

# Multiple emulsions containing amazon oil: açai oil (*Euterpe oleracea*)

Márcio Ferrari,<sup>\*1,2,3</sup> Pedro Alves da Rocha-Filho<sup>3</sup>

<sup>1</sup>Departamento Farmacêutico, Universidade de Cuiabá, Brazil,

<sup>2</sup>Departamento de Farmácia, Universidade Federal do Rio Grande do Norte Brazil,

<sup>3</sup>Departamento de Ciências Farmacêuticas, Universidade de São Paulo, Brazil.

**Abstract:** The aim of this work was to formulate O/W/O multiple emulsions containing açai oil as a model system and to evaluate their physical stability and *in vivo* Sun Protection Factor (SPF). Multiple emulsions are complex dispersion systems, known also as, “emulsions of emulsions”. These emulsion systems, have significant potential in the cosmetic industry. *Euterpe oleracea* Mart., Arecaceae, popularly known in Brazil as “açai”, is an economically important plant. Açai oil has been used as antioxidant and as anti-inflammatory activities. The multiple emulsions were prepared using a two-step procedure. The investigated formulations were characterized and their stability over time was evaluated by preliminary and accelerated stability. O/W/O multiple emulsions containing the same concentration of sunscreens with and without açai oil were evaluated by the International Sun Protection Factor Test Method. The samples containing 70% (w/w) of primary emulsion, 5% (w/w) PEG-30-dipolyhydroxystearate, 10% (w/w) of açai oil and 5% (w/w) of sucrose polybehenate have been found to be stable. The rheological measurements revealed that the samples exhibited non-Newtonian pseudoplastic flow behavior and thixotropy. To conclude, no statistical difference could be observed on the *in vivo* SPF to both multiple systems with or without açai oil.

## Introduction

The multiple emulsions present many interesting potential applications in the cosmetic, pharmaceutical, food and agricultural industries (Tedajo et al., 2005; Bonnet et al., 2009; Elshafei et al., 2010; Lima et al., 2010). They are also known as “emulsions of emulsions” (Becher, 1965; Florence & Whitehill, 1982) since they are systems in which both types of emulsions (W/O and O/W, or O/W and W/O) exist simultaneously (Omotosho, 1990), and they can be either W/O/W or O/W/O (Erös et al., 1990).

They can encapsulate substances in different phases, protect them, modulate their release rate and even possibly combine incompatible substances within their phases (Nakhare & Vyas, 1996; Vasiljevic et al., 2006). However, despite their potential, the application of multiple emulsions has been limited by their inherent thermodynamic instability (Florence & Whitehill, 1981; 1982; Geiger et al., 1998).

The stability of the emulsions is affected by several factors such as the composition, size of the globules, viscosity, volume of phases, pH, presence of electrolytes, osmotic gradient and the properties of the

interfacial film formed (Baillet et al., 1994; Gallardo et al., 1999; Özer et al., 2000; Rajinder, 2008; Schmidts et al., 2009). The majority of studies about multiple emulsions are confined to the W/O/W type. Even though O/W/O emulsions are expected to be used in the controlled release and stabilization of lipophilic actives, research on them is limited because of the practical difficulty in preparing a stable outer W/O emulsion (Yoshida et al., 1999).

Exposure to solar radiation has a variety of harmful effects on human health. Some of these effects include the induction of photosensitization, immunosuppression, photocarcinogenesis and photoaging (Schauder & Ippen, 1997; Kullavanijaya & Lim, 2005). However, the ultraviolet (UV) dose absorbed by the skin can be reduced by limiting exposure to the sun, by wearing protective clothing and through the use of photoprotective agents such as sunscreens (van der Pols et al., 2006; Ghazi et al., 2010).

Due to the benefits of products containing natural compounds and the recent desire of the consumers for these products, the use of natural compounds that can absorb UV radiation is of great interest. Previous studies have presented evidence for

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the ability of extracts and vegetable oils to provide UV absorption or photoprotection (Katiyar & Elmets, 2001; Moquio et al., 2005; Souza et al. 2005; Ferrari et al., 2007; 2008; Velasco et al., 2008; Violante et al., 2008; Violante et al., 2009).

*Euterpe oleracea* Mart., Arecaceae, locally known in Brazil as “açai” is a slender, multistemmed, monoecious palm distributed in the Amazon estuary floodplains (Muñiz-Miret et al., 1996). Due to its high consumption and commercialization, açai had long been restricted to a regional level in Brazil; however, increased international and expanded distribution has made the açai, and various products made from açai, widely available to the general public (Pacheco-Palencia et al., 2007).

Considerable interest has been generated by the açai's high antioxidant capacity, attributed to its polyphenolic compounds (Lichtenthäler et al., 2005; Pacheco-Palencia et al., 2007; Kang et al., 2010). Anthocyanins are the predominant polyphenols and account for more than 90% of açai's total polyphenolic contents (Pacheco-Palencia et al., 2009). Other non-anthocyanin polyphenol and phenolic acids were also present (Gallori et al., 2004; Lichtenthäler et al., 2005; Pacheco-Palencia et al., 2009; Tonon et al., 2010). On the other hand, anthocyanins, which belong to one class of polyphenols, may serve as antioxidants and play a photoprotective role by directly eliminating the reactive oxygen species during photooxidative stress (Zhang et al., 2010).

The anti-inflammatory activity caused by the inhibitory effects on nitric oxide (NO) production by activated macrophage cell was reported by Matheus et al., (2006). The oral contrast agent used in magnetic resonance imaging and the vasodilator effect have been reported (Córdova-Fraga et al., 2004; Rocha et al., 2007). Ribeiro et al. (2010) also observed that açai pulp was not genotoxic in mouse bone marrow or liver and kidney cells and plays a role in inhibiting the genotoxicity induced by the antitumoral agent DXR.

The aim of this work was to formulate O/W/O multiple emulsions containing açai oil as a model system and to evaluate their physical stability and *in vivo* Sun Protection Factor (SPF).

## Materials and Methods

### Materials

Acrylates/C10-30 alkyl acrylate crosspolymer and carbomer (BFGoodrich, Dinaco SA, São Paulo, Brazil), caprylic/capric triglyceride, oleyl alcohol, sucrose polybehenate, and ethylhexyl stearate were gifts from Croda do Brasil Ltda (Campinas, Brazil), BHT, EDTA, and triethanolamine were purchased from Henrifarma Ltda (São Paulo, Brazil), octocrylene, 2-ethylhexyl salicylate,

2-ethylhexyl 4- methoxycinnamate and phenoxyethanol and caprylyl glycol were gifts from ISP Corp. (São Paulo, Brazil), bis-ethylhexyloxyphenol methoxyphenyl triazine were gifts from Ciba Specialty Chemicals Inc. (São Paulo, Brazil), mineral oil and propylene glycol were acquired from Volp Ltda (São Paulo, Brazil), PEG-30 dipolyhydroxystearate and *Euterpe oleracea* fruit oil were gifts from Beraca Ingredients Co. (São Paulo, Brazil), and distilled water.

### Method of preparation and formulations

Multiple emulsions were prepared using a two-step procedure (Florence & Whitehill, 1982). In the first step, the O/W emulsion (F1) was prepared by heating the oil phase to 50±2 °C and then adding it to the aqueous phase (Table 1), followed by agitation for 15 min at 600 rpm (mechanic mixer Fisaton Mod.713D). An O/W emulsion (F2) without açai oil was also prepared (Table 1). In the second step, the O/W emulsion (primary emulsion) was added into the oil phase, heated to 70±2 °C (Table 2), while the system was stirred at 500 rpm. After the complete introduction of the primary emulsion, stirring was maintained for 15 min.

### Evaluation techniques

The appearance, homogeneity and organoleptic characteristics were evaluated by macroscopic analyses over a course of ninety days (Ferrari et al., 2008).

Microscopic analyses (Olympus BX50, Olympus Optical Co., Ltd, Tokyo, Japan) of investigated samples were conducted to check information about the properties and multiple character of the multiple emulsions after carefully diluting samples with liquid paraffin (Ferrari et al., 2008).

### Stability studies

The physical stability of the multiple emulsions was evaluated by preliminary and accelerated stability tests (Lima et al., 2008; Lima et al., 2010). The centrifugation and determination of percent phase separation were used to investigate the preliminary stability.

The centrifugation test (Fanen, mod. Excelsa Baby II, São Paulo, Brazil) was performed on freshly prepared and 24 h old multiple systems at 3000 rpm for 30 min. The percent phase separation was calculated using the equation according to Nakhare & Vyas, (1996). These tests were carried out at 25 °C.

To evaluate the accelerated stability, the samples were stored at different conditions: 4±2 °C, 25±2 °C and 37±2 °C and 45±2 °C either 75±5% relative humidity (RH). All samples were evaluated using

macroscopic and microscopic analyses and rheological behavior 24 h after preparation as well as after thirty, sixty and ninety days of storage (Lima et al., 2008).

Physical stability was assessed through rheological determinations that were obtained using a model DV-III Brookfield rotational rheometer (Stoughton, MA, USA) with a cone-plate configuration and connected to a Brookfield software program (Rheocalc version v3.0). Rheological parameters were determined at  $25 \pm 2$  °C using a CP 52 spindle ( $d=12$  mm,  $\theta=3.0^\circ$ ) and 0.5 g of each sample. Rheogram curves constructed with ascendant and descendant segments were obtained with rotation speeds increasing progressively (1-50 rpm) as well as gradually decreasing (50-1 rpm). The measurements were made in intervals of 10 rpm, remaining at each speed of rotation for for 10 s. With the obtained values from our results the consistency index, flow index and hysteresis area were calculated. Three batches were prepared, and the results were obtained with three evaluations (Lima, et al., 2008).

Statistical analyses of accelerated stability data were performed using the univariate analysis of variance and the post hoc test was examined by using Tamhane's test ( $p < 0.05$ ) (Lima et al., 2008).

#### In vivo sun protection factor determination

O/W and O/W/O emulsions containing the same concentration of sunscreens with or without açai oil were evaluated. The *in vivo* SPF was determined according to the International Sun Protection Factor Test Method (Colipa et al., 2006). The CTFA/JCIA standard formulation was used to standardize this procedure. The protocol of research was approved by the Human Experimentation Committee from the University of Cuiabá, under the protocol #0307-171. Wilcoxon no parametric tests were used to determine statistical significance ( $p < 0.05$ ).

## Results and Discussion

#### Characteristics of multiple emulsions

A stable primary emulsion is essential for the overall stability of multiple emulsions (Su et al., 2006). The stable O/W primary emulsion, standardized by Ferrari (unpublished results) (Table 1) was used to formulate the O/W/O multiple emulsions, containing in the external oil phase: lipophilic surfactant, açai oil, mineral oil, sunscreens and the additive for their stabilization (Table 2).

Macroscopically, the O/W/O multiple emulsions showed white and homogenous creams.

When diluted with liquid paraffin, homogenous mixtures were obtained and it was observed that the

external phase was oily. Microscopic analysis was performed to show the multiplicity of multiple emulsions. The multiplicity was also checked microscopically during the different steps of experiments.

**Table 1.** O/W primary emulsions composition.

Components	Formulation 1 (F1) (g%)	Formulation 2 (F2) (g%)
Propylene glycol	3.00	3.00
Dissodium EDTA	0.10	0.10
Triethanolamine (sol. 50%)	0.60	0.60
BHT	0.10	0.10
<i>Euterpe oleracea</i> fruit oil	10.00	----
Octocrylene	6.00	6.00
2-Ethylhexyl salicylate	5.00	5.00
2-Ethylhexyl 4- methoxycinnamate	7.50	7.50
Bis-Ethylhexyloxyphenol methoxyphenyl triazine	5.00	5.00
Caprylic/capric triglyceride	3.00	3.00
Oleyl alcohol	3.00	3.00
Ethylhexyl stearate	3.00	3.00
Acrylates/C10-30 alkyl acrylate crosspolymer	0.30	0.30
Carbomer	0.20	0.20
Phenoxyethanol and caprylyl glycol	1.00	1.00
Distilled water	52.20	62.20

**Table 2.** O/W/O multiple emulsions compositions.

Components	Formulation 3 (F3) (g%)	Formulation 4 (F4) (g%)
O/W Primary emulsion	70.00	70.00
BHT	0.03	0.03
<i>Euterpe oleracea</i> fruit oil	3.00	----
Octocrylene	1.80	1.80
2-Ethylhexyl salicylate	1.50	1.50
2-Ethylhexyl 4- methoxycinnamate	2.25	2.25
Bis-Ethylhexyloxyphenol methoxyphenyl triazine	1.50	1.50
Caprylic/ capric triglyceride	5.00	5.00
PEG-30 dipolyhydroxystearate	5.00	5.00
Sucrose polybehenate	5.00	5.00
Mineral oil	4.92	7.92

#### Stability tests of multiple emulsions

The samples did not show any changes in aspect and homogeneity when stored over a period of ninety days.

According to Nakhare & Vyas (1996), this formulation showed no significant percent phase separation (2.8%). Centrifugation can be used to predict the stability of the multiple emulsions (Özer et al., 2000)

because the centrifugation accelerates the effects of gravity. When the freshly prepared and 24 h old multiple emulsions were centrifuged, no phase separation was detected.

There are different factors affecting the stability of multiple emulsions, including the concentration of the primary emulsion as well as the concentration and type of emulsifier.

The increase in the concentration of the primary emulsion increases the viscosity and decreases the droplet size. This effect can lead to the increase of stability (Baillet et al., 1994; Su et al., 2006). The O/W/O multiple emulsions were prepared with a 70% (w/w) concentration of the primary emulsion.

The use of polymeric surfactants in multiple emulsions has been superior to monomeric emulsifiers because the monomeric types tend to migrate from the inner interface and from the outer interface and/or solubilize in one of the phases (Magdassi et al., 1985; Benichout et al., 2007; Vasiljevic et al., 2005; Vasiljevic et al., 2006). The results enable us to affirm that, for the preparation of physically stable multiple emulsions, a 5% (w/w) concentration of polymeric surfactant was sufficient.

Based on Stokes' Law, by increasing the viscosity of the external phase, we can achieve improved emulsion stability (Klein, 2001). By adding 5% (w/w) of sucrose polybehenate, we were able to increase the viscosity of the external phase of the multiple emulsions and improve the stability of the emulsion.

Rheological measurements of the formulations play an important role in the physical stability (Tadros, 1994; Rajinder, 2007; Rajinder, 2008). It is also essential to carry out rheological measurements as a function of temperature to obtain information on the stability and consistency of the formulations (Tadros, 2004).

The values of minimal apparent viscosities, flow index and hysteresis area values determined for formulations 24 h (first day) and ninety days after preparation at different temperatures are given in Table 3.

**Table 3.** Minimal apparent viscosity, flow index and hysteresis area values of O/W/O multiple emulsions (F3) during accelerated stability at different temperatures.

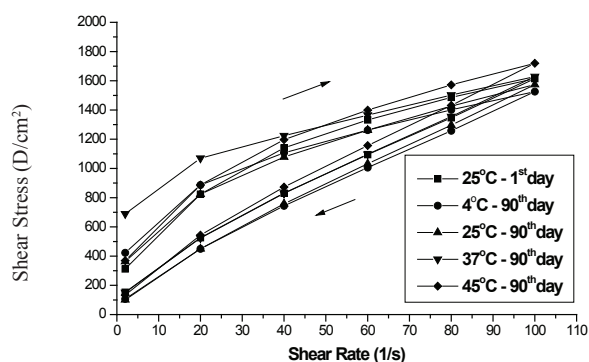
Time (days)	Temperature (°C)	Minimal apparent viscosity (cP)*	Flow index	Hysteresis area (d/cm <sup>2</sup> .s)
1	25	1606.0±144.1	0.63±0.05	21776.7±3909.9
	4	1537.1±78.7	0.61±0.01	24199.3±2132.5
90	25	1587.5±89.0	0.64±0.02	26488.2±4302.4
	37	1639.1±35.6	0.63±0.01	29610.0±1375.1
	45	1711.9±106.1	0.62±0.03	23696.3±1896.3

Data were expressed as mean±S.D. (n=9). \**p*<0.05.

Constant apparent viscosities values during aging indicate good stability of this sample. It is known that the apparent viscosity and consistency index normally decreases or increases during storage, indicating instability (Tadros, 2004) however, in this case no was indicated because there was no significant decrease or increase observed in apparent viscosities when compared with the first day (24 h). However, the decrease of this index could be explained by the breaking of multiple droplets and/or the increase of the globules size (Tadros, 1994; Nakare & Vyas, 1996). There were no significant differences in the apparent viscosity among the three batches formulated during the period and within the stress conditions investigated (Table 3).

The flow index (Table 3) was below 1.0 in all formulations indicating non-Newtonian pseudoplastic flow behavior, which is a desirable rheological property in these formulations (Guaratini et al., 2006). In this case, the viscosity decreases with the increase of the shear rate (Tadros, 2004). When the globules are aggregated, the reaction of the aggregates to shear can result in a shear-thinning (pseudoplastic) flow. At low shear rates, the aggregates may be deformed but remain intact. As the shear rate is increased, the aggregates may be broken down to individual globules, decreasing friction and therefore viscosity (Yazan et al., 1995).

The rheograms (Figure 1) showed that the descending curve is below that of the ascending curve, indicating thixotropy in the systems (Tadros, 2004). Thixotropic behavior means a reduction in structural strength during the shear load phase, and the more or less rapid, but complete structural regeneration, during the subsequent rest phase (Tadros, 2004).



**Figure 1.** Flow behavior for a stable multiple emulsions 24 h and ninety days after preparation at different temperatures.

#### In vivo SPF determination

From the statistical analysis, it can be observed that the *in vivo* SPF values were not statistically significant different when both O/W/O multiple

emulsions with or without açai oil were compared (Table 4).

**Table 4.** *In vivo* sun protection factor (SPF) values determination of formulations.

Formulations	<i>In vivo</i> SPF	
	With açai oil	Without açai oil
O/W/O multiple emulsions	31.80±6.10	33.00±4.40
CTFA/JCIA standard formulation		17.30±2.60

Data were expressed as mean ± S.D. (n=9).

The increase of the *in vivo* SPF value was not observed when açai oil was incorporated into the emulsions containing sunscreens. In accordance with Acevedo et al. (2005) probably due to low concentration of the photoprotective compounds in the oil. This association did not show synergism because it did not lead to higher SPF values (Ramos et al., 1996; Souza et al., 2005; Ferrari et al., 2007; Ferrari et al., 2008).

These results support the hypothesis that the açai oil does not have photoprotection activity.

## Conclusion

The O/W/O multiple emulsions been found to be stables, but no difference could be observed in the *in vivo* SPF to both systems with and without açai oil. These oil-in-water-in-oil multiple emulsions may find potential applications as a system to entrap sunscreens and an interesting vehicle for topical uses. The açai oil cannot be used as a natural sunscreen because it does not absorb the UV radiation, and it does not increase the SPF value. However, it can be used in cosmetic products because of its antioxidant, anti-ageing and anti-inflammatory activities.

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**\*Correspondence**

Márcio Ferrari  
Universidade Federal do Rio Grande do Norte  
Rua Gustavo Cordeiro de Farias, s/n. Petrópolis. 59012-570  
Natal-RN, Brazil  
ferrarimarcio@ufrnet.br  
Tel.: +55 84 33429812  
Fax: +55 84 32084798