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Development and sun protection factor of emusionated formulation containing Brazil nut oil

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Abstract: Brazil nut (*Bertholletia excelsa* H.B.K) is oil rich and contains compounds with bioactive properties wich grants potential for usage in new formulations with photo-protective potential, as well as other pharmaceutical and cosmetic applications. The aim of this study was the development and *in vitro* determination of the sun protection factor of emulsionated formulations containing Brazil nut oil, targeting its use as a photo-protective carrier. Brazil nut oil was extracted with hexane using the ultrasound assisted method. After the oil quality against oxidation was evaluated, it was incorporated into the formulations. The emulsions were evaluated for their preliminary stability, accelerated stability, rheological characterization, mean globule diameter, *in vitro* SPF determination and potential antioxidant activity by the DPPH radical scavenging method. The same emulsified systems containing a chemical sunscreen were also evaluated, looking for synergism possibilities. The emulsions containing 20 and 30% of Brazil nut oil, with and without the chemical sunscreen, were characterized as oil-in-water type, showing elastic/solid rheological characteristics, and presented better stability characteristics in the assessed parameters. However, the presented photo-protective activity was attributed to the chemical sunscreen itself, rather than the base formulation and the antioxidant activity was considered low. The base formulation developed with Brazil nut oil presented adequate characteristics for incorporating sunscreen, opening up possibilities for incorporating several active substances, like the sunscreen and many others.
Keywords: *Bertholletia excelsa H.B.K*; Emulsion; Sun protection factor (SPF); Brazil nut oil.

Introduction

The need for cosmetic products based in natural ingredients, specially from vegetable sources, is in constant expansion, taking researchers on a quest for new substances that fulfill the consumers and industry needs, allowing minimum ecological impact in order to explore the Brazilian biodiversity in a rational way (Chen, 2009, Joshi & Pawar, 2015).

Amongst natural products, the Brazil nut (*Bertholletia excelsa* H.B.K) stands out due to its selenium and vitamin E contents, being linked to its antioxidant activity (Montenegro et al., 2015; Pacheco & Scussel, 2007). The nut holds high lipid content and proteins (Felberg et al., 2009; Santos et al., 2011) besides some bioactive compounds such as essential fatty acids, aminoacids, fiber and minerals (Ca, Cu, Fe, P, Mg, Mn, K e Zn) (Kluczkovski et al., 2015; Michalak & Kieltyka-dadasiewicz, 2018). Belonging to the *Lecythidaceae* family, the Brazil nut tree is one of the most valuable national species from Brazil (Prance & Mori, 1978).

Active substances extracted from plants have been increasingly used in pharmaceutical, cosmetic and cosmeceutical products. These active substances found mostly as plant extracts and fixed or volatile oils, are added to formulations in order to increase its absorption, cooperating with the expected activity (Torres et al., 2018).

Sunscreen are formulations that filter UVA and UVB rays, also known as photo-protectors, have as its purpose the decrease of the damages caused by ultraviolet rays from the sun, preventing solar burns, premature aging and cancer (Rocha et al., 2011). Usually, they can be found as emulsions and nanoemulsions, which allow the adding of hydrophilic and hydrophobic sunscreens and bioactive substances to them, due to fact they are constituted of both aqueous and oily phases (Deltreggiaet al., 2019).

Within that context, the aim of this paper was to perform the *in vitro* determination of the sun protection factor (SPF) of different emulsified formulations containing Brazil nut oil, targeting its use as a photo-protective carrier.

Methods

Brazil nut oil obtainment

The nuts were provided by Borello Alimentos LTDA from the municipality of Sinop-MT. The nuts were milled in an industrial blender and turned into flour, this flour was stored in refrigerator at -5 ^{0}C until the moment of extraction. The oil extraction was performed by the ultrasound assisted method, using hexane as solvent, according to Schons et al. (2017). In order to check the oil quality against oxidation, the quality control laboratory evaluated its acidity and peroxide values according to the methodology established by the Adolfo Lutz Institute standards (IAL, 2008).

Emulsified system composition

The emulsified systems were formulated with Brazil nut oil, distilled water, sorbitan monooleate (Span 80®), HLB = 4.3 and polysorbate 80 (Tween 80®), HLB = 15.0. The amounts of each component ranged from 10 to 80%.

36 formulations were produced, then analyzed after 24 hours at room temperature (25°C) for the construction of a pseudoternary diagram, using the Sigma Plot 8.0 software, in adapted methodology to check whether the emulsified systems were formed **(**Fiori et al., 2017; Silva et al., 2015). The systems categorized as emulsions were once again prepared and taken to quality control, that assessed preliminary and accelerated stability, incorporated the UV-filter octyl-methoxycinnamate (OMC), determined the hydrodynamic diameter, performed the rheological characterization and determined the solar protection factor (SPF) in vitro and the potential antioxidant activity.

Preliminary stability of the formulations

In order to evaluate the preliminary stability, the emulsified systems were divided in 2 groups (E and G), where group E was kept at 40 ±1 ºC in an oven, and group G was kept 5 ± 1 ºC in a refrigerator. The systems went through alternated cycles of 5 ± 1 ºC and 40 ± 1 ºC every 24 h, completing the cycles at the 14th day. The pH, refraction index and electrical conductivity tests were performed and then, the systems that remained thermally stable went through the accelerated stability study (Brasil, 2004; Fiori et al., 2017; Gustmann et al., 2017; Tozetto et al., 2017).

Accelerated stability of the formulations

For the accelerated stability study, the emulsified systems were divided in three groups (E, A and G). The group E was kept at 40 ± 1 ºC, group A at 25 ± 1 ºC and group G at 5 ± 1 ºC. All groups were evaluated during a time gap of 90 days. And, for every 30 days, the formulations went through the same physicochemical analyzes performed at the preliminary stability part (Brasil, 2004; Chorilli et al., 2009; Raiser et al., 2018b; Tozetto et al., 2017).

Quality control

The verification of some physicochemical parameters aimed to test the adequacy of the formulations, as well to characterize them. These tests were performed by checking the quality parameters of the formulations after 24 h of preparation and at the end of the study cycles. The assessed parameter were: organoleptic characteristics, pH, refractive index determination and electrical conductivity determination (Isaac et al., 2008;Pianovski et al., 2008; Amorim et al., 2015; Fiori et al., 2017; Gustmann et al., 2017).

Rheological evaluation

The rheological evaluation was carried out through a partnership with the UFMT / CUA Bioassays laboratory, Barrado Garças-MT, using a Modular Compact Rheometer - MCR 102. The tests of viscoelasticity as a function of the shear stress frequency were performed with an oscillatory stress amplitude equal to 0.5 Pa, varying the frequency in the range of 100 to 0.1 Hz (Nikiforidis et al., 2012; Tozetto et al., 2017).

The experiments were carried out by applying a sufficient amount of emulsion on the surface of the reading plate and subsequently removing the excess. Readings were performed with permanent control of the measurement gap at 0.099 mm, CP 50 measurement cell and temperature control, using the Rheoplus V3.61 software (Tozettoet al., 2017).

Chemical sunscreen incorporation

The OMC was incorporated into the formulations that presented stable after the 90 days period, at the concentration of 1%. After that, these formulations were put through a new accelerated stability cycle, as describe in item 2.4.

Hydrodynamic diameter determination

The analyzes to determine the globules hydrodynamic diameter of the emulsified formulations were obtained through microscopy, according to an adapted methodology to determine the diameter of particles under electron microscopy (Soler et al., 2011). The emulsions prepared with and without the OMC were applied on slides in a microscope with an attached camera and photographed at 40x magnification. A Neubauer Chamber was used as a reference scale. Subsequently, the images were treated equally regarding the color filter

(Matrix: 30; Saturation: 10 and Brightness: 20). The globules mean diameter (DM), its standard deviation (σ) and coefficient of variation (CV%) were attained from the adjustment of the data obtained from the microscope with the assistance of the ImageJ software (Java®). This methodology was adapted from the one used to determine the HLB of vegetable oil used by Fiori et al. (2017).

SPF in vitro determination

The *in vitro* sun protection factor (SPF) of the Brazil nut oil, the emulsified formulations, the OMC and the emulsified formulations containing the UV-filter was determined according to the protocol established by Mansur et al. (1986). The solutions used to determine the SPF were prepared at a concentration of 0.2 $mgmL^{-1}$ in ethylic alcohol. The equation used to establish the SPF is given as follows:

$$FPS = FC. \sum EE(\lambda) \, . \, I(\lambda) \, .Abs(\lambda)$$

Where:

FC = correction factor (equal to 10);
EE (λ) = erythemogenic effect of the wavelength λ radiation;
I (λ) = sunlight intensity at the wavelength λ;
Abs (λ)= spectrophotometric reading of the solution absorbance at the wavelength (λ).

The analysis of variance (ANOVA) was performed to assess the significant difference in the potential solar photo-protection factor, followed by comparison between the groups by Tukey tests using the BioEstat 5.0 software. The analyzes were considered significant when the p values were inferior to 0.05 ($p < 0.05$).

Potential antioxidant activity

The potential antioxidant activity analysis of the developed formulations was carried out using the DPPH radical scavenging method (2,2 – diphenyl-1-picryl-hydraziy) as established by Rufino et al. (2007), with adaptations. The samples were solubilized in ethanol and diluted in methanol, obtaining a concentration of 4.00 $mgmL^{-1}$. From this solution, dilutions were prepared in order to obtain concentrations in the range between 0.50 and 1.00 $mgmL^{-1}$.

The absorbance was determined at the wavelength of 515 nm in a spectrophotometer (PG Instruments®, T80 UV / VIS), after keeping the samples in the dark for 1 hour. Then, the EC_{50} value (emulsion concentration necessary to reduce 50% of the DPPH radical) was calculated.

Results and discussion

The Brazil nut oil extraction was performed by the ultrasound assisted method and provided a yield of 57.85% oil, in accordance with that found by Schons et al. (2017) and corroborating that this method is fast, efficient and presents low cost for oil extraction.

The acid and peroxide values were evaluated in order to verify whether the oil was within the quality parameters required by legislation (BRASIL, 2005) in order to use it. The total acid value was of 1.35 $mgNaOHg^{-1}$, corresponding to 0.41% in oleic acid and the peroxide value of 1.53 meq · kg^{-1}, indicating low risk of oxidation and consequently good oil quality.

Thirty-six formulations were developed using the surfactants mixture (4.67: 5.33 v/v) SP/TW combined with Brazil nut oil and distilled water in pre-established proportions. With that, a pseudoternary diagram was constructed (Figure 1), resulting in 36 points with divergent balance characteristics.

From the 36 developed formulations (Figure 1), 8 (3C, 4C, 5C, 11C, 12C, 18C, 23C and 28C) formed emulsions, whilst 28 presented phase separation (PS), or formation of liquid and gel microemulsion (LME and GME, respectively). The 8 obtained emulsified formulations went on to the quality control tests. The emulsified formulations (3C, 4C, 5C, 11C, 12C, 18C, 23C and 28C) were prepared in triplicate and after 24 hours they proceeded to the preliminary stability analysis for a period of 14 days. The results of the evaluations are shown in Table 1.

The organoleptic characteristics such as appearance, color and odor did not vary. At the end of the preliminary stability test, the pH values of the emulsions remained between 6.00 and 6.40, an appropriate pH for emulsified formulations.

The found refractive index results remained between 1.33 to 1.42, with a slight decrease in the results numbers when comparing the before and after of the preliminary stability test. The electrical conductivity showed values above 1.30 μScm^{-1}, classifying the emulsions as O/W type (Gustmannet al, 2017).

At the end of 14 days of the preliminary stability test, all formulations remained stable and showed no visual changes. Thus, all emulsions submitted to the preliminary stability test proceeded to the accelerated stability tests.

The accelerated stability was performed for a period of 90 days and the results of the physicochemical analyzes are shown in Table 2.

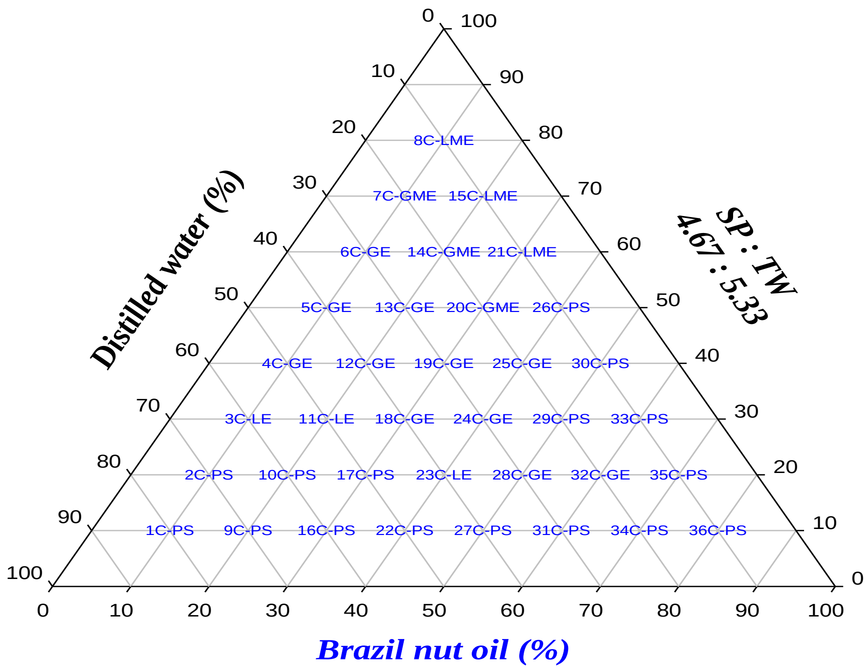

Figure 1 - Pseudoternary diagram of the SP/TW system formulations (4.67:5.33) containing Brazil nut oil *(Bertholletia excelsa)* and distilled water (%).
*Abbreviations: Liquid microemulsion (LME), Gel microemulsion (GME), Liquid emulsion (LE), Gel emulsion (GE) and Phase separation (PS).

Table 1 - Physicochemical and organoleptic characteristics analysis of the emulsions containing Brazil nut oil during the preliminary stability, after 24 hours of preparation (Before) and after 14 days (After), analyzed at room temperature (25 ºC).

Formulation	pH (*Before*)	(*After*)	Refraction index (Before)	(*After*)	Conductivity µScm^{-1} (*Before*)	(*After*)	Aspect, color and odor
3C	6.09	6.25	1.38	1.33	157.03	88.51	N
4C	6.34	6.14	1.39	1.34	117.10	33.64	N
5C	6.33	6.20	1.42	1.34	20.98	9.30	N
11C	6.31	6.15	1.39	1.34	109.97	50.47	N
12C	6.31	6.05	1.41	1.33	31.43	30.71	N
18C	6.40	6.07	1.42	1.35	62.69	61.89	N
23C	6.24	6.08	1.41	1.35	98.23	75.34	N
28C	6.19	6.00	1.42	1.35	53.83	48.03	N

*Abbreviation: N= Normal.

The 3C, 5C, 11C, 23C and 28C emulsions showed significant variation in electrical conductivity after the 30th day, variation in organoleptic characteristics and phase separation, being excluded from the tests. Meanwhile, formulations 4C, 12C and 18C remained stable at the end of the 90 days.

There was a decrease in the pH values, from 6.20 to 5.00, however, variations between 4.50 and 7.00 are acceptable for topical formulations, depending on the applying region of the body and / or age (Ali&Yosipovitch, 2013; Leonardi et al., 2002; Souza et al., 2013).

The refraction index values during the 90 days for formulations 4C, 12C and 18C was of 1.30 ± 0.1. This result indicates that there was no variation when the values on the day of preparation and after the 90 days are compared, suggesting the maintenance of the oil quality in the emulsion. The determination of the electrical conductivity is an important parameter in the evaluation of the emulsion, since it contributes to the monitoring of its stability. The formulations presented values greater than 1.30 µScm^{-1} throughout the test period, maintaining the characteristic of oil in water (O / W) emulsions.

Formulations 4C, 12C and 18C had their rheological aspects evaluated and Figure 2 shows their vibrational modules.

Table 2 - Physicochemical analysis and organoleptic characteristics of the emulsions containing Brazil nut oil during accelerated stability tests, analyzed at room temperature (25 ºC).

Formulation	*Time (days)* 0	30	60	90
		Aspect, color and odor		
3C	N	N	M	-
4C	N	N	N	N
5C	N	N	N	M
11C	N	N	M	-
12C	N	N	N	N
18C	N	N	N	N
23C	N	N	N	M
28C	N	N	M	-
		pH		
3C	6.43 ± 0.13	6.46 ± 0.09	-	-
4C	6.24 ± 0.72	6.15 ± 0.65	5.39 ± 1.30	5.06 ± 1.20
5C	6.43 ± 0.44	6.26 ± 0.31	5.48 ± 1.16	-
11C	6.55 ± 0.12	6.60 ± 0.21	-	-
12C	6.32 ± 0.29	6.27 ± 0.26	5.45 ± 0.91	5.53 ± 1.11
18C	6.52 ± 0.24	6.38 ± 0.05	5.52 ± 0.79	5.56 ± 0.93
23C	6.33 ± 0.28	6.31 ± 0.36	5.42 ± 0.94	-
28C	6.26 ± 0.25	6.28 ± 0.23	-	-
		Refractive index		
3C	1.38 ± 0.01	1.38 ± 0.00	-	-
4C	1.39 ± 0.00	1.40 ± 0.00	1.39 ± 0.00	1.41 ± 0.00
5C	1.45 ± 0.03	1.42 ± 0.02	1.45 ± 0.02	-
11C	1.37 ± 0.00	1.39 ± 0.01	-	-
12C	1.42 ± 0.03	1.42 ± 0.03	1.42 ± 0.03	1.43 ± 0.03
18C	1.40 ± 0.00	1.41 ± 0.00	1.41 ± 0.00	1.42 ± 0.03
23C	1.42 ± 0.04	1.42 ± 0.02	1.42 ± 0.02	-
28C	1.43 ± 0.03	1.44 ± 0.03	-	-
		Electrical Conductivity (μScm^{-1})		
3C	157.03 ± 4.87	113.70 ± 20.80	-	-
4C	117.10 ± 6.53	95.84 ± 34.70	62.17 ± 7.30	60.71 ± 7.75
5C	20.98 ± 3.39	39.98 ± 17.70	74.58 ± 40.00	-
11C	109.97 ± 20.24	99.71 ± 7.80	-	-
12C	31.43 ± 7.70	20.69 ± 4.98	16.34 ± 4.98	5.09 ± 3.77
18C	62.69 ± 2.23	35.42 ± 3.50	29.73 ± 8.90	5.29 ± 4.06
23C	98.23 ± 3.43	68.45 ± 47.70	47.71 ± 45.00	-
28C	53.83 ± 14.36	17.64 ± 23.40	-	-

*Abbreviation: N= Normal; M= Modified and (-) eliminated of the assay.

In oscillatory rheology, the higher the value of G'(storage module), the greater the solid character, therefore, its deformations are classified as elastic. Thus, the opposite can be infered for G” (loss module), which indicates the liquid character of the material (Nikiforidis et al., 2012; Shakerardekani et al., 2013). I.e., when G' is absent and there is a predominance of G”, it indicates that the samples behave as viscous liquids, the reverse is true, since with predominance of G' against G” or absence of the latter, the system presents a solid behavior (Ribeiro et al., 2016).

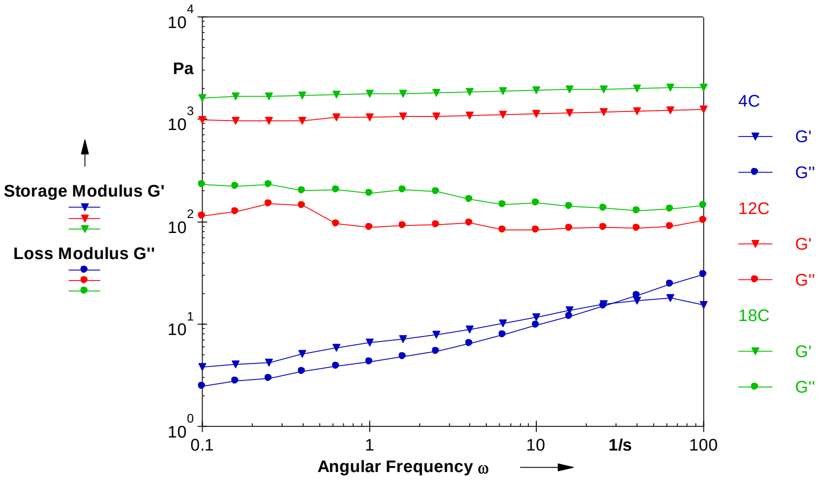

Figure 2 - Evolution of storage (G') and loss (G") modules of the stable emulsions versus shear stress frequency.

According to Figure 2, the curve obtained for formulation 4C showed that the results of G 'are close to those of G", however the values of G' were slightly higher in the variable frequency range, except at high frequency, as there is an occurrence of inversion of the modules, which can be an indicative of instability of the formulation in more extreme conditions.

Similar results were found by Tozetto et al. (2017) and Vianna Filho (2009) when studying emulsified systems, where values of G' are greater than G" with modular inversion at increased frequencies, indicating that the formulation is in transition from the elastic/solid state to viscous/liquid.

With emulsions 12C and 18C, there was a predominance of the G' module against the G" in the established frequency range (0.1 to 100 Hz), with no inversions of modules being noticed, thus, the emulsion presented an elastic/solid behavior, with stable characteristics even in extreme conditions.

Shakerardekani et al. (2013) reports in their studies on the stability of cosmetic emulsions that emulsions with values of G '(elastic module) greater than G" (viscous module) react as solid viscoelastic materials.

Therefore, in the face of the rheology test, formulation 4C was discarded, and formulations 12C and 18C remained with adequate characteristics and stability.

Formulations 12C and 18C were prepared and incorporated with the UV-filter OMC at a concentration of 1%. These formulations were named 12COMC and 18COMC. This OMC was chosen because it is one of the most used in sun protection products and can be incorporated up to a maximum concentration of 10% (Brasil, 2006; Montenegro, 2019).

After the preparation of the formulations (12COMC and 18COMC), they were evaluated during a period of 90 days through the accelerated stability tests. The results of the analyzes are shown in Table 3.

The organoleptic characteristics of the two formulations (12COMC and 18COMC) remained stable over the 90 days. They presented pH values between 6.50 and 6.90, remaining within the limit established for topical formulations, since the stability, safety and efficacy of the product are associated with this parameter (Brasil, 2004). Similar results were found in a study with pequi oil in cosmetic emulsions, with pH values around 5.5 and 6.5 (Raiser et al., 2018a). Still, the OMC presence increased the pH when compared to emulsions without the OMC.

The refractive index results remained around 1.41 and 1.43 for the 12COMC and 18COMC emulsions, respectively, maintaining the values found in the emulsions without the OMC (12C and 18C) in the different storage conditions as time went by, which shows the stability of these emulsions (Raiser et al., 2018b). The electrical conductivity values were higher than 1.30 μScm^{-1}in the formulations with the OMC, as well as in the emulsions without it, indicating the continuity of their stability.

In the rheological analysis of the 12COMC and 18COMC emulsions (Figure 3), it was verified that the G' remained higher than the G", during the entire extension of the shear stress frequency.

After the incorporation of OMC in the emulsions, there was no change in the rheological profile, maintaining the elastic/solid character found in the formulations without the OMC, which demonstrates the stability of the formulations.

The mean hydrodynamic diameter, as well as the respective coefficient of variation, of Brazil nut oil emulsions, with and without OMC, are shown in Table 4.

Table 3 - Physicochemical parameters and organoleptic characteristics of the emulsified systems containing Brazil nut oil and OMC(12COMC and 18COMC), evaluated in the accelerated stability studies on days 0, 30, 60, and 90 and analyzed at room temperature (25 °C).

Formulation	***Time (days)***			
	0	**30**	**60**	**90**
		Aspect, color and odor		
12COMC	N	N	N	N
18COMC	N	N	N	N
		pH		
12COMC	6.73 ± 0.15	6.84 ± 0.17	6.76 ± 0.16	6.73 ± 0.17
18COMC	6.79 ± 0.15	6.79 ± 0.09	6.70 ± 0.36	6.60 ± 0.50
		Refractive index		
12COMC	1.43 ± 0.00	1.42 ± 0.01	1.41 ± 0.01	1.41 ± 0.00
18COMC	1.41 ± 0.01	1.42 ± 0.00	1.41 ± 0.00	1.41 ± 0.00
		Electrical Conductivity (μScm^{-1})		
12COMC	22.61 ± 1.72	27.01 ± 1.74	27.60 ± 1.83	32.78 ± 4.27
18COMC	24.83 ± 3.83	27.75 ± 4.58	36.96 ± 2.05	40.04 ± 8.18

*Abbreviation: N= Normal.

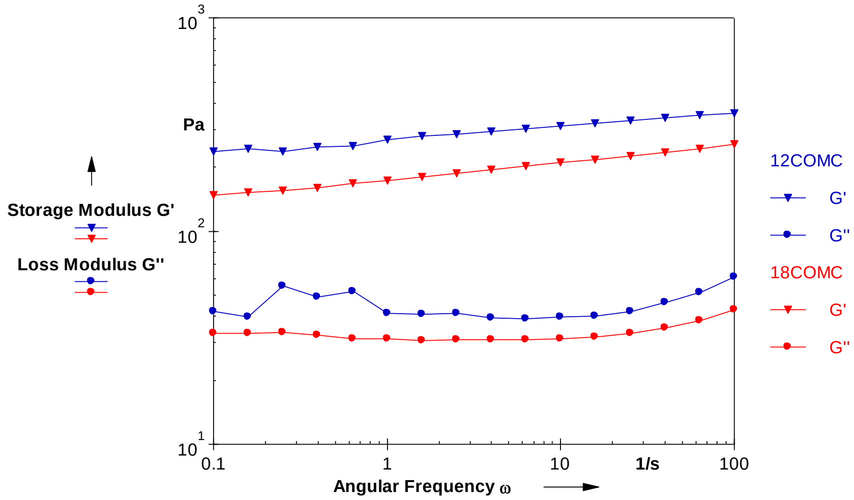

Figure 3 - Evolution of storage (G') and loss (G") modules of stable emulsions versus shear stress frequency. * Abbreviations: Emulsions 12C and 18C containing octyl methoxycinnamate (12COMC and 18COMC).

Table 4. Hydrodynamic diameter of the emulsified formulations containing Brazil nut oil (12C and 18C), and added with OMC (12COMC e 18COMC).

Formulation	Hydrodynamic diameter (μm)	Coefficient of variation
12C	6.34 ± 0.75	8.43
18C	9.92 ± 1.82	5.46
12COMC	4.67 ± 0.78	6.00
18COMC	7.67 ± 1.05	7.29

The emulsified formulations containing Brazil nut oil (12C) and added with OMC (12COMC) had smaller hydrodynamic diameter. The decrease in the size of the globules is probably due to the increase in the surfactant concentration in these systems. This occurs due to the ability of the surfactant to involve the globules forming smaller droplets (Akbari; Nour, 2018; Leal-Calderon et al., 2007). This increase in the surfactant concentration reducing the size of the globules is seen as an advantage, since it can increase the emulsion stability (Akbari & Nour, 2018).

The adding of OMC to the systems (12COMC and 18COMC) also favored the reduction of their globules mean diameters, without compromising their homogeneous distribution. The 12COMC emulsion had a lower coefficient of variation, showing that thesevalue indicate higher uniformity of the globules and stabilityof the globules and stability of the formulation (Shaw, 1991).

Emulsification is usually a result of a polydisperse system where small and large droplets coexist (Nielloud&Marti-Mestres, 2000) and the diameter of dispersed globules varies around 0.1 and 100 µm approximately (Aulton, 2005; Evans et al., 1999; Mcclements, 2012). Thus, the results of the diameters found for the developed formulations are in consonance with an emulsified system (Regattieri et al., 2016; Shaw, 1991).

The results of the potential *in vitro* sun photo-protection factor of the Brazil nut oil, the OMC and the emulsions developed with and without OMC are shown in Figure 4.

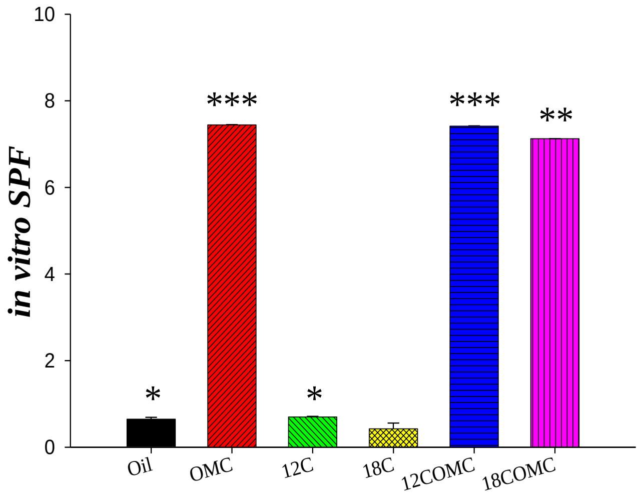

Figure 4 - Determination of the *in vitro* sun protection factor (SPF) value of Brazil nut oil, sunscreen (OMC), emulsions (12C and 18C) and emulsions incorporated with the sunscreen (12COMC and 18COMC). (ANOVA) - * Comparison between groups. (F = 10654.92 and p = <0.0001).

Brazil nut oil showed an SPF of 0.67, while formulations 12C and 18C showed values of 0.70 and 0.36. The SPF value for the pure OMC was 7.44 and formulations added with the OMC presented values of 7.42 for formulation 12COMC and 7.13 for 18COMC (Figure 4).

The hydrodynamic diameter of formulation 12C was smaller than 18C and may have helped providing a greater contact surface, which consequently provided a higher SPF value. This parameter was maintained after the incorporation of the OMC in the formulations 12COMC and 18COMC. After the incorporation of the OMC, the formulations 12COMC and 18COMC presented similar SPF results to that of the pure OMC, therefore the emulsions without the sunscreen did not influence in a greater sun protection.

The extracted oil, as well as the emulsions without chemical sunscreen, did not reach significant SPF values, which according to the Brazilian legislation RDC nº 237 of August 2002, must be equal to or greater than 2.00. Also, based on the SPF values, the formulations developed and incorporated with OMC (12COMC and 18COMC) did not promote synergism. The results agree with Polonini et al. (2012) who evaluated the Brazil nut oil and emulsions containing the oil and titanium oxide at a concentration of 10%, verifying that the SPF of the oil was low and that the SPF of the emulsion was due to the added physical sunscreen. Ferrari et al. (2007), using andiroba oil in O/W emulsions, also did not observe photoprotective action in emulsified systems. It is worth mentioning, however, that vegetable oils that presented low SPF may eventually potentiate the photo-protective action in emulsified formulations, as verified by Moraes et al. (2017) in emulsified formulations with rutin succinate oil.

Based on the fact that the SPF of formulation 18COMC is slightly lower than 12COMC, the potential antioxidant activity was determined only with formulation 12 (12C and 12COMC).

The assay for potential antioxidant activity showed EC50 values of 7.41 mgmL^{-1} and 5.73 mgmL^{-1} for emulsions 12C and 12COMC, respectively (Figure 5).

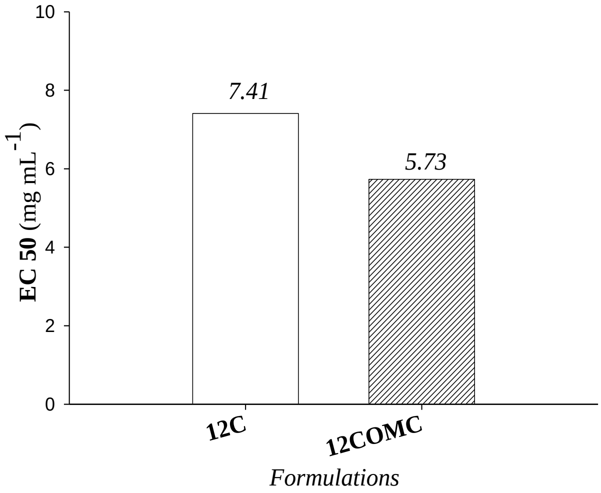

Figure 5 – Potential antioxidant activities (EC_{50}) of the emulsions containing Brazil nut oil: Emulsion without the OMC (12C) and emulsion with the OMC (12COMC).

The obtained EC_{50} result showed that the formulation 12CMCO presented a superior result than 12C, possibly due to the OMC. However, it must be taken into account that, in order to obtain satisfactory antioxidant activity, high amounts of the formulation would be necessary.

The potential antioxidant activity of emulsions does not depend only on the vegetable oil used in the formulation, but on the other constituents as well, and synergism may occur and potentiate the action as observed by Raiser et al. (2018a, b) in pequi and munguba emulsions.

Conclusion

Emulsified formulations developed containing 20 and 30% of Brazil nut oil showed adequate characteristics and stability in the evaluated parameters, but nophoto-protective activity was observed. When OMC was incorporated into these formulations, they remained stable, however, the photoprotective action was exclusively due to the OMC added and the potential antioxidant activity was low. The base formulation developed with Brazil nut oil showed adequate characteristics for incorporating a sunscreen, opening possibilities for incorporating active substances such as sunscreen and many others.

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