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Article

Development of O/W emulsions containing *Euterpe oleracea* extract and evaluation of photoprotective efficacy

Cláudia Cecilio Daher[1,2], Ipojucan Silva Fontes[2], Rayllan de Oliveira Rodrigues[2], Gabriel Azevedo de Brito Damasceno[2], Daiane dos Santos Soares[2], Cícero Flávio Soares Aragão[3], Ana Paula Barreto Gomes[3], Márcio Ferrari[2,*]

[1]Faculty of Pharmacy, Potiguar University, Natal, RN, Brazil, [2]Laboratory of Research and Development of Cosmetic Products, [3]Laboratory of Drug Quality Control, Faculty of Pharmacy, Federal University of Rio Grande do Norte, Natal, RN, Brazil

Euterpe oleracea Mart. is a palm tree popularly known as açai, which is primarily found in northern Brazil. The açai's fruits contain anthocyanins, a class of polyphenols to which antioxidant properties have been attributed. The aim of this work was to develop O/W sunscreens emulsions containing açai glycolic extract (AGE) and to evaluate both their physical stability and photoprotective efficacy. Emulsions containing AGE and sunscreens were formulated using different types and concentrations of polymeric surfactant (acrylates/C 10-30 alkyl acrylate crosspolymer and sodium polyacrylate). The influence of two rheology modifiers (polyacrylamide (and) C13-14/isoparaffin (and) Laureth-7 and Carbomer) on the stability was also investigated. Physical stability was evaluated by preliminary and accelerated studies. Emulsions with 1.0% sodium polyacrylate were stable and exhibited non-newtonian pseudoplastic behavior and thixotropy. Photoprotective efficacy was evaluated by *in vivo* Sun Protection Factor (SPF) and determination of Protection Factor of UVA (PF-UVA). When AGE was added to the sunscreen emulsion, no significant increase in the *in vivo* SPF value was observed. The emulsion containing AGE showed PF-UVA = 14.97, 1.69 of the SPF/PF-UVA ratio and a critical wavelength value of 378 nm, and may therefore be considered a sunscreen with UVA and UVB protection.

Uniterms: O/W emulsion/development. *Euterpe oleracea*/pharmacognosy. *Euterpe oleracea*/glycolic extract/photoprotective efficacy. Sunscreens/development. Sun Protection Factor.

Euterpe oleracea Mart. é uma palmeira popularmente conhecida como açaí, encontrada no norte do Brasil. O fruto do açaí apresenta em sua composição antocianinas, uma classe de polifenóis à qual é atribuída propriedade antioxidante. Os objetivos desse trabalho foram desenvolver emulsões fotoprotetoras O/A contendo extrato glicólico de açaí (AGE), avaliar a estabilidade física e avaliar a eficácia fotoprotetora. Emulsões contendo AGE e filtros solares foram formuladas utilizando diferentes tipos e concentrações de tensoativo polimérico (acrilates/C 10-30 alquil acrilato polímero cruzado e polilacrilato sódico). A influência de dois modificadores reológicos (poliacrilamida (e) C13-14/isoparafina (e) Laureth-7 e Carbomer) na estabilidade foi avaliada. A estabilidade física das emulsões foi avaliada por meio de estudos de estabilidade preliminar e acelerada. Emulsões com 1,0% poliacrilato sódico foram estáveis, exibiram comportamento não-newtoniano pseudoplástico e tixotrópico. A eficácia fotoprotetora foi avaliada pelo teste *in vivo* de Fator de Proteção Solar (FPS) e pela determinação do Fator de Proteção UVA (FP-UVA). Quando adicionado o AGE na emulsão contendo filtros solares, não se observou aumento significativo no valor do FPS. A emulsão contendo o AGE apresentou FP-UVA=14,97, a razão FPS/FP-UVA = 1,69 e o comprimento de onda crítico igual a 378 nm, podendo ser considerado um protetor solar com proteção UVA e UVB.

Unitermos: Emulsão O/A/desenvolvimento. *Euterpe oleracea*/farmacognosia. *Euterpe oleracea*/extrato glicólico/eficácia fotoprotetora. Protetores Solares/desenvolvimento. Fator de Proteção Solar.

***Correspondence:** M. Ferrari. Laboratório de Pesquisa e Desenvolvimento de Produtos Cosméticos, Universidade Federal do Rio Grande do Norte. Rua Gustavo Cordeiro de Farias, s/n – Petrópolis, 59012-570 – Natal - Rio Grande do Norte, Brasil. E-mail: ferrarimarcio@uol.com.br

INTRODUCTION

In recent years, the incidence of ultraviolet radiation-related diseases and disorders has grown, particularly in the form of skin cancers. According to estimates by the National Cancer Institute, for Brazil, there will be 182,000 cases of non-melanoma skin cancer and 5,890 new cases of melanoma in 2014 (INCA, 2014). Ultraviolet light is the most important carcinogen for the genesis of skin cancers (El Ghissassi *et al.,* 2009) and excessive sunlight exposure, has been implicated as the main environmental agent responsible for skin cancer (Gandini *et al.,* 2005).

A valuable tool for attenuating the deleterious effects of UV radiation (UVR) is photoprotection. Methods of photoprotection include actions aimed at education of population, change in habits, sun avoidance, seeking shade, use of protective clothing and correct use of sunscreens (Balakrishnan, Narayanaswamy, 2011; Lohézic-Le Dévéhat *et al*., 2013). Sunscreen use is the most common photoprotection alternative used by the population, and so these products should offer improved protection with broad-spectrum, UVA and UVB protection (Lohézic-Le Dévéhat *et al*., 2013).

The long-term effects of UV radiation include photoaging, immunosuppression, and photocarcinogenesis. UVA causes DNA damage via an oxidative process increasing the level of reactive oxygen species accompanied by cell membrane lipid peroxidation eventuating in cutaneous inflammation (Kullavanijaya, Lim, 2005).

Natural agents, with antioxidant potential and the ability to exert inhibitory effects on diverse cellular and molecular events are gaining considerable attention for the prevention of UV-induced skin damage (Afaq, Mukhtar, 2006; Afaq, 2011). Some substances naturally occurring in vegetal extracts are capable of absorbing ultraviolet radiation via chromophoric molecules associated with antioxidant activity (Sousa *et al.*, 2007).

Polyphenolic compounds, such as flavonoids and anthocyanins are compounds that have antioxidant activity (Cliff, King, Schlosser, 2007).

Vegetal extracts containing flavonoids (e.g., *Achillea millefolium, Hamamelis virginiana, Matricaria chamomilla, Mentha piperita* and *Salvia officinalis*), reported in previous studies, were shown to be capable of absorbing ultraviolet radiation (Bobin, Raymond, Martini, 1994; Souza *et al.*, 2005).

Green tea, *Hamamelis virginiana, Matricaria recutita, Aesculus Hippocastanum, Rhamnus purshiana* and *Cinnamomum zeylanicum*, and aromatic compounds isolated from lichens are examples of natural substances that have been evaluated for their sunscreen properties (Ramos *et al.*, 1996; Katiyar *et al.*, 2001; Rancan *et al.*, 2002; Bianchi, Marchetti, Scalia, 2011).

Açaí (*Euterpe oleracea* Mart.) is a palm native to the Amazon. Its fruits are of economic importance in the Brazilian state of Pará and contain in addition to essential nutrients, anthocyanins, a class of polyphenols to which antioxidant properties have been attributed (Kuskoski *et al.*, 2006; Kang *et al.*, 2011; Gordon *et al.*, 2012; Inácio *et al.*, 2013). Anthocyanins are the predominant polyphenols and account for more than 90% of açai´s total polyphenolic contents (Pacheco-Palencia *et al.*, 2009). However, anthocyanins may also serve as antioxidants and play a photoprotective role by directly eliminating reactive oxygen species during photoxidative stress (Zhang *et al.,* 2010). Schauss *et al.* (2006) demonstrated the antioxidant capacity of açai pulp against superoxide anions and peroxyl radicals, exhibiting enhanced capacity, compared to any other fruit previously reported in the literature.

In a review article, Afaq and Mukhtar (2006) suggested that the use of plants with antioxidant activity (*Punica granatum, Camellia sinensis*) or isolated compounds (resveratrol and genistein) in combination with UV filters might be an effective approach for reducing free radicals generated by UV radiation.

Because of these properties, natural agents are gaining popularity in skin care products. The use of natural agents with antioxidant activities in sunscreen products represent an approach for preventing UV-damage (Afaq, 2011).

Sunscreen products have been formulated mainly as emulsions. However, emulsions are thermodynamically unstable systems and in technological development, the aim is to delay phase separation for as long as possible. Stability is affected by several factors, such as the composition and size of the globules, viscosity, volume of the phases, pH, presence of electrolytes, osmotic gradient and the properties of the interfacial film formed (Ferrari, Rocha-Filho, 2011; Poyato *et al.*, 2013). Changes in these rheological properties may represent important early predictors of impending failure of the products.

The aim of this work was to develop O/W sunscreens emulsions containing açai glycolic extract and, evaluate both their physical stability and the photoprotective efficacy by the testing *in vivo* Sun Protection Factor (SPF) and determination of Protection Factor of UVA (PF-UVA).

MATERIAL AND METHODS

Material

Diethylamino hydroxybenzoyl hexyl benzoate, *bis*-ethylhexyloxyphenol methoxyphenyl triazine and

ethylhexyl triazone from BASF SA (São Paulo - SP, Brazil), Octocrylene, benzophenone – 3, ethylhexyl methoxycinnamate, sodium polyacrylate, and phenoxyethanol (and) caprylyl glycol were donated by Ashland Inc. (São Paulo - SP, Brazil), Acrylates/C 10-30 alkyl acrylate crosspolymer, Carbomer (Lubrizol of Brazil Ltda Additives, São Paulo -SP, Brazil), polyacrylamide (and) C 13-14 isoparaffin (and) Laureth-7 (Chemyunion Chemical Ltda, Sorocaba - SP, Brazil), caprylic/capric triglyceride, ethylhexyl stearate, ethylhexyl palmitate, PPG-3 benzyl ether myristate were donated by Croda of Brazil Ltda (Campinas – SP, Brazil), propylene glycol, disodium EDTA, BHT, and triethanolamine, (DEG Import of chemicals, Sorocaba -SP, Brazil), açai glycolic extract (*Euterpe oleracea* Fruit Extract, batch PR0D016027) was donated by Mapric Farmacocosméticos Products Ltda (São Paulo - SP, Brazil), and distilled water.

Method of preparation and formulations

The emulsion preparation techniques were dependent on the polymeric surfactant used.

For emulsions formulation containing acrylates/C 10-30 alkyl acrylate crosspolymer (P1 - P3, Table I): polymeric surfactant dispersion was performed using an indirect method, i.e., the acrylates/C 10-30 Alkyl Acrylate Crosspolymer was added into the oil phase (Lima *et al.*, 2008; Pianovski *et al.*, 2008). The oil and water phases were then heated separately to 70 ± 2 °C. The oil phase was added to the aqueous phase, followed by agitation at 500 rpm (IKA, mod. Digital RW 20, Germany) until the system was naturally cooled to 40 ± 2 °C. The açai glycolic extract was added to the formulation and the system homogenized (500 rpm) for 5 min.

For preparation of the formulations containing acrylates/C 10-30 alkyl acrylate crosspolymer with the Carbomer (PC1 - PC3 - Table I): the Carbomer was dispersed in the aqueous phase by under mechanical stirrer (Ika, mod. digital RW 20, Germany) at 900 rpm at ambient temperature. The oil phase (containing acrylates/C 10-30 alkyl acrylate crosspolymer) was heated to 60 ± 2 °C, and added to the aqueous phase. After complete phase oil introduction, stirring was maintained for 15 min at 900 rpm. The açai glycolic extract was added and the system maintained under stirring at 5 min to 900 rpm. The same preparation method was performed for formulations containing acrylates/C 10-30 alkyl acrylate crosspolymer with polyacrylamide (and) C 13-14 isoparaffin (and) Laureth-7 (PS1 - PS3 - Table I).

For sodium polyacrylate (R1 - R3, Table I) emulsion preparations: the polymeric surfactant was dispersed in the aqueous phase by a mechanical stirrer (Ika, mod. digital RW 20, Germany) at 500 rpm at ambient temperature for 25 min. The oil phase was heated to 50 ± 2 °C, and added to the aqueous phase. After complete phase oil introduction, stirring was maintained for 10 min at 900 rpm. The açai glycolic extract was added and the system maintained under stirring at 5 min to 900 rpm.

The formulations were produced in three batches.

Preliminary stability tests

Centrifugation test: centrifugation was performed on 24h- old preparation at 3000 rpm (Fanen, mod. 206 BL, Brazil) for 30 min. at room temperature (Lima *et al*., 2008). The appearance, homogeneity and organoleptic characteristics were evaluated by macroscopic analyses (Ferrari, Rocha-Filho, 2011).

Thermal stress: emulsions were submitted to a heated thermostatic bath (Logen Scientific, mod. LSBMLS 2006-2, Brazil) set for the temperature range of 40 to 80 °C, with temperature increase at intervals of 5 °C, and holding at each temperature for 30 min. The organoleptic characteristics, pH value determination, electrical conductivity measures, and viscosity, were obtained to evaluate the formulations before and at the end at 80 °C, after the natural cooling of the samples at room temperature (25 ± 2 °C) (Braconi *et al*., 1995).

Freeze-defrost cycles: samples were subjected to 4 ± 2 °C/24 hours (Mabe, mod. REMB 460, Brazil) and then 45 ± 2 °C/24 hours (Fanen, mod. 515, Brazil), thus completing a cycle. The organoleptic characteristics, pH value determination, electrical conductivity measures, and viscosity were obtained to evaluate the formulations before and at the end of the sixth cycle (12th day) (Lima *et al*., 2008).

pH value determination: 1.0 g of emulsion and 9.0 g of distilled water were placed in a test tube and homogenized. The pH value (Hanna Instruments, mod. HI 21, Brazil) was determined by inserting the electrode directly into the aqueous dilution 1:10 (w / w) of the sample (Davis, 1977).

Electrical conductivity determination: electrical conductivity measures (Logen Scientific, mod. CD-300-K1, Brazil) were evaluated at a temperature of 25 ± 2 °C by inserting the electrode directly into the sample (Pianovski *et al.,* 2008).

Viscosity determination: viscosity determinations were obtained using a model DV-III Brookfield rotational rheometer (Stoughton, MA, USA) with cone-plate configuration and linked to a Brookfield software program

TABLE I - O/W emulsions compositions with açai glycolic extract (%, w/w)

COMPOSITIONS	P1	P2	P3	PC1	PC2	PC3	PS1	PS2	PS3	R1	R2	R3
Propylene glycol	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Dissodium EDTA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Triethanolamine (sol. 50%)]	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	---	---	---
BHT	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Acrylates/C10-30 alkyl acrylate crosspolymer	0.1	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	---	---	---
Sodium polyacrylate	---	---	---	---	---	---	---	---	---	0.5	1.0	1.5
Polyacrylamide (and) C 13-14 isoparaffin (and) Laureth-7	---	---	---	---	---	---	0.5	1.0	1.5	---	---	---
Carbomer	---	---	---	0.1	0.2	0.3	---	---	---	---	---	---
Caprylic/capric triglyceride	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Ethylhexyl stearate	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
PPG-3 benzyl ether myristate	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Ethylheyl palmitate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Bis-ethylhexyloxyphenol methoxyfeniltriazine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Benzophenone-3	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Diethylamino hydroxybenzoyl hexyl benzoate	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Ethylhexyll triazone	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Octocrylene	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2-Ethylhexyl methoxycinamate	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Phenoxyethanol (and) caprylic glycol	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Açai glycolic extract	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Disttiled water	64.1	64.0	63.9	63.8	63.7	63.6	63.4	62.9	62.4	64.3	63.8	63.3

(Rheocalc version 3.0). Rheological parameters were determined at 25 ± 2 °C using a CP 52 spindle (d = 12 mm, θ = 3.0°) and 0.5 g of each sample. Rheogram curves constructed with ascendant and descendant segments were obtained with progressively increasing (1 – 10 rpm) as well as gradually decreasing (10 – 1 rpm) rotation speeds. The measurements were made at intervals of 2 rpm, remaining at each speed of rotation for 10 s. For accelerated stability tests, the values obtained were used to calculate the flow index and hysteresis area. Three batches were prepared and measurements repeated three times for each sample (Lima *et al*., 2008).

Accelerated stability tests

The samples considered stable by preliminary tests were stored under different conditions: 4 ± 2 °C (Mabe, mod. REMB 46, Brazil); 25 ± 2 °C (ambient temperature), 37 ± 2 °C and 75 ± 5% relative humidity (RH) (Nova Etica, mod. 520-CLDTS 150, Brazil) and 45 ± 2 °C and 75 ± 5% RH (Nova Etica, mod. 520-CLDTS 150, Brazil). The samples were maintained under these conditions for 90 days. The macroscopic analyses (appearance, homogeneity and organoleptic characteristics), pH value determinations and rheological behavior (minimal apparent viscosity, rheogram curves, flow index and hysteresis area) were evaluated at different time intervals (24 h after preparation of formulations and on 30^{th}, 60^{th} and 90^{th} days) (Ferrari, Rocha-Filho, 2011).

Statistical analyses

Statistical analyses of the preliminary and accelerated stability data were performed using the analysis of normality of the sampling distribution, evaluated by parametric analysis of variance (ANOVA) as well as the Tukey test ($p<0.05$) (Ferrari, Rocha-Filho, 2011) using SPSS 13.0 for Windows.

In vivo Sun Protection Factor (SPF) determination

The O/W emulsions containing the same concentration of sunscreens with and without açai glycolic extract were evaluated.

The *in vivo* SPF was determined according to the International Sun Protection Factor Test Method – 2006 (Colipa, 2006). A standard sunscreen formulation of methodology (Colipa, 2006) containing 7% of PABA and 3% of benzophenone 3 was used as control.

The results were expressed by the mean and standard deviation of the analyses of ten volunteers.

The research protocol was approved by the Human Experimentation Committee of the University of Cuiabá, under protocol # 2011-123. Wilcoxon non parametric tests were used to determine statistical significance ($p<0.05$).

In vitro UVA Protection Factor (FP-UVA) determination

O/W sunscreen emulsions with and without açai glycolic extract were applied (1.3 mg/cm^2) to roughened polymethylmethacrylate PMMA Helioplates HD6 plates (50 x 50 x 2.5 mm, S_a = 2µm, Helioscience, France) and distributed evenly over the whole surface using a coated finger. For each formulation, three plates were prepared and measurements repeated three times for each sample. The plates were left to equilibrate in a dark place under ambient temperature for 15 min. UV transmission measurements between 290 and 400 nm were performed using a spectrophotometer equipped with an integrating sphere (UV Transmittance Analyzer UV – 1000S, Labsphere, USA). The *in vitro* PF – UVA, critical wavelength (λ_c) and SPF/PF-UFA were evaluated according to the *In vitro* method for determination of the UVA protection factor and "critical wavelength" values of sunscreen products (Colipa, 2011; Brasil, 2012).

Comparison of the formulations with and without the açai glycolic extract was performed using the Student t-test ($p<0.05$) for independent variables (Statistic Program version 9.0).

RESULTS AND DISCUSSION

Preliminary stability tests

The preliminary stability studies are used to delineate the initial phase of product development. These studies use extreme and different conditions to accelerate the potential reactions between components and demonstrate the most common instability processes of emulsions (phase separation, flocculation and creaming) (Tcholakova *et al.,* 2006). The conditions of these studies are not intended to estimate product shelf-life, but to provide the formulation screening (Lima *et al*., 2008).

In the present study, formulations were prepared containing polymeric surfactants acrylates/C 10-30 alkyl acrylate crosspolymer (P1-P3) or sodium polyacrylate (R1-R3) at different concentrations (Table I). These polymers have hydrophobic groups with similar amphoteric structures as surfactants and act by an electrosteric stabilization mechanism, which represent a

combination of electrical repulsion and steric stabilization (Lochead *et al.*, 1986; Hemker, 1990).

Macroscopically the P1 and P2 emulsions showed phase separation within 24 hours of preparation. The P3 (0.3% acrylates/C 10-30 alkyl acrylate crosspolymer) emulsion and formulations containing sodium polyacrylate (R1, R2 and R3; 0.5, 1.0 and 1.5% respectively) proved macroscopically stable. Previous studies have reported the use of polymeric surfactants in stabilizing emulsified systems due to their conformation, stabilization mechanisms, good skin compatibility and use at low concentrations (Tadros, 2004; Lima *et al.*, 2008; Pianovski *et al.*, 2008).

The influence of the two thickeners and rheology modifiers on P3 stability was investigated using polyacrylamide (and) C13-14/isoparaffin (and) Laureth-7 (PS1-PS3) and Carbomer (PC1-PC3) (Table I). All these formulations were stable on macroscopic analyses.

The emulsions (Table I) had beige color, açai glycolic extract odor and a homogenous creams consistency.

The centrifugation test was performed for the macroscopically stable emulsions. No phase separation or organoleptic characteristic changes were observed after centrifugation for any of the samples (Table II).

The addition of rheology modifiers can increase the stability of emulsions. Ferrari and Rocha-Filho (2011) demonstrated that the association between the polymeric surfactants and rheology modifier improved the stabilization of emulsion on the centrifugation test. According to Tadros (2004) the absence of rheology modifier in the formulations resulted in phase separation after centrifugation because there was an insufficient increase in the viscosity of the external phase of the vehicles to maintain the integrity of the emulsions.

The emulsions tested in this study were then subjected to thermal stress and freeze-defrost cycle (Table II).

The PC2, PS1 and R3 formulations showed statistically significant differences ($p < 0.05$) after the freeze-defrost cycle when compared with initial pH values (Table II).

Monitoring the pH value is crucial for determining the stability of emulsions. In fact, pH changes indicate the occurrence of chemical reactions. Changes in pH may

TABLE II - Results of preliminary stability tests of emulsions containing açai glycolic extract

PARAMETERS	P3	PC1	PC2	PC3	PS1	PS2	PS3	R1	R2	R3
AFTER 24h										
Centrifugation	N	N	N	N	N	N	N	N	N	N
pH value	6.84 (±0.33)	6.36 (±0.33)	6.19 (±0.21)	6.03 (±0.13)	6.93 (±0.12)	6.75 (±0.16)	6.82 (±0.08)	6.91 (±0.20)	6.82 (±0.22)	6.66 (±0.11)
Electrical conductivity (mS)	623 (±62)	594 (±40)	608 (±33)	594 (±30)	775 (±90)	977 (±25)	1,179 (±33)	929 (±81)	1,566 (±38)	1,924 (±142)
Viscosity (cP)	2,173 (±368)	3,897 (±579)	6,003 (±775)	7,844 (±1249)	4,382* (±1509)	5,389 (±917)	8,248 (±1007)	1,923 (±556)	8,678 (±419)	6,344 (±1313)
AFTER TS										
pH value	6.92 (±0.06)	6.44 (±0.32)	6.14 (±0.16)	5.85 (±0.15)	7.01 (±0.17)	6.68 (±0.11)	6.84 (±0.19)	6.84 (±0.22)	6.78 (±0.15)	6.68 (±0.18)
Electrical conductivity (mS)	605 (±35)	619 (±24)	601 (±52)	592 (±33)	801 (±48)	1,008 (±29)	1,245* (±12)	1,004* (±66)	1,634 (±42)	2,180* (±1080)
Viscosity (cP)	2,261 (±235)	3,651* (±689)	5,337 (±1953)	7,369 (±487)	3,665* (±1509)	4,796 (±708)	8,165 (±338)	2,252* (±667)	8,062 (±978)	7,026* (±918)
AFTER FDC										
pH value	6.93 (±0.12)	6.42 (±0.29)	6.09 (±0.15)*	5.78 (±0.13)	7.02 (±0.14)*	6.74 (±0.10)	6.87 (±0.07)	6.92 (±0.14)	6.81 (±0.12)	6.79 (±0.15)*
Electrical conductivity (mS)	590* (±65)	566 (±104)	645 (±22)	629 (±33)	650 (±230)	1,016 (±75)	1,224 (±94)	996* (±55)	1,623 (±123)	2,320 (±1009)
Viscosity (cP)	1,973 (±154)	3,458* (±541)	5,632 (±328)	6,956 (±390)	3,235* (±332)	4,914 (±322)	7,694 (±438)	1,863 (±594)	8,308 (±267)	6,892* (±1684)

FDC = Freeze-Defrost Cycles; TS = Thermal Stress; N= Normal. *$p < 0.05$ compared to the initial time. Data were expressed as mean ± Standard Deviation (SD). n=9.

also indicate chemical decomposition of the formulation components (Leonardi *et al.*, 2000) and promote changes in other physicochemical properties such as viscosity (Frange, Garcia, 2009). This situation most likely occurred for the formulations PS1 and R3 indicating the instability of these systems.

According to Pearce and Kinsella (1978) and Kato *et al.* (1985), electrical conductivity is often used to determine the type of emulsion and to control its stability during the storage period. The conductivity measure can be used to check the integrity of the external phase (Pianovski *et al*., 2008).

A statistically significant change in the electrical conductivity values of P3, PS3, R1 and R3 was observed (Table II). This situation may be considered a sign of the instability of the emulsion. According Masmoudi *et al.* (2005) there is not a linear relationship between the increase or decrease in conductivity and the instability, therefore other adjuvant methods should be used to evaluate the stability.

Viscosity is a parameter that determines whether a product exhibits the proper consistency and fluidity and can indicate if stability is adequate; in other words, it provides an indication of the behavior of the product over time (Anvisa, 2004).

The formulations PC1, PS1, R1 and R3, exhibited significant variations for viscosity in the preliminary stability studies (Table II).

PC1 and PS1 emulsions showed a decrease in viscosity after thermal stress and after the freeze-defrost cycle compared to the initial viscosity (Table II). Decreases in viscosity may be a sign of instability (Masmoudi *et al*., 2005). R1 and R3 emulsions showed increased viscosity after the freeze-defrost cycle and after thermal stress compared to the initial viscosity (Table II). This can occur due to evaporation of water from the formulations during storage (Leonardi *et al*., 2000).

PC3, PS2 and R2 formulations were considered stable under conditions of preliminary stability tests and were therefore evaluated by the accelerated stability tests.

Accelerated stability tests

Accelerated stability tests employ less extreme conditions compared to the previous test. The accelerated tests may be used to estimate shelf-life of the product (Baby *et al.*, 2008).

The organoleptic characteristics evaluation assesses by means of comparative analyses, the changes with respect to color, odor and appearance. These analyses indicate the similarities of the emulsions subjected to the stability tests in relation to the emulsions that have been recently formulated, independent of the conditions and storage periods (Calero *et al*., 2013). After the accelerated stability test, all of the formulations (PC3, PS2 and R2) maintained their organoleptic characteristics: beige color, açai glycolic extract odor and homogenous cream consistency.

The PS2 formulation showed significant variations in the parameters of pH and viscosity after 90 days of storage at different temperatures. Other studies (Anchisi *et al.*, 2001; Gonçalves, Maia Campos, 2009) have reported the development of stable emulsions containing this same polymer (polyacrylamide (and) C 13-14 isoparaffin (and) Laureth-7) at concentrations higher than 2%.

The pH value of the PC3 formulation remained stable throughout the duration of the study, but statistically significant changes in viscosity values were observed. According to the technical literature (Lubrizol, 2007), this polymer is more vulnerable to ions, such as the salts found in surfactants and vegetable extracts.

After application of the accelerated stability tests, only R2 remained stable. The data are summarized in Table III.

The pH of the R2 emulsion was reduced (Table III); however, the changes in these pH values were no greater than 10%, where its representative pH remained 6.2 and 6.9, values compatible with the skin. The degradation of any component of the phytocosmetic may promote a drop in pH value and promote skin irritation (Isaac *et al*., 2008). The alteration in the pH value caused no changes in organoleptic characteristics or viscosity.

No statistically significant changes in the viscosity values were evident over the duration of the experiment (90 days) indicating good stability of the samples. According to Tadros (2004) at decrease or increase in viscosity during storage indicates instability of the system. There were no significant differences in the minimal apparent viscosity among the three batches formulated during the period or among the stress conditions investigated.

The O/W emulsion developed (R2) showed non-Newtonian behavior (Figure 1) given it did not exhibit a non linear relationship between shear stress and shear rate (Tadros, 2004). In addition, the flow index values (Table II) were below 1.0 indicating pseudoplastic flow behavior (Castelli *et al.*, 2008; Mendonça *et al.*, 2009; Ferrari, Rocha-Filho, 2011), a desirable rheological property for cosmetics products (Guaratini, Gianeti, Maia Campos, 2006).

Pseudoplastic behavior is suitable for topical application products where, after shearing, the initial

TABLE III - pH value, minimal apparent viscosity, flow index and hysteresis area values of O/W emulsion (R2) during accelerated stability studies at different temperatures

Time (days)	Temperature (°C)	pH value	Minimal apparent Viscosity (cP)	Flow index	Hysteresis area (d/cm2.s)
1°	25	6.9(±0.10)	8,586 (±326)	0.35(±0.03)	298.51(±246.44)
90°	4	6.2(±0.04)*	8,933 (±676)	0.22(±0.09)	904.64(±728.41)
	25	6.3(±0.02)*	8,371 (±182)	0.26(±0.04)	330.15(±214.17)
	37	6.3(±0.03)*	8,374 (±270)	0.23(±0.08)	265.41(±177.57)
	45	6.3(±0.02)*	8,030 (±193)	0.28(±0.06)	321.08(±187.28)

*p<0.05. Data were expressed as mean ± Standard Deviation (SD). (n=9).

resistance to the emulsion flow decreases, reflected in ease of application. A decrease in viscosity shear indicates thixotropy. Thixotropy is a term used to describe an isothermal system, in which the apparent viscosity decreases under shear stress, followed by a gradual recovery when the stress is removed (Tadros, 2004; Lee, Moturi, Lee, 2009).

Thixotropic products become more fluid when subjected to an external pressure, and spread more easily in the region where they are applied, where the initial viscosity recovers when the application ceases (Tadros, 2004). The rheograms (Figure 1) showed the descending curve was below that of the ascending curve, and the hysteresis area (Table III) indicates thixotropy in the systems.

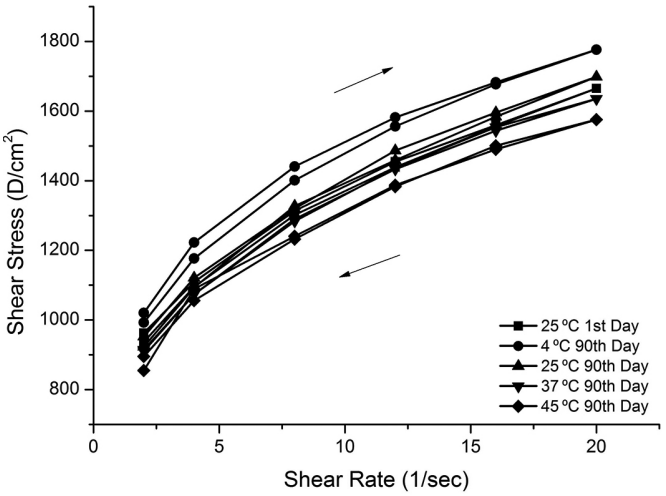

FIGURE 1 - Flow behavior for a stable O/W sunscreens emulsion (R2) 24 h and ninety days after preparation at different temperatures.

Sun Protection Factor Study (SPF)

Vegetal substances have recently been considered as potential resources for sunscreen formulations due to their UV spectrum absorption and antioxidant properties (Avila Acevedo *et al*., 2005). Determination of the antioxidant activity of plant extracts has been well described; however, the evaluation of their photoprotective capacity is usually limited by the UV spectrum characteristics and the concentration of the UV-absorbing compounds (Hupel, Poupart, Ar Gall, 2011).

Given the structural similarities between polyphenolic compounds and organic UV filters, in addition to the antioxidant activity of these bioactive compounds, they might exert photoprotective activity (Velasco *et al*., 2008a; Velasco *et al*., 2008b; Violante *et al*., 2009).

The *in vivo* SPF values of the O/W sunscreen emulsion containing 1.0% of sodium polyacrylate (R2) are summarized in Table IV.

There was no statistically significant difference in the *in vivo* SPF values obtained (p<0.05), demonstrating that the açai glycolic extract, at the concentration used, was unable to exert synergistic action when associated with UV filters. Other researchers (Dondi, Albini, Serpone, 2006; Velasco *et al*., 2008b) have reported that the association of UV filters with bioactive compounds in a complex medium, such as an emulsion, leads to a distinctive photochemical profile, different from the photochemistry of the isolated compounds.

According to Velasco *et al.* (2008b) bioactive compounds interact with UV filters and their photoprotective efficacy may be dependent on the nature of the bioactive compound and UV filter concentrations.

The SPF results obtained in our experimental study are consistent with findings reports in previous studies (Silva *et al*., 2005; Souza *et al*., 2005; Lopes *et al*., 2007; Violante *et al*., 2009; Ferrari, Rocha-Filho, 2011; Mansur *et al*., 2012). The cited studies described the difficulty in increase in the SPF value by the addition of natural active agents in the formulation containing chemical and/or

TABLE IV – The *in vivo* sun protection factor (SPF) values of the O/W sunscreen emulsion containing 1.0% of sodium polyacrylate (R2)

Formulation	SPF ($\bar{X}$)	Standard Deviation (SD)
Sunscreen emulsion with açai glycolic extract	25.3	± 4.5
Sunscreen emulsion without açai glycolic extract	22.5	±2.4
Standard Sunscreen Formulation - Control	16.7	±3.4

Data were expressed as mean ($\bar{X}$) ± Standard Deviation (SD). n=10.

physical UV filters. When the *P. incarnate* L. dry extract interacted negatively with UV filters and led to reduction if the SPF value, Velasco *et al.* (2008b) attributed this behavior to four main factors: presence of inorganic UV filter; composition of the vehicle; quantitative composition of the organic UV filter; and phytochemical composition of the extracts. These last three factors may explain why the açai glycolic extract failed to promote an the increase in the SPF value.

The antioxidant properties of açai have been previously described in the literature (Kuskoski *et al*., 2006; Kang *et al*., 2011; Gordon *et al*., 2012; Inácio *et al*., 2013). However, although incorporation of açai glycolic extracts into sunscreen emulsion did not increase SPF *in vivo*, this formulation may be effective as an antioxidant cosmetic product.

In vitro UVA Protection Factor (FP-UVA) determination

To provide protection against UVB and UVA radiation, the sunscreen products should contain not only UVB filters but also one or more UVA filters. UVA protection has been recognized as essential and has become a target for improving sunscreen efficacy (Hojerová, Medovcíková, Mikula, 2011; Sambandan, Ratner, 2011).

The *in vitro* UVA protection factor (PF-UVA) values, SPF/PF-UVA and the critical wavelength (λc) determinations are reported in Table V.

There was no significant difference ($p<0.05$) in the PF-UVA values between formulations with and without açai extract, indicating that açai glycolic extract, at the concentration used, was unable to increase the UVA protection.

Brazilian legislation (Brasil, 2012) recommends that the PF-UVA for sunscreen products should be at least 1/3 of the SPF and that SPF/PF-UVA value should be ≤ 3. Our experimental results are in accordance with the requirements of Brazilian legislation (Brasil, 2012) and European Commission Recommendations (European Commission, 2006).

According to Wang, Osterwalder, and Jung (2011), topical application of antioxidant compounds associated with UV filters may neutralize the free radicals induced by UV radiation. Wang and col. (2011) reported that a product with a high PF-UVA absorbs most of the harmful radiation in the UVA spectrum reducing the amount of free radicals generated in the skin.

Other studies have been performed with the aim of increasing UVA protection using natural products (Rancan *et al.*, 2002; Baby *et al.*, 2008; Velasco *et al.*, 2008b; Lohézic-Le Dévéhat *et al.*, 2013).

The critical wavelength value for the test product is defined as that wavelength where the area under the absorbance spectrum for the irradiated product from 290 nm to critical wavelength is 90% of the integral of absorbance spectrum from 290 nm to 400 nm (Colipa, 2011). According to Brazilian legislation (Brasil, 2012) and the European Cosmetics Association (Colipa, 2011)

TABLE V – *In vitro* UVA protection factor (PF-UVA), SPF/PF-UVA and critical wavelength (nm) determinations of the O/W sunscreen emulsion with (R2) and without açai glycolic extract

	Formulation without açai glycolic extract	Formulation with açai glycolic extract	Reference sunscreen formulation
PF-UVA	13.38 (±0.52)	14.97 (±0.44)	14.02 (±0.16)
SPF/PF-UVA	1.68 (±0.06)	1.69 (±0.05)	1.14 (±0.01)
Critical wavelength	378	378	---

Data were expressed as mean (n=3) ± Standard Deviation (SD).

the critical wavelength should be greater than or equal to 370 nm. As shown in Table V, the critical wavelength values for the two formulations were consistent with those required by Brazilian legislation and the European Cosmetics Association (Colipa, 2011).

When the sunscreen product has a PF-UVA of at least 1/3 of the SPF, an SPF/PF-UVA value ≤ 3 and a critical wavelength value ≥ 370nm, it may be labeled as providing "broad spectrum" UVA and UVB protection (Colipa, 2011). The O/W sunscreen emulsion developed showed these characteristics, so it may therefore be considered a sunscreen with UVA and UVB protection.

CONCLUSIONS

The O/W sunscreen emulsion with 1% of sodium polyacrylate (R2) remained stable after preliminary and accelerated stability tests and showed pseudoplastic and thixotropy behavior, important characteristics for a sunscreen product. When açai glycolic extract was added in the emulsion sunscreen (R2), no significant increase in the *in vivo* SPF value was observed. The sunscreen emulsion containing açai glycolic extract showed a PF-UVA = 14.97, 1.69 of the SPF/PF-UVA ratio and a critical wavelength value of 378 nm. This emulsion may therefore be considered a sunscreen with UVA and UVB protection, according to Brazilian legislation and the European Cosmetics Association. There was no significant difference in the parameters between the formulations with and without açai glycolic extract. The results obtained in this study do not rule out the use of açai glycolic extract in sunscreen formulations. As previously reported in the literature, açai has antioxidant activity, so may be used to protect the skin against damage caused by free radicals induced by UV radiation. However, studies with isolated compounds or dry extract of açai should be performed to evaluate photoprotective activity.

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