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Separation and identification of fatty acids in cosmetic formulations containing Brazil nut oil by capillary electrophoresis

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ABSTRACT

An analytical method based on capillary electrophoresis (CE) with a partially aqueous electrolyte system was developed to enable the free fatty acids of Brazil nut oil to be identified in cosmetic formulations. In this study, a gel cream formulation was developed and its oil phase was extracted with a mixture of chloroform-methanol-water (1:2:0.8 v/v/v). The chloroform layer was saponified with a methanolic solution of NaOH (0.5 mol L^{-1}) at 75-80 °C for 25 minutes. Experiments were carried out on a Beckman PACE/MDQ CE system (Fullerton, CA, USA) equipped with an on-column, diode-array detection system set at 254 nm and at 25ºC. The electrolyte consisted of 12.5 mmol L^{-1} sodium tetraborate buffer pH 7.0, 12.5 mmol L^{-1} polyoxyethylene 23-lauryl ether, 7.5 mmol L^{-1} sodium dodecylbenzenesulfonate (used as chromophore for indirect UV detection) and acetonitrile (35% v/v). The proposed method allowed the separation and identification of the fatty acids of Brazil nut oil in a cosmetic gel cream, as well as enabling possible interference by the oily phase components in the formulation to be identified.

Keywords: Bertholletia. Fatty acids. Cosmetics. Capillary electrophoresis. Nut oil.

INTRODUCTION

The 'Brazil nut' tree, one of the tallest in the Amazon rainforest, reaches 30 to 50 meters in height (Ferreira et al., 2005) and can live for over 500 years. Its large fruits, known locally as *ouriço* or 'hedgehog', weigh about one pound and contain 15 to 24 seeds (Cunha et al., 2004), which are released, eaten and planted by a wild rodent, the agouti.

The 'Brazil nut' (*Bertholletia excelsa*) is a seed native to the Amazon basin in South America and extracted mainly from wild forest. It is well known for its nutritional value due to its high lipid (70% w/w) and protein (20% w/w) contents. The seeds are a source of fat, protein and selenium, which is an important antioxidant. The oil from the seeds contains vitamins A and E, which are essential for skin protection. Owing to its chemical characteristics and high concentration of essential fatty acids, mainly oleic and linoleic, the oil can be used in various cosmetic formulations, such as creams, lotions, shampoos, conditioners and soaps (Croda, 2011). Table 1 shows the fatty acids detected in 'Brazil nut' oil by gas chromatography, according to studies by various authors.

Table 1. Fatty acid contents in Brazil nut oil according to various authors

Fatty Acid (%)	Elias & Bressani (1961)	Melo & Mancini (1991)	Gutierrez et al. (1997)	Solis (2001)	Venkatachalam & Sathe (2006)	Ryan et al. (2006)
Palmitic	13.9	14.5	13.9	15.20	15.11	13.50
Stearic	10.3	8.3	8.1	11.60	9.51	11.77
Oleic	30.5	27.2	29	34.50	28.75	29.09
Linoleic	44.9	49.9	48.8	37.80	45.43	42.8

According to Carvalho et al., (1994), after the decline of rubber latex extraction, the Brazil nut became the main exported product from northern Brazil, although now threatened in many areas by tree felling. The fruit is produced during the rainy season or Amazon 'winter', from December to June.

The number of cosmetic products used for skin care and hair treatments, based on herbal extracts and essential oils, has increased remarkably. Considering the complex nature of such matrices, well-developed methods, for quality control during the industrial processing and storage of such extracts, as well as to reveal adulteration of raw materials and final products, are much needed and desirable (Tomás-Barberán, 1995).

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Vegetable oils are natural products composed of a mixture of esters of glycerol, wherein the fatty acid chains have from 8 to 24 carbon atoms, with varying degrees of unsaturation. Therefore, the analysis of fatty acid composition is the first step in the preliminary assessment of the quality of crude oil and/or its transformation products. This can be achieved by many analytical methods, such as gas chromatography, liquid chromatography, nuclear resonance magnetic spectroscopy (Moretto & Fett, 1998).

In this context, standardized natural extracts are being studied widely with a view to their use in cosmetic products (Baby et al., 2005).

In cosmetic emulsions, plant oils are constituents of the oily phase with properties such as low viscosity and molecular weight. These qualities make them less occlusive than mineral oils. They also show good skin penetration and compatibility and the ability to carry therapeutic agents, besides providing nutrients such as tocopherols, carotenoids and essential fatty acids. They can dissolve many hydrophobic substances, such as oils, phenols, terpenes and aromatic acids. Leaves, seeds, kernels and plant extracts, being rich in nutrients, can be incorporated into other emollients, fragrances, dyes and vehicles for skin drug applications (Silva, 1997).

Pereira (2008) reported data on vegetable oils with high contents of fatty acids, especially linoleic acid. These extracts, when incorporated into nonionic and anionic emulsions, promote highly emollient properties and other desirable rheological characteristics, such as stability and easy industrial processing. As Brazil nut oil exhibits a high concentration of linoleic acid, the same properties should be observed when this extract is incorporated into gel cream cosmetic preparations.

In the last decade, capillary electrophoresis (CE) has been used to advantage as an alternative technique for the determination of fatty acids in oils and fats. CE is a versatile separation technique that has been increasingly employed in the analysis of complex natural matrices, such as food (Garcia-Cañas & Cifuentes, 2008) and biological systems (Song et al., 2008), as it offers high separation efficiency and unique selectivity.

Fatty acids do not possess strong chromophore groups in their structures, ruling out their direct photometric detection. One solution to this problem was to use indirect UV and indirect fluorescence detection in CE (**Yeung, 1995)**. Many chromophoric agents have been proposed, such as *p*-anisate (Miwa et al., 1987) and diethylbarbiturate (Roldan-Assad & Gareil, 1995).

The use of capillary zone electrophoresis (CZE) for free fatty acid (FFA) determination has been limited by the poor aqueous solubility of lipids and low UV absorbance of the fatty acids. Aqueous electrolytes can be used to solubilize lipids with C_2–C_{14} chains before determination by CZE. However, most fatty acids with more than 17 carbon atoms have been analyzed by micellar electrokinetic chromatography (MEKC) (Roldan-Assad & Gareil, 1995; Drange & Lundanes, 1997).

Oliveira et al., (2003) (also Oliveira, 2003; thesis) developed a novel capillary electrophoretic method using UV indirect detection (224 nm) for the analysis of trans fatty acids in hydrogenated oils, among them, Brazil nut oil. The electrolyte consisted of a pH 7 phosphate buffer at a concentration of 15 mmol.L^{-1} containing 4 mmol.L^{-1} sodium dodecylbenzenesulfonate, 10 mmol.L^{-1} polyoxyethylene 23 lauryl ether (Brij 35), 2 wt% 1-octanol and 45 wt% acetonitrile. According to the authors, the main fatty acids in Brazil nut oil are linoleic (42.2 ± 0.87 wt%), followed by oleic (37.4 ± 0.54 wt%), palmitic (13.4 ± 0.35 wt%) and stearic (7.00 ± 0.51 wt%) acids.

Moraes (2003) studied the hydrolysis of Brazil nut oil and assayed the fatty acids present in the oil by capillary electrophoresis, with indirect UV detection (224 nm). The electrolyte used consisted of a 15 mmol.L^{-1} phosphate buffer (pH 7), 4 mmol.L^{-1} sodium dodecylbenzenesulfonate, 10 mmol.L^{-1} polyoxyethylene 23 lauryl ether (Brij 35) and 2 wt% *n*-octanol. The linoleic acid was found to be 22.3 wt% of the oil, oleic acid was 17.6 wt%, palmitic acid 8.1 wt% and stearic acid 2.2 wt%.

Bannore et al., (2008) demonstrated the suitability of CE with a partially aqueous electrolyte system to analyze the free fatty acids present in peanut seeds. The behavior of five fatty acids common to most oils, *viz.* palmitic, stearic, oleic, linoleic and linolenic acids, was investigated by CE in various non-aqueous electrolyte systems and an aqueous solvent, in order to achieve separation of these acids. The partially aqueous electrolyte system consisted of 40 mM Tris, 2.5 mM adenosine-5-monophosphate (AMP) and 7 mM α-CD in a mixture of N-methylformamide (NMF) / dioxane / water (5:3:2 v:v:v), at pH 8.9. This capillary electrophoretic method enabled the quantitative analysis of oleic and linoleic acids in peanut seeds.

No reference was found in the literature to a CE method for fatty acid separation and identification in cosmetics containing Brazil nut oil. Thus, the objective of the present study was to develop such a method to separate and identify these fatty acids in cosmetic emulsions.

MATERIAL AND METHODS

Reagents and apparatus

All reagents were of analytical grade and water was deionized in a Milli-Q® system (Millipore, Bedford, MA, USA). Ethanol, methanol and acetonitrile (ACN) were of HPLC grade (Merck, Rio de Janeiro, Brazil). Sodium tetraborate, polyoxyethylene 23-lauryl ether (Brij™35), sodium dodecylbenzenesulfonate (SDBS) and the standards of palmitic acid (C16:0), margaric acid (C17:0) (internal standard), stearic acid (C18:0), oleic acid (C18:1c), linoleic acid (C18:2cc) and linolenic acid (C18:3ccc) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The extra-virgin Brazil nut oil was obtained from the Brazilian company Inovam (Amazonas, AM, Brazil). Ethylenediaminetetraacetic acid (disodium EDTA), phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben and isobutylparaben (Phenova); glycerin, cyclomethicone, crosspolymer dimethicone, butylhydroxytoluene (BHT); caprylic/capric triglyceride, polyacrylamide, C13-14 isoparaffin and laureth-7 (Sepigel™ 305) and PEG-5 Ceteth-2 (Procetyl AWS) were obtained from Mapric Produtos Fármacos e Cosméticos LT-ME, São Paulo, SP, Brazil.

All CE experiments were conducted on a Beckman PACE/MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) equipped with an on-column diode-array detection (DAD) system set at 254 nm and a temperature control device maintained at 25 °C. The Beckman 32 Karat™ 8.0 software was used for data acquisition, electropherogram peak integration and data analysis. Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA), of total length 60.2 cm (effective length 50.0 cm) and i.d. 75 mm, were used. The samples were injected hydrodynamically, with a pressure of 0.3 psi, for 3 s. The electrophoretic system was operated under normal polarity and constant voltage conditions of +20 kV.

Preparation of the gel cream

The formulation selected for this study was a gel cream. This type of cosmetic has a low concentration of oily substances, providing the good tactile properties of the gel and consequently the sensation of freshness. It can be used on all skin types, depending, however, on the other components added to the formulation (Martini, 2005).

The gel was prepared in the laboratory and contained: 0.1% disodium EDTA; 0.5% Phenova; 3% glycerin; 3% cyclomethicone; 2.5% crosspolymer dimethicone; 0.1% BHT; 1.5% caprylic/capric triglyceride; 2.0% Sepigel™305; 0.5% Procetyl AWS; 5% *Bertholletia excelsa* seed oil and purified water to 100% (w/w).

The components of the aqueous phase were mixed at room temperature, except for disodium EDTA, which was dissolved in a small amount of purified water at a temperature of 70°C, prior to being added to the mixture. BHT was dissolved in caprylic/capric triglyceride at 70°C. This solution was added to the Procetyl AWS and *Bertholletia excelsa* seed oil. The oily phase was poured into the aqueous phase and the mixture added to the Sepigel™305. Finally the cyclomethicone and the crosspolymer dimethicone were added to the preparation.

A gel cream and a gel cream base were prepared, with and without Brazil nut oil, respectively.

Oily phase extraction of cosmetic formulation

The oily phase of gel cream samples, prepared as previously described, was extracted by the method proposed by Bligh and Dyer (1959), in which a mixture of chloroform-methanol-water (1:2:0.8 v/v/v) was homogenized with the sample. The system separated into two phases on addition of more chloroform. The bottom (chloroform) layer was collected carefully and dry under the stream in a speedvac and transferred to a 25 mL round-bottom flask, where the solvent was removed by rotary evaporator.

Saponification of Brazil nut oil, gel cream oily phase and raw materials

Commercially available samples of *Bertholletia excelsa* seed oil, the oily phases obtained by extraction of the gel cream (gel cream containing *Bertholletia excelsa* seed oil and the gel cream base) and the raw materials used in the preparation of the formulation, were saponified by adding 1 mL of a methanolic solution of NaOH (0.5 mol L^{-1}) to 2g of each sample and heating at 75–80 °C for 25 min. The solutions were immediately cooled in a bath containing a mixture of ethanol and dry ice. Aliquots of these solutions were diluted 50 times with methanol before being injected into the capillary inlet of the CE system (Oliveira, 2003).

Preparation of standard solutions

The capillary electrophoretic behavior of standard solutions of the five commonest FFAs in Brazil nut oil (Table 1), namely palmitic, stearic, oleic, linoleic and linolenic acids was also investigated.

Stock standard solutions of fatty acids were prepared by weighing 20 mg of each acid and dissolving it in methanol in a 10 mL volumetric flask. The solutions were stored in a freezer for use.

Sonication was used to aid solubilization. Sodium tetraborate buffer (pH 7) was prepared in a 100 mL volumetric flask and the volume completed with deionized water. Both sodium tetraborate buffer and Brij™35 stock solutions were kept under refrigeration to prevent mould formation. The optimized electrolyte used for CE consisted of: 12.5 mmol L^{-1} sodium tetraborate buffer pH 7.0; 7.5 mmol L^{-1} SDBS; 12.5 mmol L^{-1} Brij™ 35 and ACN (35% v/v).

RESULTS

Some electropherograms of a standard mixture of the four main FFAs in Brazil nut oil dissolved in methanol are shown in Figures 1-3. Tests with two different sample injection times (6 and 3 seconds) can be observed in Figures 1a and 1b, different sample injection pressures (0.3 and 0.5 psi) in Figures 2a and 2b and different applied voltages (20 and 25 kV) in Figures 3a and 3b.

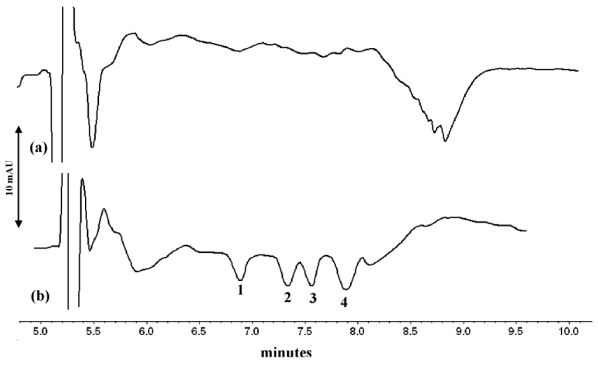

Figure 1. CE electropherograms of standard mixture of fatty acids dissolved in methanol, with nonaqueous electrolyte system of 12.5 mmol L^{-1} sodium tetraborate buffer pH 7.0, 12.5 mmol L^{-1} polyoxyethylene 23-lauryl ether (Brij™ 35), 7.5 mmol L^{-1} sodium dodecylbenzenesulfonate (SDBS) and ACN (35% v/v). Hydrodynamic injection at 0.5 psi for (a) 6 s and (b) 3 s; applied voltage +20 kV, detection at 254 nm and temperature 25 °C. Concentration of each fatty acid: 250.0 µg mL^{-1}. Peaks: (1) stearic acid, (2) oleic acid, (3) palmitic acid and (4) linoleic acid.

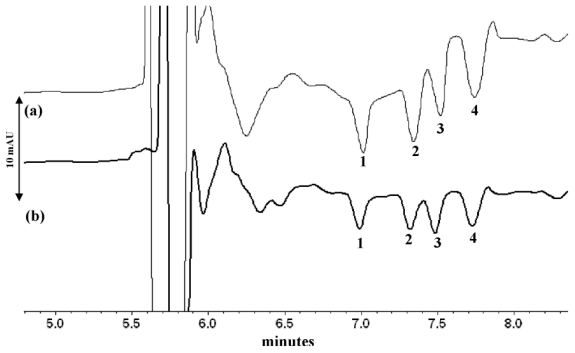

Figure 2. CE electropherograms of standard mixture of fatty acids dissolved in methanol, with nonaqueous electrolyte system of 12.5 mmol L^{-1} sodium tetraborate buffer pH 7.0, 12.5 mmol L^{-1} polyoxyethylene 23-lauryl ether (Brij™ 35), 7.5 mmol L^{-1} sodium dodecylbenzenesulfonate (SDBS) and ACN (35% v/v). Hydrodynamic injection for 3 s at (a) 0.5 psi and (b) 0.3 psi; applied voltage 25 kV, detection at 254 nm and temperature 25 °C. Concentration of each fatty acid: 250.0 µg mL^{-1}. Peaks: (1) stearic acid, (2) oleic acid, (3) palmitic acid and (4) linoleic acid.

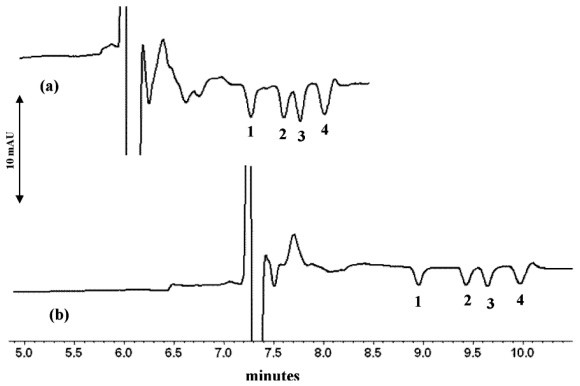

Figure 3. CE electropherograms of standard mixture of fatty acids dissolved in methanol, with nonaqueous electrolyte system of 12.5 mmol L^{-1} sodium tetraborate buffer pH 7.0, 12.5 mmol L^{-1} polyoxyethylene 23-lauryl ether (Brij™ 35), 7.5 mmol L^{-1} sodium dodecylbenzenesulfonate (SDBS) and ACN (35% v/v). Hydrodynamic injection for 3 s at 0.5 psi, with applied voltage of (a) 20 kV and (b) 25 kV; detection at 254 nm and temperature 25 °C. Concentration of each fatty acid: 250.0 µg mL^{-1}. Peaks: (1) stearic acid, (2) oleic acid, (3) palmitic acid and (4) linoleic acid.

Table 2 shows various methodological validation parameters: efficiency of separation (theoretical number of plates in equivalent column), symmetry factor of the peak, resolution and migration time repeatability (RSD).

Table 2. System suitability parameters for optimized capillary electrophoresis method

Parameters	Fatty acids[a]					
	1	2	3	4	5	6
Theoretical plates (N) (plates/m)	1.0 x 10^5	1.4 x 10^5	2.0 x 10^5	1.5 x 10^5	1.2 x 10^5	1.5 x 10^5
Asymmetry (As)	0.90	1.15	1.36	1.50	1.02	1.25
Migration time (%RSD) [b]	0.73	0.85	1.21	1.13	1.33	1.27
Resolution (Rs)	$2.13_{(2-1)}$	$1.53_{(3-2)}$	$1.84_{(4-3)}$		$2.13_{(5-4)}$	$3.33_{(6-5)}$

[a]1: Stearic acid; 2: Margaric acid (IS); 3: Oleic acid; 4: Palmitic acid; 5: Linoleic acid; 6: Linolenic acid.

[b] Average of 10 determinations; RSD: relative standard deviation.

Figures 4-7 show, respectively, the electropherograms of the standard mixture of six FFAs dissolved in methanol, the extra-virgin Brazil nut oil sample and the oily phases of the gel cream and gel cream base.

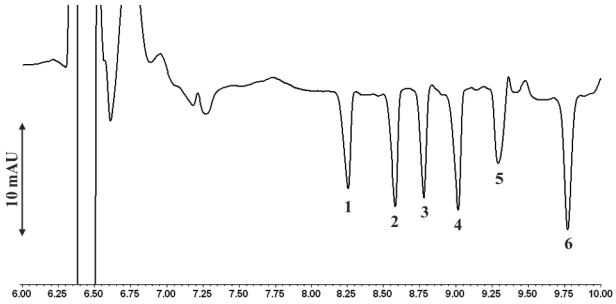

Figure 4. Electropherogram of standard mixture of six FFAs dissolved in methanol. Peaks: (1) stearic acid, (2) margaric acid (internal standard, IS), (3) oleic acid, (4) palmitic acid, (5) linoleic acid and (6) linolenic acid; using an electrolyte system constituted of 12.5 mmol L^{-1} sodium tetraborate buffer pH 7.0, 12.5 mmol L^{-1} polyoxyethylene 23-lauryl ether (Brij™ 35), 7.5 mmol L^{-1} sodium dodecylbenzenesulfonate (SDBS) and ACN (35% v/v). Hydrodynamic injection for 3 s at 0.3 psi; applied voltage of +20 kV, detection at 254 nm and temperature at 25 °C. Concentration of each fatty acid: 250.0 µg mL^{-1}.

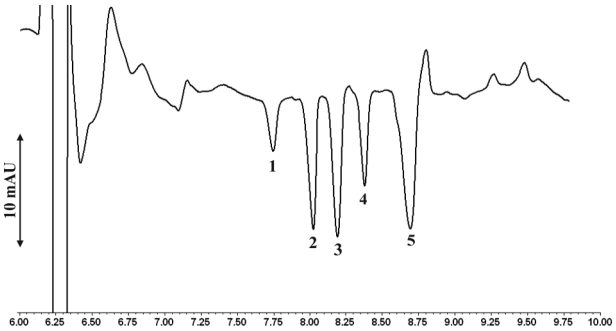

Figure 5. Electropherogram of extra-virgin Brazil nut oil sample. Peaks: (1) stearic acid, (2) margaric acid (internal standard, IS), (3) oleic acid, (4) palmitic acid and (5) linoleic acid; electrolyte system: 12.5 mmol L^{-1} sodium tetraborate buffer pH 7.0, 12.5 mmol L^{-1} polyoxyethylene 23-lauryl ether (Brij™ 35), 7.5 mmol L^{-1} sodium dodecylbenzenesulfonate (SDBS) and ACN (35% v/v). Hydrodynamic injection for 3 s at 0.3 psi; applied voltage +20 kV, detection at 254 nm and temperature 25 °C.

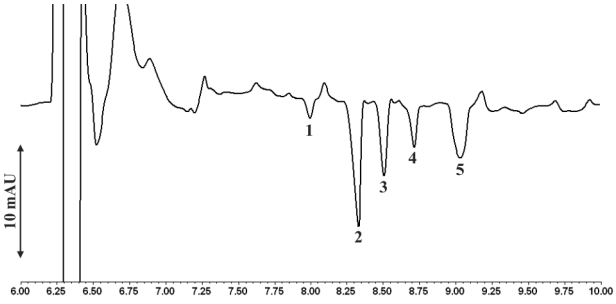

Figure 6. Electropherogram of oily phase of gel cream. Peaks: (1) stearic acid, (2) margaric acid (IS), (3) oleic acid, (4) palmitic acid and (5) linoleic acid; electrolyte system of 12.5 mmol L^{-1} sodium tetraborate buffer pH 7.0, 12.5 mmol L^{-1} polyoxyethylene 23-lauryl ether (Brij™ 35), 7.5 mmol L^{-1} sodium dodecylbenzenesulfonate (SDBS) and ACN (35% v/v). Hydrodynamic injection for 3 s at 0.3 psi; applied voltage +20 kV, detection at 254 nm and temperature 25 °C.

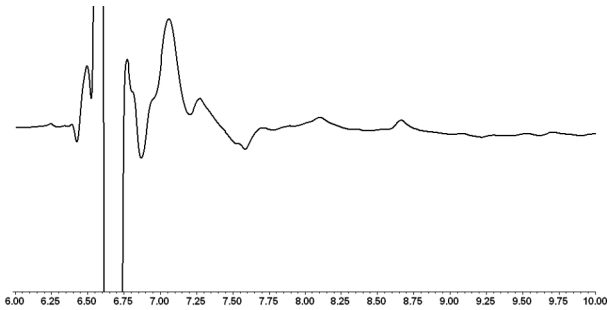

Figure 7. Electropherogram of oily phase of gel cream base; electrolyte system of 12.5 mmol L^{-1} sodium tetraborate buffer pH 7.0, 12.5 mmol L^{-1} polyoxyethylene 23-lauryl ether (Brij™ 35), 7.5 mmol L^{-1} sodium dodecylbenzenesulfonate (SDBS) and ACN (35% v/v). Hydrodynamic injection for 3 s at 0.3 psi; applied voltage +20 kV, detection at 254 nm and temperature 25 °C.

Table 3 shows the fatty acid composition of Brazil nut oil calculated from the electropherogram.

Table 3. Fatty acid contents in Brazil nut oil calculated from CE results in this study

Fatty acid	Relative content (%)
Palmitic (C16:0)	14.95 ± 0.05
Stearic (C18:0)	6.81 ± 0.03
Oleic (C18:1)	30.77 ± 0.08
Linoleic (C18:2)	47.47 ± 0.15

Figure 8 shows the electropherograms of the excipients used in the preparation of the cosmetic formulation.

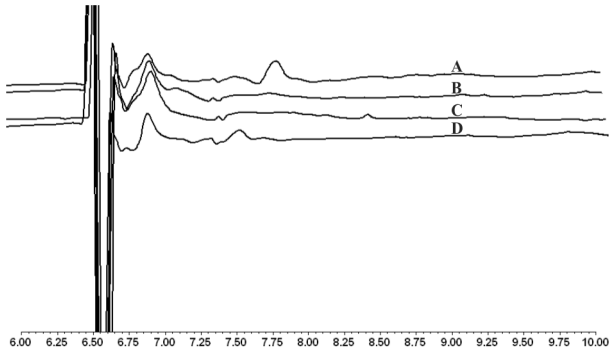

Figure 8. Electropherograms of gel cream excipients dissolved in methanol: (A) Procetyl AWS, (B) SepigelTM 305, (C) Phenova and (D) caprylic/capric triglyceride; electrolyte system: 12.5 mmol L-1 sodium tetraborate buffer pH 7.0, 12.5 mmol L-1 polyoxyethylene 23-lauryl ether (BrijTM 35), 7.5 mmol L-1 sodium dodecylbenzenesulfonate (SDBS) and ACN (35% v/v). Hydrodynamic injection for 3 s at 0.3 psi, applied voltage 20 kV, detection at 254 nm and temperature 25 °C.

DISCUSSION

Accelerated stability tests were performed on the cosmetic formulations (gel cream and gel cream base) as stipulated by the Brazilian federal sanitation authority, ANVISA (Brasil, 2004). It was observed that they maintained their physical and organoleptic characteristics, pH and viscosity without detectable change.

Oily phase extraction from cosmetic formulation

The extraction procedure described by Bligh and Dyer (1959) was used on the cosmetic formulation samples in this study. According to Brum et al., (2009), an advantage of the Bligh and Dyer method is the use of a solvent system based on a mixture of three solvents (chloroform, methanol and water). This method afforded a high oil extraction yield because methanol is a more powerful solvation agent than other solvents used to extract lipids (polar and nonpolar) from both lipid and non-lipid matrices (Brum et al., 2009) and no better extraction procedure has yet been described in the literature.

Development of capillary electrophoresis method

Capillary selection

After preliminary tests with several uncoated fused capillaries, 30, 40 and 50 cm in effective length (to the detector), it was found that the best results were achieved with the longest capillaries. Thus, the capillary chosen had a total length of 62.0 cm (50.0 cm effective length) and i.d. of 75 μm. Shorter capillaries gave poor resolution and overlapping peaks.

Selection of the electrolyte pH

Tests were carried out in order to determine the optimum pH for separation of fatty acids. The pH significantly influences the electroosmotic flow, modifying the migration time and selectivity. Theoretically, for the best resolution the optimum pH is close to the pKa of the investigated solutes, so that the differences in ionization lead to different migration times. The buffer composition can modify the retention time and the selectivity; thus, the different ions interact with the inner surface of the capillary wall and can modify the electroosmotic flow (Oliveira, 2003).

The experiments were performed at pH 7.0, using 12.5 mmol L^{-1} sodium tetraborate buffer. At this pH, the FFAs are deprotonated, since their pKa is around 5. Hence, the fatty acids behave predominantly as anions and tend to migrate in the direction of the anode. However, at this pH, the electroosmotic flow is fast enough to sweep the negatively charged FFAs toward the cathode (located after the detection window), in spite of their charge.

The concentration of each fatty acid used was adjusted to 250.0 μg mL^{-1} in all experiments.

Selection of the chromophore

Fatty acids do not have strong chromophores in their structures and, therefore, exhibit low absorption in the UV. It was necessary to carry out a study to determine the best chromophore for the analysis of the selected compounds.

Experiments were done with imidazole, quinine, benzene, phenol, histidine and SDBS. The best results were achieved with SDBS.

Addition of organic solvents

The addition of organic solvents alters the electroosmotic flow by changing the viscosity of the electrolyte. The effects of adding acetonitrile (ACN), methanol, 1-butanol, isopropanol (IPA) and tetrahydrofuran (THF) at various concentrations were tested. The final choice of solvent was made to give both good separation and high solubility of fatty acids in the electrolyte.

ACN was chosen for this study, as it enabled good separation of fatty acids. Nevertheless, it was necessary to make adjustments to its concentration, since increasing its concentration led to an increase in the baseline noise and in the migration time. Consequently, complete separation of the analytes was not achieved. This observation corroborates a study by Oliveira (2003) in which fatty acids were separated by using ACN as solvent. The present authors optimized an electrolyte whose solvent concentration was 45%, since, above this concentration, the separation of these compounds was impaired. According to Oliveira (2003), ACN (>45%) reduced the electroosmotic flow and improved the sharpness of the peak. In the present research, methanol, 1-butanol, THF and IPA were rejected because they did not lead to a successful separation of fatty acids.

Injection time

In preliminary tests, the hydrodynamic injection pressure was set to 0.5 psi, in order to test the injection time variable. It was observed that when the injection time was longer than 3 s, only a partial separation of fatty acids occurred. At 6 s, for instance, all four acids tested at first (palmitic, stearic, oleic and linoleic) were co-eluted. By comparing Figures 1a and 1b, it can be observed that, when the injection time was increased, the total separation of the compounds was not satisfactory. The voltage used was +20 kV.

Fixing the injection time at 3 s (Figure 1b) and voltage at +25 kV, the next step was to determine the injection pressure. According to the electropherograms shown in Figure 2, the stability of the baseline was poorer, and oleic and palmitic acids still co-eluted, when a pressure of 0.5 psi was used (Figure 2b).

Determination of voltage

The voltage was adjusted to maintain good separation of compounds with a short retention time, without interfering with other parameters that might reasonably affect the separation results. The conditions of 3 s and 0.3 psi were fixed for the assessment of the voltage variable.

It was observed that in the experiments made at +25 kV, the oleic and palmitic acids co-eluted (Figure 3 a). Under these conditions, the experiments made at +20 kV (Figure 3b) allowed the separation of all fatty acids. The current was approximately 50 μA. As expected, a lower voltage produced longer retention times, while a higher voltage could result in significant loss of resolution.

Optimized electrolyte

After varying all parameters, the optimized electrolyte system consisted of: 12.5 mmol L^{-1} sodium tetraborate buffer (pH 7.0), 12.5 mmol L^{-1} Brij ™ 35, 7.5 mmol L^{-1} SDBS as chromophore and ACN (35% v/v).

As pointed out in the introduction, some authors have already published papers in which CE is used for fatty acid analysis (Oliveira et al., 2003; Moraes, 2004; Bannore et al., 2008). Nevertheless, in these methods, expensive reagents were used and the fatty acids were not separated with good resolution. Furthermore, linolenic and palmitic acids co-eluted and the separation time was around 20 min (Bannore et al., 2008). Oliveira et al., (2003) and Moraes (2003) used a higher concentration of acetonitrile and obtained lower resolution than in the present study.

System suitability testing was performed to ensure that the instruments produced accurate and reproducible data. System suitability criteria were established as a part of the method validation. According to the FDA, the tailing factor (asymmetry) should be not more than 2, number of theoretical plates higher than 2000 and resolution higher than 2. Table 2 shows the results obtained for the analysis of the fatty acid standard solutions under the optimized conditions (concentration of each fatty acid: 250.0 $\mu g.mL^{-1}$). Results are within FDA recommendations and USP requirements (U.S. Pharmacopeia 2010; FDA 2011).

Figure 4 shows the separation of the standard mixture of fatty acids contained in Brazil nut oil.

Application

To demonstrate the applicability of the proposed method and to identify the FFAs, samples of both extra-virgin Brazil nut oil and the oily phase of the gel cream were analyzed.

As observed in Figure 5 and Table 3, the extra-virgin Brazil nut oil has linoleic acid (C18:2cc) in highest percentage, followed by oleic (C18:1c), palmitic (C16:0) and stearic (C18:0) acids. These results seem to confirm those reported by other authors (Table 1), who used gas chromatography to determine the Brazil nut oil fatty acid profile.

The fatty acids identified in the extracted oily phase of gel cream were stearic (C18:0), oleic (C18:2), palmitic (C16:0) and linoleic (C18:2) (Figure 6).

The base formulation of the gel cream oily phase (Figure 7) did not interfere in the detection of the fatty acids by the method.

The excipients used in the preparation of the cosmetic formulation (gel cream) were subjected to saponification and injected into the CE system, in order to identify any possible interference of these materials with the method. It was observed that the excipients used in the gel cream did not cause any such interference (Figures 8A, 8B, 8C and 8D). This result implies that it is possible to identify the fatty acids in Brazil nut oil in cosmetic formulations by the proposed method.

CE was performed with a partially aqueous electrolyte system in order to achieve a baseline separation for these FFAs. The optimum electrolyte was applied to the separation and identification of FFAs of Brazil nut oil, in order to develop a CE method for their determination in cosmetic formulations.

Concluding remarks

In this paper, a method for the separation and identification of fatty acids in Brazil nut oil and in cosmetic formulations, using CE with indirect UV detection, is reported. The objective was achieved, since the proposed method enabled the identification of the desired compounds. The proposed electrophoretic method, based on the partially aqueous electrolyte system, allowed the rapid separation and identification of the fatty acids studied, which differ from each other by just one carbon atom and/or one unsaturated bond in their structures. CE proved to be a suitable and efficient technique for this purpose; thus, small amounts of solvents were used, in comparison with other techniques, such as high performance liquid chromatography (HPLC). Another advantage is the decrease in time of analysis. The proposed CE method can be used as a tool for quality control in cosmetic industries.

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RESUMO

Separação e identificação de ácidos graxos em formulações cosméticas contendo óleo de castanha do Brasil por eletroforese capilar

Um método de análise por eletroforese capilar com sistema de eletrólito parcialmente aquoso foi desenvolvido para identificar os ácidos graxos livres do óleo de Castanha do Brasil em formulações cosméticas. No presente trabalho foi desenvolvida uma formulação cosmética (gel creme) cuja fase oleosa foi extraída com uma mistura de clorofórmio-metanol-água (1:2:0.8 v/v/v). A camada de clorofórmio, foi saponificada com solução de NaOH em metanol (0,5 mol L^{-1}) a 75-80 °C durante 25 minutos. Os experimentos foram realizados em sistema de eletroforese capilar Beckman PACE/ MDQ (Fullerton, CA, USA), com detecção de arranjo de diodos a 254 nm e a 25 ºC. O eletrólito utilizado foi 12,5 mmol L^{-1} de tetraborato de sódio tampão a pH 7,0, 12,5 mmol L^{-1} de éter de polioxietileno 23-lauril, 7,5 mmol L^{-1} de dodecilbenzenosulfonato de sódio (utilizado como agente cromóforo para detecção UV indireta) e acetonitrila (35% v/v). O método proposto permitiu a separação e a identificação dos ácidos graxos do óleo de Castanha do Brasil em formulações cosméticas, bem como possibilitou a identificação de interferências presentes na fase oleosa da formulação.

Palavras-chave: Bertholletia. Ácidos graxos. Cosméticos. Eletroforese capilar. Óleo de castanha.

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