Mean SPF	*UVA/UVB Ratio*	λ_c *(nm)*
Control (10% TiO_2)	16 ± 2.1	0.584	379
Açaí oil	1 ± 0.2	0.545	378
Açaí oil + 10% TiO_2	15 ± 1.7*	0.99	374
Andiroba oil	1 ± 0.1	0.668	377
Andiroba oil + 10% TiO_2	15 ± 0.3*	0.498	373
Buriti oil	1 ± 0.1	0.693	374
Buriti oil + 10% TiO_2	15 ± 0.2*	0.509	374
Castanha do Brasil oil	1 ± 0.0	0.587	374
Castanha do Brasil oil + 10% TiO_2	14 ± 0.1*	0.498	373
Patauá oil	1 ± 0.1	0.587	370
Patauá oil + 10% TiO_2	16 ± 0.3*	0.474	372
Pequi oil	1 ± 0.1	1.441	352
Pequi oil + 10% TiO_2	15 ± 0.5*	0.487	372
Pracaxi oil	1 ± 0.0	0.071	360
Pracaxi oil + 10% TiO_2	18 ± 0.9*	0.485	372
Tucumã oil	1 ± 0.0	0.404	364
Tucumã oil + 10% TiO_2	15 ± 0.9*	0.522	374

SPF = Sunburn Protection Factor; λ_c = Critical Wavelength. *no statistical difference with the control (p= 0.05).

In addition, other parameters for the plant species were determined, which can also be seen in Table 1. It is observed that the species have an almost even UVA protection because of the proximity of the results found – none has an UVA protection greater than the UVB protection. Besides, some species have shown Critical Wavelength values greater than 370 nm. The λc is considered the wavelength value at which the integral of the area under the absorbance spectrum of the sample reaches 90% of the total absorbance, from 290 to 400 nm (Colipa, 2011), being the wavelength value of 370 nm the borderline for a product to be considered as a broad spectrum sunscreen. Despite these values, we can not affirm that these species can be used as raw materials for sunscreens.

Another point of our work was the determination of the main fatty acids components of these studied oils, which are listed in Table 2. The chromatograms can be visualized in Figure 2. As one can see, the majority components of these oils are the oleic and linoleic acids, which possess roles in oxidative processes, and so they can act diminishing the inflammatory response derived from exposure of the human skin to the solar radiations.

Carapa guianensis Aubl., known to have in its oil composition substances as oleic, palmitic, stearic and linoleic acids (Anbrozin *et al.*, 2006), presented oleic acid as its main oil compound in our study, which accounts for 53.7% of the whole oil. *Astrocaryum vulgare* Mart., for its turn, presented oleic and palmitoleic acids as major components, what is consistent with previously published findings for these specie (Oboh & Oderinde, 1988).

Table 2. Main constituents of Amazon native oils.

Oil	*Main constituents (%)*		
Açaí	C18:1 – 60.7	C16:0 – 22.8	C16:1 – 4.2
Andiroba	C18:1 – 53.7	C16:0 – 28.3	C18:2 – 9.0
Buriti	C18:1 – 75.5	C16:0 – 18.3	C18:2 – 2.8
Castanha do Brasil	C18:1 – 42.6	C18:2 – 30.2	C16:0 – 15.3
Patauá	C18:1 – 79.1	C16:0 – 11.8	C18:2 – 4.5
Pequi	C18:2 – 41.3	C18:1 – 33.5	C16:0 – 16.3
Pracaxi	C18:1 – 53.3	C18:2 – 25.5	C22:0 – 5.0
Tucumã	C18:1 – 66.6	C16:0 – 21.2	C18:2 – 5.3

Acids: C16:0 – palmitic; C16:1 – palmitoleic; C18:1 – oleic; C18:2 – linoleic; C22:0 – behenic.

For *Bertholletia excelsa* Bonpl., a previous study (Ferreira *et al.*, 2006) have reported that its oil possess 34% of linoleic acid, approximately the same value we found (30.2%). However, this study found 51% of oleic acid, while we found 42.6%. We can hypothesize that this occurred due to differences in the method of extraction of the oil, as well as in the harvest period.

The results found for *Oenocarpus bataua* Mart. are also in accordance with the ones from scientific literature: we found 79.1% of oleic, 11.8% of palmitic and 4.5% of linoleic acids, while Darnet *et al.* (2011) found 76.8%, 15.5% and 3.9% for the same substances, in the same order, and Rodrigues *et al.* (2010) found 75.5% and 18.7% for oleic and palmitic acids, respectively.

The composition of buriti oil (*Mauritia flexuosa* L.f.) has already been determined, by Albuquerque *et al.* (2005), who found oleic and linoleic as the main unsaturated fatty acids (73.3 - 78.3% and 2.4-3.9%, respectively) and palmitic as the main saturated fatty acid (17.34 - 19.2%). In our work, we found. In the same sequence, 75.5%, 2.8% and 18.3%, results that corroborate the referred previously work.

With respect to *Caryocar villosum* (Aubl.) Pers., our research goes against another one previously published (Marx *et al.*, 1997), once ours found 41.3% of linoleic acid and theirs found palmitic acid as the main component (33.5%).

It was not found any publication regarding the fatty acids content of *Euterpe oleraceae* M., highlighting the uniqueness of our work for this specific specie. It of our believe that we did not find such information because açaí is seldom used as oil, being its consumption is done more through its crude berry pulp or its dry extract. Similarly, we did not find any previous data from composition of *Pentaclethra macroloba* (Willd.) Kuntze.

CONCLUSION

In conclusion, the SPF data permit to confirm that, despite the wide range of absorption in the UV region, these species absorb so weakly that they do not seem to be adequate for use on the photoprotection matter. However, their great amounts of saturated and unsaturated acids permit that they can be used for other purposes in cosmeceuticals products, since they can act as emollients, moisturizers and lubricants.

ACKNOWLEDGMENTS

This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa e Desenvolvimento de Minas Gerais (FAPEMIG).

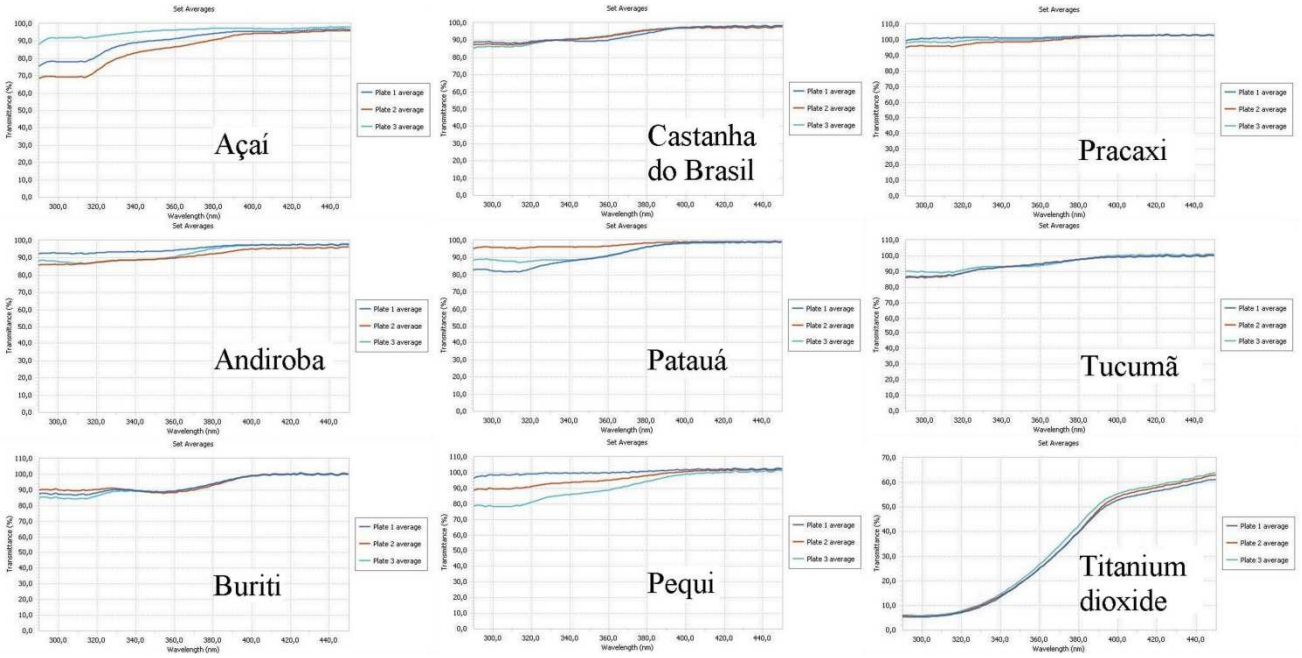

Figure 1**.** Diffuse transmittance spectral data from the oil-free base emulsions containing 10% Amazon oils and 10% titanium dioxide (control)

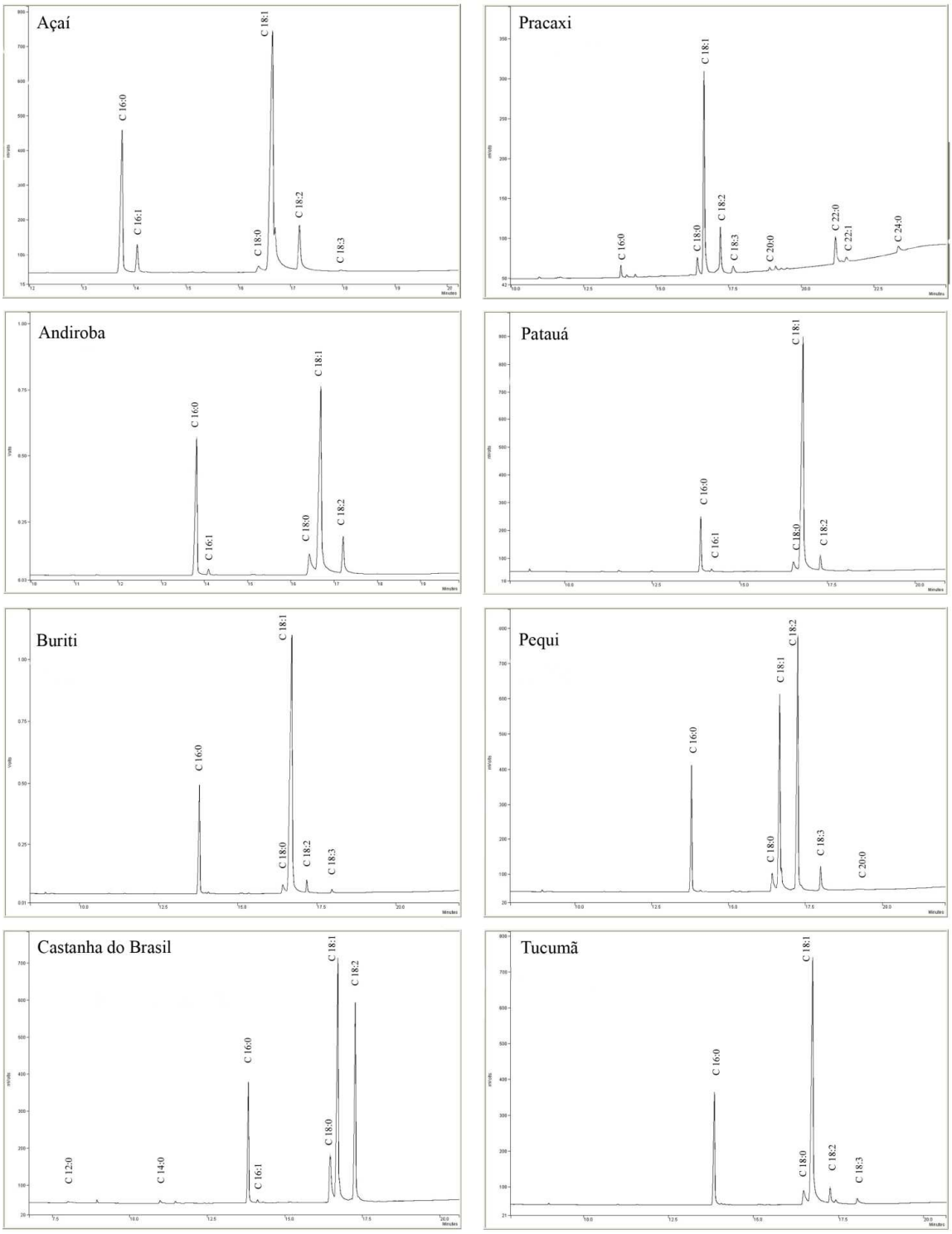

Figure 2. Gas chromatograms of the Amazon oils studied. Acids: C12:0 – lauric; C14:0 – myristic; C16:0 – palmitic; C16:1 – palmitoleic; C18:0 – stearic; C18:1 – oleic; C18:2 – linoleic; C18:3 – linolenic; C20:0 – eicosanoic; C22:0 – behenic; C22:1 – erucic; C24:0 – lignoceric

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