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DEVELOPMENT AND EVALUATION OF LIQUID CRYSTAL O/W EMULSION SYSTEMS CONTAINING PEQUI OIL (*CARYOCAR BRASILIENSE* CAMB.) AND BIOPEPTIDES

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ABSTRACT

Pequi (*Caryocar brasiliense*) is an oleaginous fruit containing high amounts of fatty acids. It also contains carotenoids and other phenolic compounds, which possess anti-oxidant and anti-inflammatory actions. Emulsions containing liquid crystals are stable systems which interact efficiently with the skin due to their biomimetic properties. Nowadays, biomimetic peptides have been widely used in the cosmetic industry as they act via different mechanisms in order to stimulate fiber renewal. This work aimed to develop and evaluate the physicochemical stability of four liquid crystal O/W emulsion systems containing pequi oil and biopeptides. The obtained results evidenced the formulations had a microstructural organization with formation of liquid-crystal phases which remained stable during stability assays at several heat and time variations. Formulations named A, B, C and D had the same organoleptic characteristics. Formulations developed with [Cetearyl Alcohol (and) Glyceryl Stearate (and) PEG-40 Stearate (and) Ceteareth-20] had a more homogeneous internal structure due to addition (formulation D) or not (formulation B) of active compounds pequi oil or biopeptides.

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INTRODUCTION

The use of vegetal oils in the cosmetic industry is common practice mostly due to their content of saturated/unsaturated fatty acids. Vegetal oils act as humectants and emollients and interact very favorably with skin tissue. They promote tissue repair, improve the absorption of other active compounds and protect the skin (Todorova and Tamburic, 2016; Boock, 2007). Pequi (*Caryocar brasiliense* Camb.) is a fruit originated from cerrado ecosystems, one of the most diversified Brazilian biomes. All parts of the plant possess some sort of activity, the fruit and the almond oils being the most explored by the industry. Both possess analogous properties and similar chemical compositions, differing slightly in the amount of fatty acids and in the content of certain vitamins and secondary metabolites. Such oils are commonly used in cosmetics and food products (Escobar *et al*., 2016; Pianovski *et al*., 2008). Biopeptides are biomolecules composed of short sequences of aminoacids, which have similar structure to peptides found in human physiology and thus are compatible with skin tissue (Draelos, 2015). During development of emulsions, technologies used and choice of raw materials are vital for development of high performing products, either due to proper application on skin tissue or due to their final stability (Magalhães, 2010).

Among emulsions used in cosmetic industry, liquid crystal emulsions, due to their biomimetic properties, are good emollients and interact well with skin tissues (Todorova and Tamburic, 2016; Zhang and Liu 2013). Thus, for treatment of skin disfunctions, such as stretch marks, the use of this kind of emulsion can improve the action of additives, as it remains longer on the tissue and keeps it hydrated for longer. Thus, the aim of this work was to develop and evaluate the physicochemical stability of four liquid crystal oil in water (O/W) emulsions containing pequi oil and biopeptides for application on stretch marks in order to attenuate such skin dysfunction. Cosmetic bases developed were **A** - Cetearyl Alcohol (and) Cetyl Palmitate (and) Sorbitan Palmitate (and) Sorbitan Oleate associated with Cetearyl Olivate (and) Sorbitan Olivate and **B** - Cetearyl Alcohol (and) Glyceryl Stearate (and) PEG-40 Stearate (and) Ceteareth-20.

MATERIAL AND METHODS

Development of emulsion systems: The four liquid crystal O/W emulsion systems were developed using **(A)** - Cetearyl Alcohol (and) Cetyl Palmitate (and) Sorbitan Palmitate (and) Sorbitan Oleate in association with Cetearyl Olivate (and) Sorbitan Olivate with no additives; **(C)** – Cetearyl Alcohol (and) Cetyl Palmitate (and)

Sorbitan Palmitate (and) Sorbitan Oleate in association with Cetearyl Olivate (and) Sorbitan Olivate with addition of 10.0% (w/w) of *Caryocar brasiliense oil* + 2.0% (w/w) of Biopeptide a + 2.0% (w/w) of Biopeptide b; (B) - Cetearyl Alcohol (and) Glyceryl Stearate (and) PEG-40 Stearate (and) Ceteareth-20 with no additives; (D) - Cetearyl Alcohol (and) Glyceryl Stearate (and) PEG-40 Stearate (and) Ceteareth-20) with addition of 10.0% (w/w) of *Caryocar brasiliense oil* + 2.0% (w/w) of Biopeptide a + 2.0% (w/w) of Biopeptide b. Biopeptides a and b are shown in Table 1. The following parameters were considered for further assessment of the emulsions systems: mixing speed, helix type and whether there was or not inversion of phases.

Preparing of emulsions: The emulsions were prepared using different compounds/excipients grouped in three different "steps". Compounds from Step 1 were heated in an electric oven (Layr, Brazil) at 86-87 °C; then, compounds from Step 2 were heated up to 85 °C. Next, compounds from Step 2 were poured over the mixture obtained in Step 1. The resulting mixture was agitated in a microprocessor high-torque mechanical stirrer Q250M (700 rpm) for 15 minutes and then heated for 5 minutes. After the 15 minutes period, the mixture was further stirred (800 rpm) for 1 hour. When the system reached 40-45 °C, compounds from Step 3 were added. This procedure was carried out for all formulations developed, A, B, C and D (Table 1).

Stability studies of the emulsion systems

Preliminary stability test

Centrifuge test: A total of 5.0 g of formulation were placed in conical plastic tubes, which were then centrifuged at 3000 rpm at room temperature (25.0 ± 2.0 °C) for 30 minutes, using a Q222T centrifuge (Quimis) (Agência Nacional de Vigilância Sanitária, 2004).

Thermal stress: The formulations were placed in glass tubes so that most of the formulation in the tubes had contact with external water in a water bath Q334M (Quimis). The samples were kept under 40 ± 2°C, 50 ± 2°C and 60 ± 2°C, for 30 minutes for each temperature tested. The samples were assessed for surface dryness, separation of phases or precipitation of any compounds after each heating cycle (Agência Nacional de Vigilância Sanitária, 2004).

Normal Stability Test (NST): This assay aims to generate data regarding product stability, shelf-life and compatibility between formulation and packaging materials. Improving formulation stability is essential for development of products either at laboratorial or industrial scales. The conditions tested in this assay are less severe in comparison to those evaluated in preliminary stability tests, which are preditive for assessing product shelf-life (Agência Nacional de Vigilância Sanitária, 2004). After the formulations had passed preliminary stability tests, normal stability tests were carried out. For this assay, 10 g of samples were placed in 15 g white propilene tubes, so there would be garanteed head-space available. The samples were prepared in duplicates and kept either in incubator (40 ± 2°C), fridge (5 ± 2°C), freezer (–5 ± 2°C) or at room temperature (25 ± 2°C) for a period of 90 days. During these periods, the samples were assessed at the starting time (T_0) and at subsequent days T_{15},Tt_{30}, T_{60} and $T_{90.}$ During these periods, pH and organoleptic properties of the samples were verified; samples were also assessed under polarized light microscopy (Agência Nacional de Vigilância Sanitária, 2004; Baby *et al*., 2004). Formulation samples stored at room temperature protected from light and moisture (25.0 ± 2.0 °C) were considered as standard samples, as their organoleptic and physico-chemical properties should suffer negligible or at the very least only minor changes (Draelos, 2015). Experimental conditions and pre-established days of analysis were as follows:

- **t0 (day 0)**—24 h after preparation of the four formulations at room temperature (25.0 ± 2.0 °C);
- **t15, t30, t60 and t90** (days 15, 30, 60 and 90)—set amount of days and trialed under conditions of: (a) 45.0 ± 0.5 °C and 75% relative humidity (RH); (b) 25.0 ± 2.0 °C under indirect sunlight and influence of UV radiation; (c) and 5.0 ± 0.5 °C and (d) – 5.0 ± 0.5 °C.

Variations of parameters considered acceptable for approval of formulation samples were as follows (Draelos, 2015):

- **Appearance, color and odor:** normal (N) when stored under direct or indirect sunlight at room temperature. Slight modifications (SM) were considerable acceptable for formulations stored at higher temperatures;
- **pH value:** variability range up to 0.3 pH units;
- **Polarized light microscopy:** analysis of formation of liquid-crystal structures.

Physicochemical properties of the formulations

Determination of pH

The average pH values of the samples were determined via direct potenciometry using a digital pHmeter Q400RS (Quimis). For pH assessment, 10% w/v emulsions were prepared in three separate replicates using freshly distilled water, which were then homogenized and had their pH evaluated at room temperature (25.0 ± 2.0 °C) (Agência Nacional de Vigilância Sanitária, 2004).

Assessment of liquid-crystal formation: Samples were spread over glass slides, mounted under coverslips and observed in a light microscope (BX41TF – Olympus) (Zhang and Liu, 2013). This assay was carried out at days T_0, $T_{7,}$ T_{15}, T_{30}, T_{60} e T_{90} for all formulations that remained stable. Samples were observed using a 100x lens.

Statistical Analysis: For statistical analyses, the following variables were considered: 5 time periods (T_0, T_{15}, T_{30}, T_{60} e T_{90}), 4 different formulations (A, B, C and D) and 4 different tested conditions (room temperature, fridge, freezer and incubator). Data were analyzed using non-parametric tests with the aid of Statistica® 7.0 software (Sat SoftTM, Tulsa, Okla, USA). Variances were assessed for homogeneity using the Harley test (α=0.05) (Granato *et al*., 2010). These analyses were performed by comparing all data placed in the same row in the table considering a formulation sample and the conditions to which each sample was subjected during the 90-day period of analysis.

RESULTS AND DISCUSSION

Development of formulations and preliminary stability test: All formulations were developed using the same methodology regarding temperature and time/speed of stirring. In order for liquid crystals to be formed and to assess how the hydrophilic phase of the emulsifier would interact with the aqueous phase, the emulsion systems were subjected to temperature variations ranging from 80 to 87 °C. It was verified that the formulations became stable due to proper interaction between molecules when temperature reached 80 °C for 5 minutes (Quirino-Barreda *et al*., 2017). Speed also influenced uniformity of the formulations, as rotations between 900 and 2000 rpm allowed aeration of the formulations and impaired formation of liquid crystals. At rotations below 800 rpm, liquid crystal structures became properly organized and there was less bubbling (Quirino-Barreda *et al*., 2017). A naval helix was used instead of an anchor helix, as it sheared and dispersed the formulation homogenously. It was also verified whether pouring the compounds from Step 1 on Step 2 or vice-versa would be better, and in either case it there were no alterations on the formation of liquid crystal structures. The use of emulsifiers at low concentrations made the formulations not stable enough; therefore, emulsifiers were used at the highest concentration recommended by manufacturers. Processing time for standardization of the amount of emulsifier was of 1 hour.

Table 1. Composition of the four liquid crystal O/W emulsions systems without (A and B) or with active compounds added (C and D) (pequi oil and biopeptides)

	Percentage of components in each formulation (% w/w)			
Components	A	B	C	D
Step 1				
Distilled water qs. To	89.60	89.10	73.30	72.80
Dissodium PCA	_	_	2.00	2.00
Xanthan gum	0.20	0.20	0.20	0.20
Glicerin	2.00	2.00	2.00	2.00
Methylparaben	0.15	0.15	0.15	0.15
Dissodium EDTA	0.20	0.20	0.20	0.20
Step 2				
Cetearyl Olivate and Sorbitan Olivate	4.00	_	4.00	_
Cetearyl Alcohol and Cetyl Palmitate and Sorbitan Palmitate and Sorbitan Oleate	3.50	_	3.50	_
Cetearyl Alcohol and Glyceryl Stearate and PEG-40 Stearate and Ceteareth-20	_	8.00	_	8.00
Pequi Oil	_	_	10.00	10.00
Propylparaben	0.15	0.15	0.15	0.15
BHT	_	_	0.20	0.20
Step 3				
Butylene Glycol (and) Aqua (and) Cetyl Hydroxyethylcellulose (and) Rutin (and) Palmitoyl Tripeptide-1 (and) Palmitoyl Tetrapeptide-7 (and) Phaseolus Lunatus (Green Bean) Seed Extract (Biopeptide a)	_	_	2.00	2.00
Betaine (and) Sodium PCA (and) Sorbitol (and) Serine (and) Glycine (and) Glutamic Acid (and) Alanine (and) Lysine (and) Arginine (and) Threonine (and) Proline (and) Methylparaben (and) Propylparaben (Biopeptide b)	_	_	2.00	2.00
Water, Olea Europaea Leaf Extract	0.20	0.20	0.20	0.20
Neutralizing agent qs. To	pH 6.0-6.3			

Legend: (A) - Cetearyl Alcohol (and) Cetyl Palmitate (and) Sorbitan Palmitate (and) Sorbitan Oleate in association with Cetearyl Olivate (and) Sorbitan Olivate with no additives; (B) - Cetearyl Alcohol (and) Glyceryl Stearate (and) PEG-40 Stearate (and) Ceteareth-20 with no additives; (C) – Cetearyl Alcohol (and) Cetyl Palmitate (and) Sorbitan Palmitate (and) Sorbitan Oleate in association with Cetearyl Olivate (and) Sorbitan Olivate with addition of 10.0% (w/w) *Caryocar brasiliense oil* + 2.0% (w/w) of Biopeptide a + 2.0% (w/w) of Biopeptide b; (D) - Cetearyl Alcohol (and) Glyceryl Stearate (and) PEG-40 Stearate (and) Ceteareth-20) with addition of 10.0% (w/w) *Caryocar brasiliense oil* + 2.0% (w/w) of Biopeptide a + 2.0% (w/w) of Biopeptide b.

Table 2. Value of pH of the formulations developed without active (A and B) and with pequi oil and biopeptides (C and D) during the normal stability test

Time (days)		**T_0**	**T_{15}**	**T_{30}**	**T_{60}**	**T_{90}**
Formulation	**Condition**	**pH (dimension less ± DP)**				
Formulation C	RT	5.94[a]	6.07 ± 0.2[a]	5.96 ± 0.2[a]	6.06± 0.2[a]	5.93± 0.2[a]
	E		6.00 ± 0.2[a]	5.84 ± 0.2[a]	6.02± 0.2[a]	5.76± 0.2[a]
	G		6.02 ± 0.2[a]	6.00 ± 0.2[a]	6.06± 0.2[a]	5.68± 0.2[a]
	F		5.96 ± 0.2[a]	5.90 ± 0.2[a]	6.06± 0.2[a]	5.65± 0.2[a]
Formulation A	RT	5.94[a]	5.95 ± 0.2[a]	5.93 ± 0.2[a]	6.08± 0.2[a]	6.02± 0.2[a]
	E		6.01 ± 0.2[a]	5.90 ± 0.2[a]	6.06± 0.2[a]	6.02± 0.2[a]
	G		6.08 ± 0.2[a]	5.93 ± 0.2[a]	5.88± 0.2[a]	5.99± 0.2[a]
	F		5.98 ± 0.2[a]	5.98 ± 0.2[a]	6.06± 0.2[a]	5.93± 0.2[a]
Formulation D	RT	5.81[a]	5.70 ± 0.2[a]	5.75 ± 0.2[a]	5.78± 0.2[a]	5.61± 0.2[a]
	E		5.74± 0.2[a]	5.64 ± 0.2[a]	5.83± 0.2[a]	5.45± 0.2[a]
	G		5.71 ± 0.2[a]	5.60 ± 0.2[a]	5.80± 0.2[a]	5.90± 0.2[a]
	F		5.71 ± 0.2[a]	5.75 ± 0.2[a]	6.06± 0.2[a]	5.92± 0.2[a]
Formulation B	RT	6.06[a]	5.94± 0.2[a]	5.95 ± 0.2[a]	5.97± 0.2[a]	6.02± 0.2[a]
	E		6.02 ± 0.2[a]	5.78 ± 0.2[a]	6.01± 0.2[a]	5.67± 0.2[a]
	G		6.08 ± 0.2[a]	5.95 ± 0.2[a]	5.96± 0.2[a]	6.03± 0.2[a]
	F		6.02 ± 0.2[a]	5.96 ± 0.2[a]	6.06± 0.2[a]	6.00± 0.2[a]
P (ANOVA) *			< 0.05	< 0.05	< 0.05	< 0.05

Legend: RT: 25.0 ± 2.0º C under indirect lighting; G: 5.0 ± 0.5º C; E: 45.0 ± 0.5º C and 75% relative humidity; F: -5.0 ± 0.5º C. Mean ± standard deviation (n = 2).

The preliminary test allows for selection of samples of better integrity. Thermal stress accelerates chemical reactions in a product through extreme temperature variation, which allows for evaluation of physicochemical properties. The centrifuge test places the sample under stress by simulating an increase of gravity which displaces particles and detects possible instability indicators. After stressing the sample, it is verified which conditions lead to precipitation, separation of phases, *caking*, coalescence, among other modifications. Should any of these modifications be detected, the formulation must be rejected and redone from scratch (Agência Nacional de Vigilância Sanitária, 2004). It was verified that all samples remained stable at 40 °C when placed in water bath, but it was perceptible that the color of bases C and D was darkened. At temperatures higher than 50 °C, a slight color alteration was detected in the samples that contained oil (C and D); the samples became darker, probably due to oxidation. At 60 °C, all samples had noticeable changes.

Foam was formed at the surface of both base A and base B; the color of the bases became lighter, even transparent in the case of base A. In base C there was separation of phases, regardless of addition of biopeptides. In base D oil droplets could be observed. During adequation of formulations, it was verified that at 50 °C, emulsion C suffered separation of phases when not containing gum, but such did not happen when gum was added. Separation of phases happened due to an increase in kinetic energy, which promotes collision between molecules, leading to coalescence and flocculation (Andrade, 2008). Temperature probably also induced the transition of phases of the liquid crystal to isotropic liquids (Quirino-Barreda *et al*., 2017; Neto and Salinas, 2005). The centrifuge test determined all samples remained stable after the gravity stress. Base C had bubbles on its surface, which happened probably due to aeration of the samples.

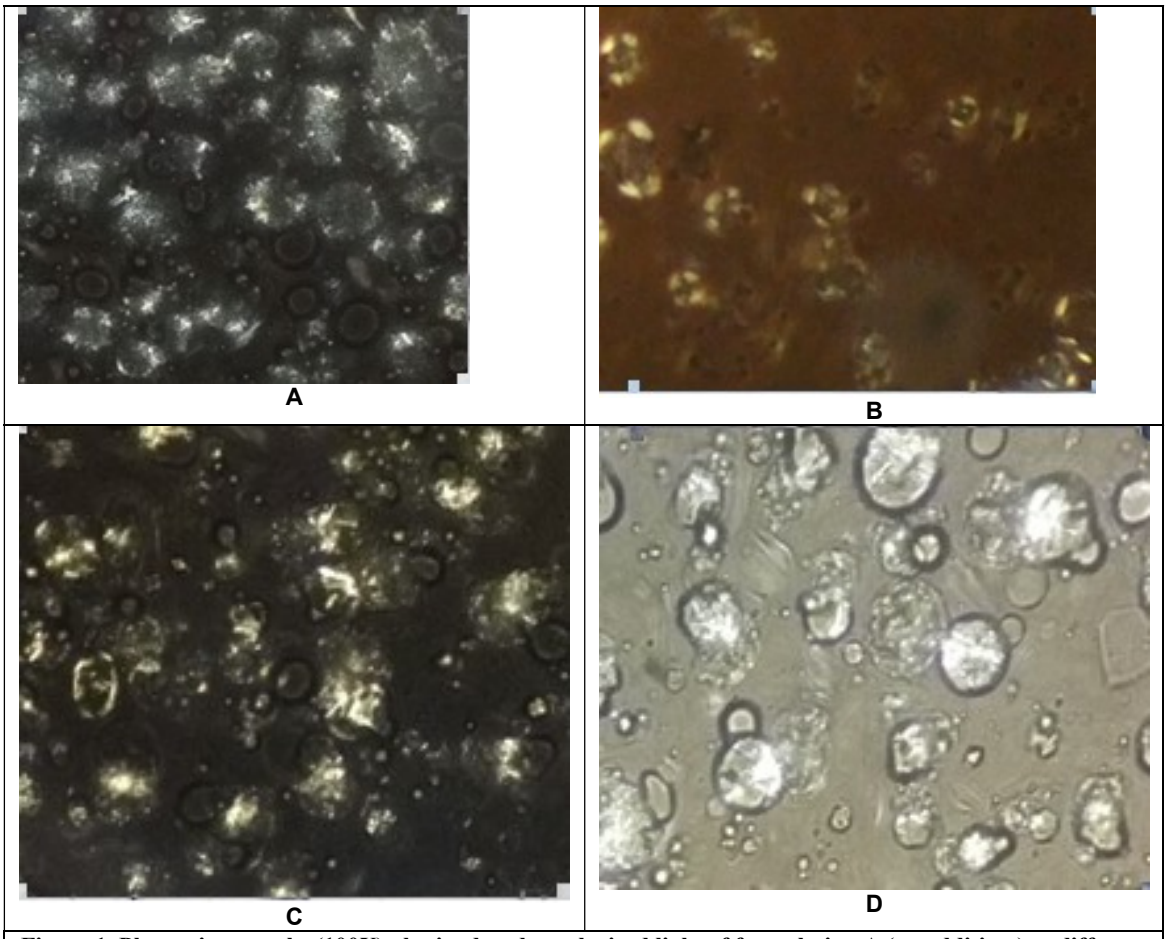

Figure 1. Photomicrographs (100X) obtained under polarized light of formulation A (no additives) at different storage conditions: A) t7; B) t30; C) t60; D) t90

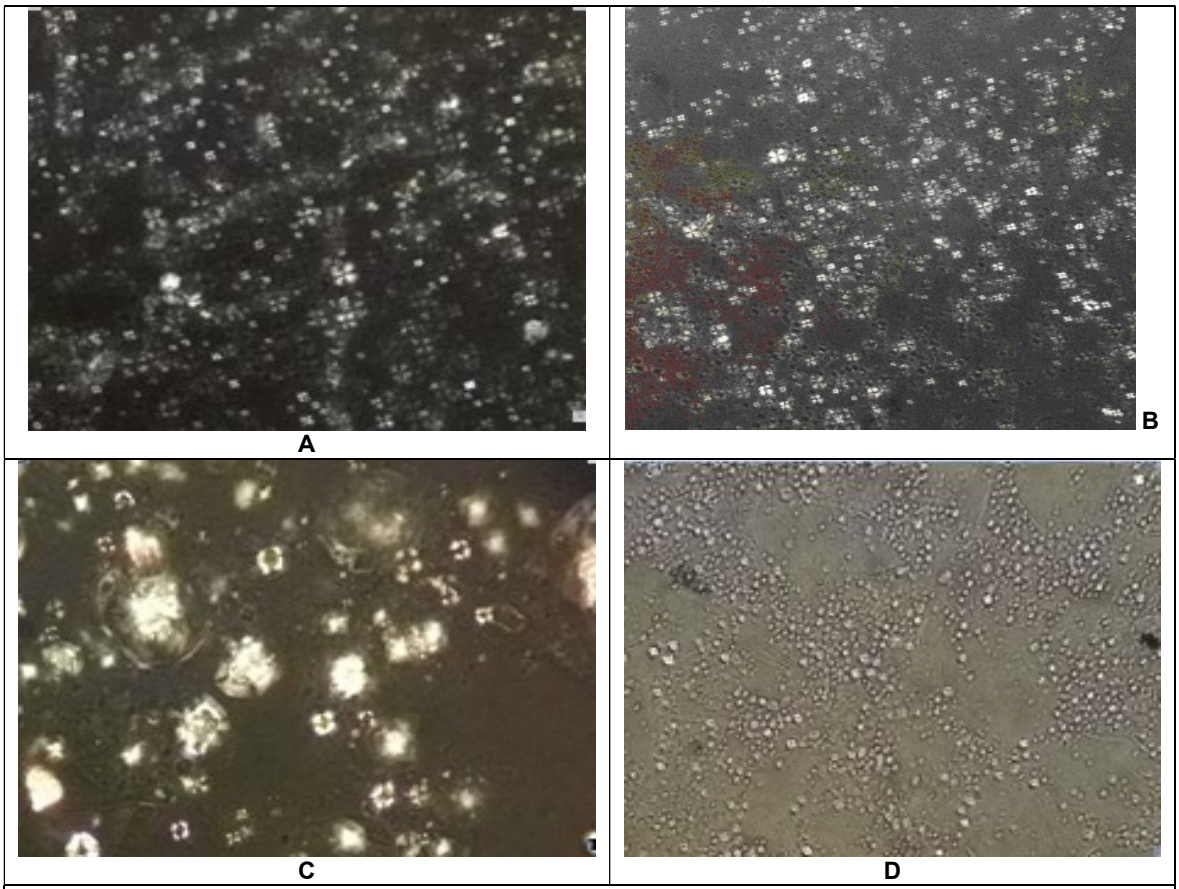

Figura 2. Photomicrographs (100X) obtained under polarized light of formulation B (no additives) at different storage conditions: A) t7; B) t30; C) t60; D) t90

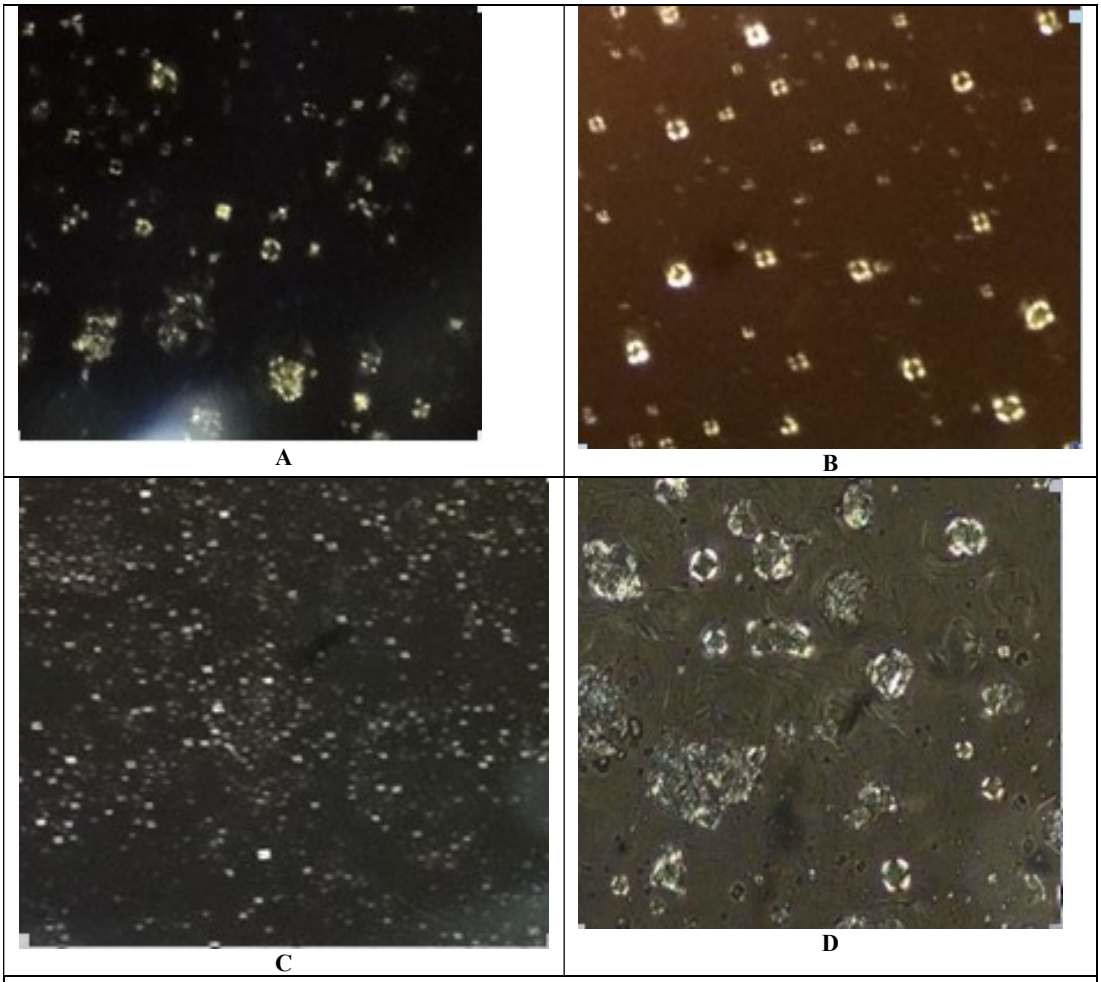

Figura 3. Photomicrographs (100X) obtained under polarized light of formulation C (containing additives) at different storage conditions: A) t7; B) t30; C) t60; D) t90

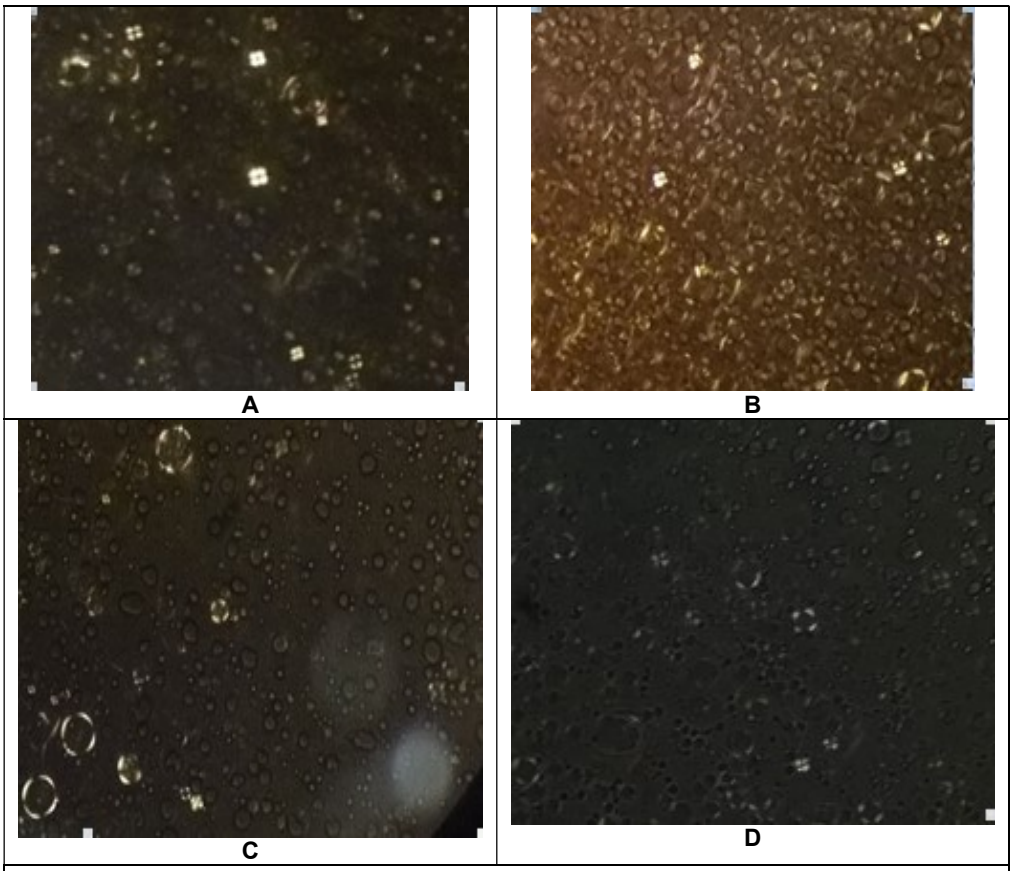

Figura 4. Photomicrographs (100X) obtained under polarized light of formulation D (containing additives) at different storage conditions: A) t7; B) t30; C) t60; D) t90

During trialing of the samples, base B suffered separation of phases when there was no addition of thickener, but its addition, even at small amounts, led to stability of the formulation. Even though there was separation of phases in base C during the thermal stress test, it was decided for accelerated stability tests to be carried out, as the centrifuge test indicated this base was stable. Some authors do not consider certain preliminary stability tests to be indicated, as exposure to high temperatures does not mean the samples remain stable three months under such conditions (Tadros *et al*., 2007). The only alteration considered valid was the addition of a thickener; it was tested whether the emulsions would be stable enough to not need a thickener, but separation of phases made not adding a thickener not advisable. Even though the addition of gum mitigated to some extent the issue, base C still suffered from separation of phases at 60 °C. It would be possible to add other thickener, increase the concentration of gum or even add oil phase thickeners which could "suppress" the emulsifiers.

Normal Stability Test (NST)

***Determination of pH*:** The pH is a physicochemical parameter which allows for identifying of possible chemical reactions (usually caused by intrinsic factors) which not always can be detected via organoleptic changes (Agência Nacional de Vigilância Sanitária, 2004). The chemical reactions which influence pH alterations in formulations are usually originated from the breaking of double bonds from unsaturated fatty acids, constituents of oily substances used in formulations. This process can be identified due to the unpleasant smell generated and other alterations which compromise the safety of use of the product (Andrade, 2008; Ramalho and Jorge, 2006). The pH ranges from all samples remained stable between 5.8 and 6.1. The additives in formulations C and D led to a pH variation ranging from 5.45 to 6.07. Up to 60 days of the stability test, all pH readings showed variations no higher than 0.3 units. After this period, pH readings of formulation D showed variations of up to 0.4 units at 45 °C (incubator condition) (Table 2). The reduction of pH values was not statistically significant. Some variations in pH readings were probably due to the presence of several fatty acids in the formulations, mostly emulsifiers and pequi oil, which possess palmitic and oleic acids at high concentrations. Acidification of the sample is due to oxidation of fatty acids which leads to formation of hydroperoxides or to hydrolysis of triglycerides generating free fatty acids (Masmoudi *et al*., 2005). The presence of carotenoids in pequi oil also probably led to low pH readings, as depending on the amount of oxygen in the sample, acidification can be accelerated due to high temperatures, presence of unsaturated lipids and exposure to light and metals (Jaeschke, 2013).

Organoleptic properties: Organoleptic characteristics provide general information of a formulation in a quick manner by comparing these characteristics to those of a standard (reference sample). These parameters add upon others such as separation of phases, precipitation and turbidity (Agência Nacional de Vigilância Sanitária, 2004). The formulations which had no additives (A and B) were homogenous, their color being slightly yellowish (milky). Formulations containing additives (C and D) were also homogenous but their color was of an intense yellow due to the presence of pequi oil, which also modified the odor of the emulsions. In the first 15 days the formulations suffered no modifications. However, after 30 days small organoleptic modifications could be verified; such alterations persisted throughout 90 days of analysis. The samples that remained in the incubator had a different, more intense odor. At room temperature, the two emulsions which contained additives suffered changes: emulsion A had its color intensified, while emulsion B had its odor pronounced. Either in freezer or in a fridge, both emulsions with additives had their odor intensified. The color and odor changes the formulations suffered can be attributed to the modifications of fatty acids and carotenoids found in the oil added. Therefore, addition of a higher amount of antioxidants, following regulations, could prevent the changes in color and odor (Jaeschke, 2013; Masmoudi *et al*., 2005). As for apparent instability of samples kept in the freezer, it happened probably due to the quick changes the liquid crystal structures suffered due to formulations being placed under extremely low temperatures.

Polarized light microscopy of the analyzed formulations: Microscopy allowed for visualization of the liquid crystal structure of the samples and also the behavior of the structures under different test conditions. Liquid crystal structures reflect light in a birefringent manner, and the anisotropic behavior of light confirmed such structures had been formed. Such structures can also be detected visually as they have "malta crosses" shapes (Zhang and Liu, 2013). By assessing photomicrographs obtained during microscopy evaluation of the samples it was possible to observe the anisotropic lamellar structures had remained stable throughout all 90 days of testing at extreme storage conditions. Formulation C, which contained additives (pequi oil and biopeptides), had better lamellar structures in comparison to the formulation with no additives, such as formulation A (Figure 1). Base A [Cetearyl Alcohol (and) Cetyl Palmitate (and) Sorbitan Palmitate (and) Sorbitan Oleate in association with Cetearyl Olivate (and) Sorbitan Olivate] had a positive influence from the additives (base C), mostly from the addition of pequi oil, which improved the emulsifying process and aided in maintaining liquid crystal structures stable (Figure 3). On the other hand, the addition of actives pequi oil and biopeptides in formulation D (Figure 4) was only able to preserve the liquid crystal structure previously organized in formulation B [Cetearyl Alcohol (and) Glyceryl Stearate (and) PEG-40 Stearate (and) Ceteareth-20] (Figure 2).

The influence of time and reduction of temperature after the emulsifying process influenced the formation of liquid crystal structures, increasing the visual amount of anisotropic lamellar structures (Zhang and Liu, 2013). The incorporation of xanthan gum to all formulations provided better organization of the internal structures of the formulations, which were previously large and uneven. This thickener remains in the aqueous phase of the formulation, and thus during emulsification, it tends to "jellify" the system and prevent mobility of micelles, allowing for more stable structures to be formed; the formation of liquid crystal structures also improve physicochemical stability of the system by improving its microstructures (Engels and Rybinski,1998).

Applications of the developed formulations: In previous studies, it was already reported that pequi oil improves skin repair; due to its anti-inflammatory properties, it can increase the number of fibroblasts which augment tissue healing (Bezerra *et al*., 2015; Nascimento *et al*., 2015; Lopes *et al*., 2008). Formulations containing pequi oil could be used at the beginning of the development of stretch marks, as they would regenerate tissue fibers and increase skin hydration due to being rich in fatty acids, such as oleic acid (ômega-9) (Rodrigues, 2011). Biopeptides are hydrophilic molecules and thus permeate the skin at a limited rate, which is hydrophobic. The inclusion of biopeptides in cosmetic formulations has been challenging, but research has demonstrated it is possible to add these compounds to lipophilic formulations in order to facilitate their permeation (Martinelli, 2015). The biopeptides used in this work Palmitoyl Tripeptide-1 (and) Palmitoyl Tetrapeptide-7 (Regestril®) act directly upon elastic collagen fibers, as they improve fibroblast activity. Palmitoyl Tripetide-1 (PAL GHK) is composed of palmitic acid bound to a elastin fragment, which is made of glycine, histidine and lysine. The incorporation of a fatty acid allows for better interaction of the peptide with the skin. Palmitoyl Tripeptide-1 acts upon fibroblasts in a mechanism where these cells recognize the elastin fragment as damaged, which induces their proliferation in order to make new fibers. Palmitoyl Tetrapeptide-7 (PAL GQPR) has a similar function regarding interactions with fatty acids, but is composed of glycine, glutamine, proline and arginine, which are fragments of human immunoglobulins which induce secretion of certain cytokines, such as IL-6. This peptide also acts upon fibroblasts, inducing production of collagen fibers and modulating cytokine production. The use of both peptides is approved by the FDA (Cellbone, 2021; Peptides Science, 2021; The dermatology review, 2021; Regestril, 2020; Pickart *et al*., 2015).

Liquid crystals are natural structures found in human physiology at the lipid bilayer of cell membranes and in lipid structures of the stratum corneum. When hydrated, due to natural hydration factors (NHF), such structures can be found as lamellar structures (Boock, 2007; Quirino-Barreda *et al*., 2017; Atkins and Jones, 2012; Herman, 2010). The formation of liquid crystals in emulsions improve hydration, extends dispersions of active compounds and increases stability of the emulsion system; these properties make the presence of these structures in emulsions desirable, as they improve hydration and sensorial properties when the emulsion is applied on the skin (Zhang and Liu, 2013). It is known that cosmetic formulations are incapable at penetrating the skin at its deeper layers. Simple emulsions by themselves are unable to stimulate regeneration of skin fibers damaged by stretch marks; they can only improve hydration and cutaneous texture. The development of emulsion systems with a better microstructural organization and liquid crystal phases allows for better permeabilization of biopeptides, making it possible for emulsions to have effects upon skin regeneration that has been damaged by stretch marks.

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CONCLUSION

The results obtained in this work show the formulations developed possess a microstructural liquid crystal organization, which remained stable throughout stability assays under different time and temperature conditions.

All formulations tested had the same organoleptic properties. The formulations developed with [Cetearyl Alcohol (and) Glyceryl Stearate (and) PEG-40 Stearate (and) Ceteareth-20] had a more homogenous internal organization when they had pequi oil and biopeptides added to them.

REFERENCES

Agência Nacional de Vigilância Sanitária. Guia de estabilidade de produtos cosméticos. Brasília: ANVISA, 2004. *1*, 47p. (Séries temáticas da ANVISA: Qualidade em Cosméticos, 1).

Andrade FF (2008). Desenvolvimento e avaliação de cristais líquidos obtidos em emulsões O/A a base de óleo de andiroba e éster fosfórico. Dissertation, Mestre em Ciências Farmacêuticas, Universidade São Paulo, Ribeirão Preto, São Paulo.

Atkins P, Jones L (2012). *Princípios de química: questionando a vida moderna e o meio ambiente*. 5rd ed.; Porto Alegre: Bookman, Brazil, pp. 550.

Baby AR, Maciel CPM, Zague V, Kaneko TM, Consiglieri, VO, Velasco MVR (2004). Estabilidade de produtos de aplicação tópica: ensaios aplicados aos produtos cosméticos e dermatológicos emulsionados. Int. J. Pharm. Compd., 6: 130-139.

Bezerra NKMS, Barros TL, Coelho NPMF (2015). A ação do óleo de pequi (*Caryocar brasiliense*) no processo cicatricial de lesões cutâneas em ratos. Rev. Bras. Pl. Med, 17: 875-880.

Boock KP (2007). Desenvolvimento e avaliação da estabilidade física de emulsões contendo cristais líquidos e ativos hidratantes a base de manteiga de cupuaçu (*Theobroma grandiflorum*) ou cacau (*Theobroma cacau*). Dissertation, Mestrado em Ciências Farmacêuticas, Universidade de São Paulo, Ribeirão Preto.

Cellbone Technology. Available online: http://www.cellbone.com/PeptideGQPR.htm (acessed on 07 July 2021).

Deus TN (2008). Extração e caracterização de óleo do pequi (C*aryocar brasiliensis* Camb.) para o uso sustentável em formulações cosméticas óleo/água (O/A). Dissertation, Mestrado em Ecologia e Produção Sustentável, Universidade Católica de Goiás, Goiânia.

Draelos Z D (2015). *Cosmetic dermatology: products and procedures*. 2nd ed.; Durham: John Wiley & Sons, pp 308.

Engels T, Rybinski WV (1998). Liquid crystalline surfactant phases in chemical applications**.** J. Mat. Chem, 8:1313-1320.

Escobar JL, Adrighetti CR, Ribeiro EB, *et al*. (2016). Desevolvimento de sabonetes em barra contendo óleo de pequi (*Caryocar brsiliense* Camb.). Sci. Electron. Arch. 9. Available online: http://www.seasinop.com.br/revista/index.php?journal=SEA&page=article&op=view&path%5B%5D=221&path%5B%5D=pdf_93 (accessed on 09 May 2021).

Granato D, Castro IA, Ellendersen LSN, Masson ML (2010). Physical stability assessment and sensory optimization of a dairy-free emulsion using response surface methodology. J.Food. Sci, 75:S149-155.

Herman S (2010). Liquid crystals and skin. Gci, Available online: http: http://gcimagazine.texterity.com/gcimagazine/201002?oly_enc_id=0462C9041334E8U&r=0462C9041334E8U&pg=56#pg56 (accessed on 09 May 2021).

Jaeschke DP (2013). Estudo da degradação de vitamina C e carotenoides em polpa de acerola durante aquecimento ôhmico e convencional**.** Monograph, Engenharia Química, Universidade Federal do Rio Grande do Sul, Rio Grande do Sul.

Lima A (2008). Caracterização química, avaliação da atividade antioxidante *in vitro* e *in vivo* e identificação dos compostos fenólicos presentes no pequi (*Caryocar brasiliense*, Camb.). Thesis, Doutorado em Ciência dos Alimentos, Universidade de São Paulo, São Paulo.

Lopes PS, Pinto CASO, Baby AR, Velasco MVR, Taqueda ME, Kaneko TM (2008). Avaliação in vitro da papaína e do óleo de pequi como promotores de permeação cutânea para diclofenaco de sódio em pele humana. Rev. Bras. Cienc. Farm, 44: 225-231.

Magalhães WCP (2010). Emulsões cosméticas: critérios de desenvolvimento. C&T, *22*, p. 36.

Martinelli AM (2015). Desenvolvimento de sistemas líquidos cristalinos lamelares contendo diferentes concentrações de AC-WAHX-KTTKS para o tratamento do envelhecimento cutâneo. Dissertation, Mestrado em Ciências Farmacêuticas, Universidade Estadual Paulista Júlio de Mesquita Filho, São Paulo.

Masmoudi H, Dréau YLE, Piccerelle P, Kister J (2005). The evaluation of cosmetic and pharmaceutical emulsions aging process using classical techniques and a new method: FTIR. Int. J. Pharm, 289: 117-131.

Nascimento WM, Filho ALMM, Costa CLS, *et al*. (2015). Estudo da resistência cicatricial cutânea de ratos tratados com óleo de pequi (*Caryocar brasiliense*). Com. Scientiae Saúde, 14: 449-455.

Neto AMF, Salinas SRA (2005). The physics of lyotropic liquid crystals phase transitions and structural properties**.** Monograph, Oxford University**,** New York.

Peptides Scienc. Available online: https://www.peptidesciences.com/pal-ghk (accessed on 07 July 2021).

Pianovski AR, Vilela AFG, Silva AAS, *et al*. (2008). Uso do óleo de pequi (*Caryocar brasiliense* Camb.) em emulsões cosméticas: desenvolvimento e avaliação da estabilidade física. Rev. Bras. Pl. Med, 44: 249-259.

Pickart L, Vasquez-Soltero JM, Margolina A (2015). GHK-Cu may Prevent Oxidative Stress in Skin by Regulating Copper and Modifying Expression of Numerous Antioxidant Genes. Cosmetics, *2*:236-247.

Quirino-Barreda CT, Gazga-Urioste C, Juárez-Sandoval JJ, Faustino-Vega A, Noguez-Méndez NA, Macín-Cabrera SA (2017).

Cristales líquidos liotrópicos. Nanoestructuras biomiméticas para uso tópico medicinal. *MundoNano,* 10: 7-25.

Ramalho VC, Jorge N (2006). Antioxidants used in oils, fats and fatty foods. Quím. Nova 29: 755-760.

Regestril™. São Paulo: Pharma Special, 2020. Literatura de ativo.

Ribeiro DM (2011). Propriedades físicas, químicas e bioquímicas do pequi (*Caryocar brasiliense* Camb.) de diferentes regiões do cerrado. Dissertation, Mestrado em Nutrição Humana Universidade de Brasília, Brasília.

Rodrigues HG (2011). Modulação do processo de cicatrização pelos ácidos oleico e linoleico. Thesis, Doutorado em Fisiologia Humana, Universidade de São Paulo, São Paulo.

Tadros T, Nestor J, Claire-Taelman M, Smits R (2007). Emulsões: correlação da estabilidade física de longo prazo com medições reológicas de curto prazo. C&T, 19: 60-65.

The dermatology review. Available online: https://thedermreview. com/palmitoyl- tetrapeptide-7/ (accessed on 07 July 2021).

Todorova P, Ross P, Tamburic S. (2016). Hidratação Biomimética versus tradicional**.** C&T *28*: 40-45.

Zhang W, Liu L (2013). Study on the formation and properties of liquid crystal emulsion in cosmetic**.** JCDSA, 3: 139-144.

Zielinska A, Nowak I (2014). Fatty acids in vegetable oils and their importance in cosmetics industry. Chemik**.** 68: 103-110.

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