Revista Virtual de Química

ISSN 1984-6835

Artigo

Development, Stability and Antioxidant Activity of Microemulsion Containing Pequi (*Caryocar brasiliense* Camb.) Oil

Torres, M. P. R.; Raiser, A. L.; Marcílio, M. R.; Ribeiro, E. B.; Andrighetti, C. R.; Valladão, D. M. S.*

Rev. Virtual Quim., **2018**, *10* (2), 346-361. Data de publicação na Web: 20 de março de 2018

http://rvq.sbq.org.br

Desenvolvimento, Estabilidade e Atividade Antioxidante de Microemulsão Contendo Óleo de Pequi (*Caryocar brasiliense* Camb.)

Resumo: O estudo de veículos para administração de fármacos pode potencializar a ação das substâncias veiculadas aos mesmos e a avaliação de seus parâmetros de qualidade evidencia o comportamento dos componentes da formulação. Este estudo teve como objetivo obter formulações microemulsionadas contendo óleo de pequi (*Caryocar brasiliense* Camb.) e determinar sua estabilidade, além da sua potencial atividade antioxidante. Os diagramas de fases pseudoternários foram construídos usando uma mistura dos tensoativos oleato de sorbitano (Span 80), polisorbato 80 (Tween 80) e o cotensoativo, 1-butanol, além dos triglicerídeos de ácido cáprico/caprílico (Polymol 812) e água destilada. O óleo de pequi foi incorporado na proporção de 3% (m/m). As microemulsões selecionadas foram submetidas à centrifugação, 14 dias de ciclos gela-degela e análises físico-químicas (pH, condutividade elétrica e índice de refração) e depois avaliadas. Cinco das sete formulações iniciais permaneceram estáveis. As formulações foram submetidas à estabilidade acelerada em triplicata por 90 dias. Duas microemulsões mantiveram estabilidade física e química adequada. Os testes de diâmetro hidrodinâmico mostraram que as duas formulações tinham tamanho de gotícula na faixa dos nanômetros. O perfil reológico mostrou que ambas as formulações apresentavam comportamento newtoniano e viscosidade linear. A análise da potencial atividade antioxidante mostrou que as formulações apresentaram atividade significativa e que essa atividade aumentou quando o óleo de pequi foi adicionado. Concluindo, os sistemas aprovados mantiveram uma estabilidade notável e podem ser usados como um veículo de liberação para o óleo extraído do pequi.

Palavras-chave: *Caryocar brasiliense* Camb.; Microemulsão; Atividade antioxidante.

Abstract

The study of drug delivery systems can potentiate the action of ingredients carried by these systems and the evaluation of their quality parameters offers evidences of the compounds behavior in the formulations. This study aimed to obtain microemulsified formulations containing pequi (*Caryocar brasiliense* Camb.) oil and to determine its stability, plus its potential antioxidant activity. The pseudoternary phase diagrams were constructed using a mixture of the surfactants sorbitan oleate (Span 80), polysorbate 80 (Tween 80) and cosurfactant, 1-butanol, besides the triglycerides of capric/caprylic acid (Polymol 812) and distilled water. Pequi oil was incorporated in the proportion of 3% (w/w). The selected microemulsions were subjected to centrifugation, 14 days of heating–cooling cycles, and physicochemical analysis (pH, electrical conductivity and refractive index), and then evaluated. Five out of the initial seven formulations remained stable. Therefore, the formulations were subjected to accelerated conditions in triplicate for 90 days. Two microemulsions maintained adequate physical and chemical stability. The hydrodynamic diameter tests showed that two formulations had droplet size in nanometer scale. The rheological profile showed that both formulations presented newtonian behavior and linear viscosity. The analysis of the antioxidant activity showed that the formulations had significant activity and had this activity increased when the pequi oil was added. Concluding, the approved systems kept a remarkable stability and can be used as a modified vehicle to the oil extracted from pequi.

Keywords: *Caryocar brasiliense* Camb.; Microemulsion; Antioxidant activity.

* Universidade Federal de Mato Grosso, Campus Sinop, Instituto de Ciências da Saúde, CEP 78557-267, Sinop-MT, Brazil.
mayconprt@gmail.com
DOI: 10.21577/1984-6835.20180027

Volume 10, Número 2 Março-Abril 2018

Revista Virtual de Química
ISSN 1984-6835

Development, Stability and Antioxidant Activity of Microemulsion Containing Pequi (*Caryocar brasiliense* Camb.) Oil

Maycon de Paula R. Torres,[a] Aléxia L. Raiser,[b] Márcia Regina Marcílio,[b] Elton B. Ribeiro,[b] Carla Regina Andrighetti,[b] Dênia M. S. Valladão[a]

[a] Federal University of Mato Grosso, *Campus* Sinop, Institute of Health Sciences, Graduation Program in Environmental Sciences, CEP 78557-267, Sinop-MT, Brazil.

[b] Federal University of Mato Grosso, *Campus* Sinop, Institute of Health Sciences, CEP 78557-267, Sinop-MT, Brazil.

* mayconprt@gmail.com

Recebido em 15 de outubro de 2017. Aceito para publicação em 15 de março de 2018

1. Introduction

Delivery systems based on nanostructures have been used in different areas of research for the development of modified release mechanisms of drugs, oils and extracts from vegetable sources.[1,2] Thereby, the potential of these products in microemulsions has been investigated, which are characterized as macroscopically homogeneous, translucent and thermodynamically stable dispersions of oil and water, stabilized by an interfacial film of surfactants and cosurfactant,[3–6] in which both polar and nonpolar molecules can be carried.[7,8] This kind of formulation have as an advantage over other emulsified systems the fact that they have smaller droplet size and are formed spontaneously, without the need of an input of energy. Furthermore, the number of studies regarding microemulsions has increased over the last years, signing it as a very prominent pharmaceutical tool for the delivery of active ingredients.[9]

The mechanism of microemulsion systems with modified release may change the pharmacokinetics of some orally administered drugs and may reduce their toxicity.[10–12] When administered topically, microemulsion has great facility to cross the stratum corneum of the skin,[13–15] increasing the skin penetration and allowing its transport to deep layers and underlying tissues.

Accompanying new market trends, cosmetic industry searches for options of natural compounds, such as natural antioxidants. In this context, *Caryocar brasiliense* (pequi) stands out for being a typical Brazilian tree, widely distributed throughout the Brazilian Cerrado.[16–18] From the pequi fruit, an oil is extracted, which consists mainly of oleic, palmitic and linoleic acids, vitamins, carotenoids and phenols, which are metabolites that prevent lipoperoxidation and thus prevent the formation of free radicals.[19,20] Pequi oil is used in food, cosmetic and in the therapeutic field[21,22] where it showed effectiveness as an antioxidant agent,[23,24] type of activity that may be useful in the treatment of some types of cancer, such as lung cancer.[25] In addition, due to the chemical composition of pequi oil, it has a remarkable capability of retaining water in the skin.[20]

Thus, the use of pequi oil in modified release dosage forms, such as microemulsions, may potentiate these activities. Within this context, this paper aims the development of microemulsion systems containing pequi oil to evaluate its antioxidant potential.

2. Experimental Section

2.1. System Composition

Microemulsions were formulated using distilled water, caprylic/capric triglyceride-Polymol 812 (CCT), Hydrophilic-Lipophilic Balance (HLB) = 10,8 (Henrifarma®, Brazil), Sorbitan monooleate -Span 80® (SP), HLB = 4.3 (Sigma-Aldrich®, Brazil), Polysorbate 80-Tween 80® (TW), HLB = 15.0 (Synth®, Brazil) and 1-butanol (BT), (Synth®, Brazil). Pequi oil was added as the active ingredient at a concentration of 3% (w/w).

2.2. Extraction of pequi oil

The fruits were obtained from the local market in the municipality of Sinop-MT and dried at 40 °C for 48 hours to remove the excessive water. Afterwards, the fruits were milled in an analytical mill and their oil was extracted by ultrasound with hexane in a ratio of 1: 5 for 2 hours at a frequency of 40 KHz and an average temperature of 35 °C. The extracted oil was filtered and then evaporated in a rotary evaporator at a temperature of 50 °C.

2.3. Determination of the surfactant mixing ratio

To determine the HLB of the system a mixture of surfactants and cosurfactants was used. To act as a cosurfactant, BT was used in a ratio equivalent to 10% (fraction =1) of the surfactant mixture.

BT is considered an excellent surfactant[13,26,27] for the preparation of microemulsions and at this concentration it is considered residual and non-toxic.[28] The HLB of the surfactant mixture and the composition required to achieve an HLB corresponding to the oily phase was calculated. The HLB of the SP and TW surfactants were taken into account, according to Ribeiro et al.[12]

2.4. Development of microemulsion systems

The development of the formulations utilized pre-established quantities of the components, and each component ranged from 10% to 80%. In the later stage, pequi oil was added to all formulations at a concentration of 3% (w/w).

The samples were visually rated after 72 hours at 25 °C in regions: ME (microemulsion) MEL (liquid microemulsion), LE (liquid emulsion), EG (gel emulsion), and SF (phase separation). Titrations were performed on mixtures with surfactant/oil phase mass ratios of 1:9 and 9:1 under stirring with 3% pequi oil (w/w) to obtain the delimiting points, area and classification of different systems to form the diagram regions. Adding distilled water in quantities between 0.05 mL and 0.20 mL performed the titration. During this process, the mixture was stirred both manually and mechanically. After homogenization of each titrant, the formulation volumes were visually classified.

Pseudoternary diagrams were constructed from the sample and titration data using SigmaPlot version 8.0 software. In the diagram, the superior vertex represents 100% surfactant/cosurfactant, the lower right represents 100% oily phase, and the lower left represents 100% aqueous phase.

After acquisition of the pseudoternary diagram, composition of the systems that fall into the ME liquid region can be determined. This region contained preselected points that were distributed in lines dividing the region to achieve representative samples of the studied systems.

2.5. Physicochemical Characterization

A method to verify physicochemical parameters was designed to test the suitability of the formulations to act as delivery vehicles and to perform the initial characterization of each sample. This method verified the physical and chemical parameters of the formulations 24 h after preparation and at the end of the stability study. The tests were performed in triplicate. Aliquots of some preselected vehicles were centrifuged (Quimis®, Brazil) at 360 × g for 30 minutes at room temperature. After centrifugation, the samples with visual heterogeneity were excluded. The pH of the vehicles was determined with a pH meter (Tecnopon®, Brazil), calibrated with standard pH 7 and pH 4 buffer solutions. The electrical conductivity was measured using a conductivity meter (Tecnopon®, Brazil) calibrated with a 0.1 mol/L KCl solution to identify the system type (water-in–oil (w/o) or oil-in-water (o/w)) and any tendency toward phase inversion.

2.6. Stability Studies

To perform the preliminary stability test, samples were divided into two groups according to temperature: one group refrigerated at 5 ± 1°C and the other heated at 40 ± 1°C. These systems were preliminarily

subjected to alternating cycles of 5 ± 1°C and 40 ± 1°C for 24 hours each, and the cycles were completed on the 14th day. After these cycles, it was possible to identify the most stable formulations. Afterwards, the formulations were subjected to the accelerated stability test, with exposure to extreme conditions to determine their stability over a longer period of time. The systems were divided into three groups according to temperature: 5 ± 1°C, 25 ± 1°C and 40 ± 1°C. The groups were assessed in triplicate for a period of 90 days. Every 30 days, the formulations were maintained at room temperature for 24 hours to determine their physicochemical properties.[30,31]

2.7. Rheological characterization

Rheological parameters were measured using a Modular Compact Rheometer–MCR 102 (Anton Paar Germany GmbH, Ostfildern, Germany) according to Cotrim et al.[29] In all the experiments, 600 µL of ME was placed on the reading plate surface, and the excess of sample was removed. Readings were taken with continuous control of the gap measurement with the supported TruGap™ at 0.099 mm. The measuring cell was a Toolmaster™ CP 50, and precise temperature control was achieved with T-Ready™. The data and graphics were compiled using SigmaPlot 8.0 software. The flow and viscosity curves were based on the established parameters for the control of shear stress (τ) with 0–5 Pa for the upward curve and 5–0 Pa for the downward curve. These tests were conducted under isothermal conditions at 25 °C, and 75 readings were taken for analysis.

2.8. Dynamic Light Scattering

Colloidal structures of formulation were prepared for analysis by the dynamic light scattering technique. It was then possible to investigate the hydrodynamic diameter and the polydispersity index (PDI) of the dispersed solid. The samples were prepared from a 10:1,000 dilution of the formulation in deionized water in quartz cuvette using deionized water as the reference. Dynamic light scattering analysis were performed using a Zetasizer Nano Z90® (Malvern Instruments®, United Kingdon) with excitation at 632.8 nm.

2.9. Antioxidant activity

Determination of the potential antioxidant activity was carried out using the DPPH (2,2-diphenyl-1-pricil-hydrazila) radical capture method in 96-well ELISA plates, by diluting the microemulsions in methanol. Absorbance readings were performed at the wavelength of 492 nm using methanol as blank. To evaluate the free radical scavenging activity, the percentage of DPPH radical inhibition was calculated by the equation: % inhibition of DPPH = $[(A_0 - A_1) / A_0 \times 100]$, where A_0 = control absorbance (medium with dpph and no antioxidant) and A_1 = sample absorbance.[32-34]

2.10. Statistical analysis

Data were expressed as the mean ± standard deviation. Statistically significant differences in antioxidant activity were evaluated using analysis of variance (ANOVA) followed by multiple comparisons by Tukey test. Statistical significance was considered for a P-value < 0.05.

3. Results and discussion

3.1. Surfactant mixing ratio determination

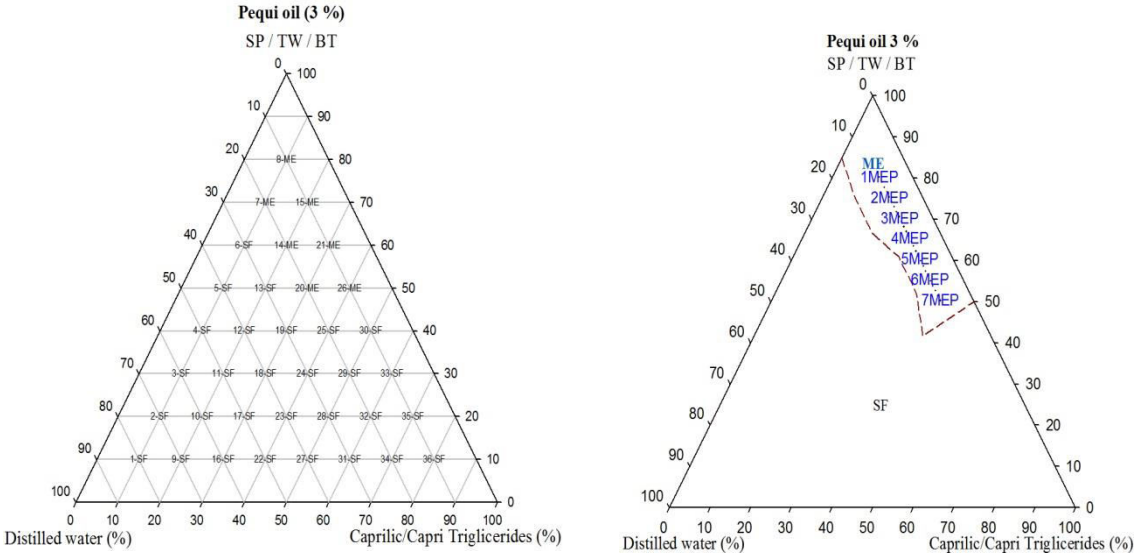
To obtain the surfactant mixing ratio, the cosurfactant concentration was fixed as 10%, and the amount of surfactants SP and TW

were calculated to achieve the required oily phase Caprylic/Capric Triglyceride HLB, which is 10,81.[12] The obtained surfactant fractions were 3.5 and 5.5 for SP and TW, respectively, resulting in the surfactant mix ratio of 3.5:5.5:1.0.

3.2. Development of microemulsion systems

Pseudoternary phase diagrams that were assembled to classify the points and domains of each region are demonstrated in Figure 1.

(a) **(b)**



* Liquid micro emulsion (MEL), Phase separation (SF)

Figure 1. Diagram of the pseudoternary classification of all 36 points in pre-established proportions (a) and domains with the points selected for further study (b).

The SP/TW/BT (3.5:5.5:1.0) surfactant mixture associated with CCT, pequi oil and distilled water in predetermined proportions resulted in diagram points with balanced characteristics. These data resulted in 36 points with distinct characteristics (Fig. 1(a)) and 7 points showed up to be homogeneous systems (ME region). The seven light-yellowish, odorless and translucent formulations (1MEP, 2MEP, 3MEP, 4MEP, 5MEP, 6MEP and 7MEP) selected from the delimited microemulsion domain of the diagram, after titrations (Fig. 2(a)), contained at least 50% of the surfactant mixture, 10% of the aqueous phase, and an oily phase in a range between 10% and 50%, besides the 3% pequi oil added to them. Formulations with surfactant amount below 50% showed instability and phase separation.

These proportions of surfactants for microemulsion formation corroborate results presented in the literature, as well as the low proportion of aqueous phase.[12,26,35] These surfactant contents are necessary for an adequate reduction in the surface tension between the oil and the aqueous phase, allowing the stabilization of the formulation, besides providing the reduced droplet size, in nanometer scale.[6,36]

3.3. Stability and physicochemical analysis

The results of the physicochemical analysis after temperature cycling for the formulations are demonstrated in Table 1. All 7 formulations showed macroscopic stability after centrifugation and were clear and homogeneous. The pH values obtained before and after the test presented a variation range between 6.4 and 8.2.

Table 1. Physicochemical parameters for the preliminary stability study of the formulations

Preliminary Stability							
Parameters Before cycles	**Formulations**						
	1MEP	**2MEP**	**3MEP**	**4MEP**	**5MEP**	**6MEP**	**7MEP**
Centrifugation	N	N	N	N	N	N	N
pH	7.47	7.83	7.90	7.67	7.45	8.20	8.10
Conductivity (µScm-1)	6.51	6.41	5.93	5.48	5.32	6.00	6.82
Refractive index	1.39	1.39	1.38	1.38	1.38	1.38	1.38
Parameters After cycles							
pH	6.78	6.84	7.29	6.40	6.43	6.90	6.56
Conductivity ($\mu S.cm^{-1}$)	2.71	3.26	1.31	4.17	1.91	1.27	5.18
Refractive Index	1.45	1.44	1.45	1.44	1.44	1.44	1.44

N= No phase separation

The assays of conductivity before the preliminary stability showed that all ME are oil-in-water (O / W) due to their values found above the electrical conductivity of water (> 1.3 µS cm $^{-1}$). After the preliminary test, formulations 3MEP and 6MEP presented conductivity below 1.3, what indicated a possible phase inversion,[36,37] therefore these formulations were eliminated from the trials.

The physicochemical analyses of the remaining formulations in accelerated stability conditions are presented in Table 2. The pH values obtained for the systems 1, 2, 4, 5 and 7 MEP showed a small variation from 6.3 to 7.3. The pH value is an important parameter of analysis, once its variation can indicate presence of impurities, occurrence of hydrolysis and decomposition of the formulation, generating instability.[38] The pH values determined were around 7.0 and did not show large range variations, indicating no degradation of the components of the formulations. Moreover, pH values have remained within a biocompatible range which is ideal for formulations in the most diverse routes of administration.[39]

Table 2. Physicochemical parameters during accelerated stability

Formulation	pH			
	Time (days)			
	0	30	60	90
1MEP	6.99 ± 0.13	6.88 ± 0.27	6.58 ± 0.45	6.52 ± 0.46
2MEP	6.86 ± 0.13	6.77 ± 0.33	6.54 ± 0.71	6.63 ± 0.54
4MEP	7.01 ± 0.14	6.60 ± 0.48	6.58 ± 0.40	7.08 ± 0.40
5MEP	7.19 ± 0.20	6.87 ± 0.29	6.95 ± 0.33	6.85 ± 0.31
7MEP	7.38 ± 0.17	7.20 ± 0.25	6.36 ± 1.39	6.83 ± 0.63
Formulation	**Conductivity (µS.cm^{-1})**			
	Time (days)			
	0	30	60	90
1MEP	5.88 ± 0.60	4.08 ± 1.86	3.81 ± 1.60	3.61 ± 1.79
2MEP	4.88 ± 0.61	3.76 ± 1.13	3.39 ± 1.22	3.13 ± 1.30
4MEP	4.10 ± 1.17	2.36 ± 1.14	2.77 ± 1.37	2.19 ± 1.00
5MEP	4.56 ± 1.14	2.69 ± 1.27	2.45 ± 1.31	2.36 ± 1.19
7MEP	6.08 ± 0.56	3.21 ± 2.07	2.58 ± 2.03	2.59 ± 2.16
Formulation	**Refractive Index**			
	Time (days)			
	0	30	60	90
1MEP	1.39 ± 0.01	1.40 ± 0.05	1.39 ± 0.05	1.40 ± 0.05
2MEP	1.39 ± 0.01	1.39 ± 0.03	1.39 ± 0.04	1.39 ± 0.04
4MEP	1.39 ± 0.02	1.39 ± 0.03	1.39 ± 0.03	1.39 ± 0.03
5MEP	1.39 ± 0.01	1.39 ± 0.04	1.39 ± 0.04	1.39 ± 0.03
7MEP	1.39 ± 0.01	1.39 ± 0.03	1.39 ± 0.04	1.39 ± 0.03

The formulations also presented a small variation on the refractive values through time. The values remained around 1.39 and 1.4, demonstrating constancy during the entire trial. Determination of the refractive index is an important physical characterization test that relates to the specific characteristics of each formulation, like chain lengths and number of instaurations of the ingredients.[40] The refractive index obtained for the developed formulations remained constant throughout the experiment, showing that there were no changes in the homogeneity of the formulations. This can also be sustained by the fact that a stable homogeneous mixture is translucent, while an unstable and heterogeneous mixture is turbid, unless the components of the mixture have identical refractive index.[41]

All formulations demonstrated decreasing electrical conductivity through time, although formulations 1MEP and 2MEP remained with values above 1.3 µS·cm^{-1}. The other formulations (4 MEP, 5 MEP and 7 MEP)

presented mean conductivity values near 1.3 μS·cm^{-1} after 90 days of tests, and some even below it, if the standard deviation values are considered. The conductivity for formulations 4, 5 and 7 MEP presented values too close to 1.3 μS cm $^{-1}$ after 90 days of tests, considering the parameter of variation within the same groups in different temperature conditions.

These results suggest that in formulations 4 MEP, 5 MEP and 7 MEP phase inversion occurred, indicating that only formulations 1MEP and 2 MEP were approved on the accelerated stability tests. Based on these results, these three formulations showed a tendency to phase inversion, which could compromise their stability, fact that was not observed in formulations 1 and 2 MEP. Thus, only 1 MEP, which was constituted by 10% of aqueous phase, 10% of oily phase and 80% of surfactant mix, and 2MEP, which was constituted by 10% of aqueous phase, 15% of oily phase and 75% of surfactant mix, maintained all the characteristics of stable O/W systems and had evaluated their rheological characteristics, light scattering and potential antioxidant activity.

3.4. Rheological characterization

Flow and viscosity curves, as a function of shear rate (), were attained for the rheological characterization. The flow curves of the pre-selected ME formulations (1MEP and 2MEP) were measured (Figure 2a). It was observed that for samples 1MEP and 2MEP, the flow curve began at the origin and exhibited linear ascending and descending behavior, indicating that these formulations behave as newtonian fluids at 25 °C. In addition, viscosity curves of samples passing the test, as a function of , are displayed in Figure 2b. It is observed that the viscosity values did not change as the shear rate increases, with values between 0.20 and 0.25 Pas.

Literature reports that microemulsified systems with a newtonian profile and linear viscosity show stability over a long period,[12,36,37] being a good parameter of evaluation and characterization of stable systems.[42] Other studies that analyzed the stability and rheological behavior of emulsion systems containing Span 83 and Tween 80 obtained systems with non-newtonian flow and higher viscosity.[43] Researches also report that the addition of cosurfactants and / or oily phases may lead to a reduction in viscosity.[35]

3.5. Droplet size analysis and particle size distribution

Light scattering is a routine technique for determining the hydrodynamic diameter of nanostructured colloidal systems.[44] Droplet size analysis and particle size distribution attained by DLS technique for samples 1MEP and 2MEP are displayed in Figure 3.

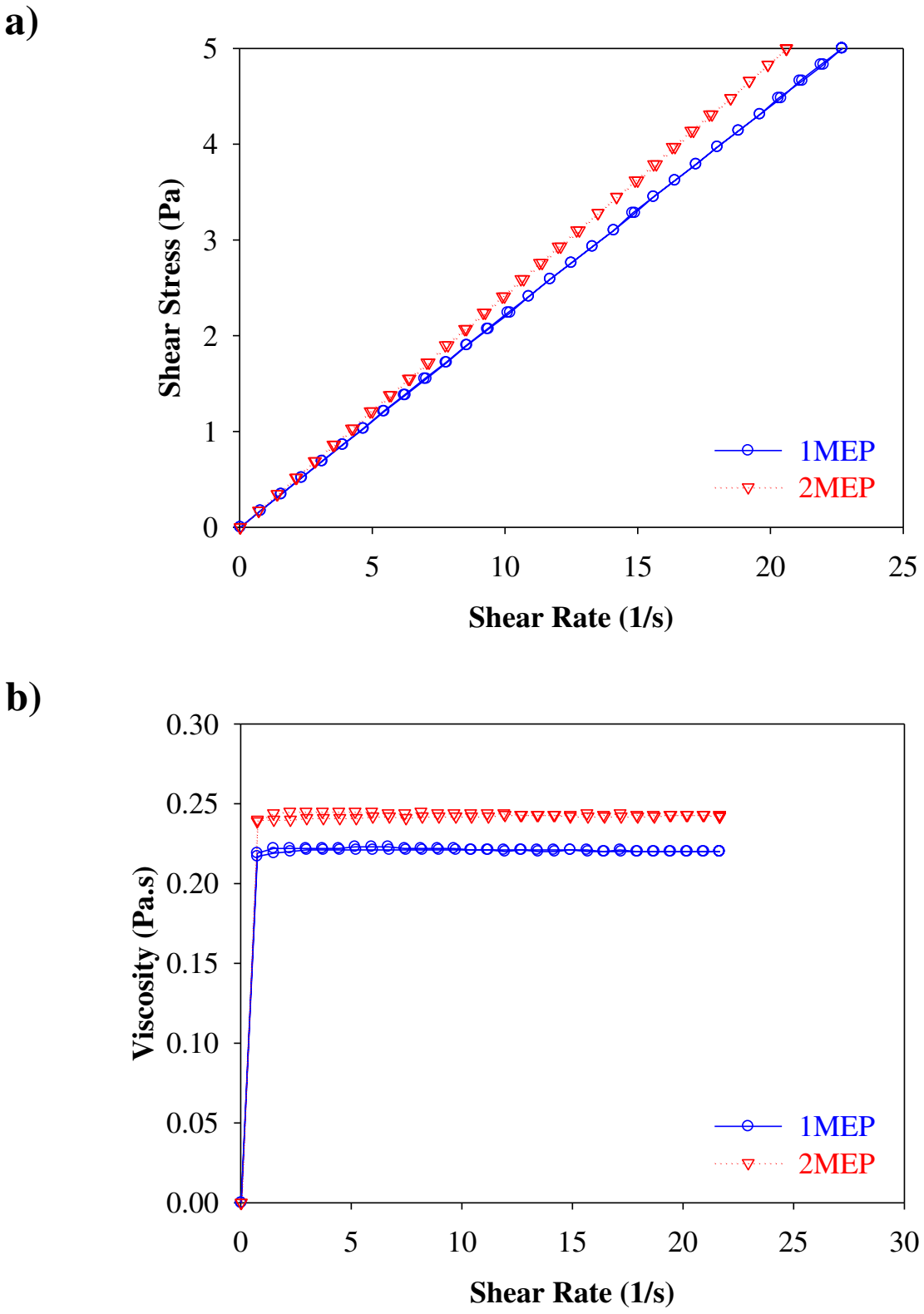


Figure 2. Flow (**a**) and viscosity curves at 25 °C of selected Pequi oil microemulsions (MEP)

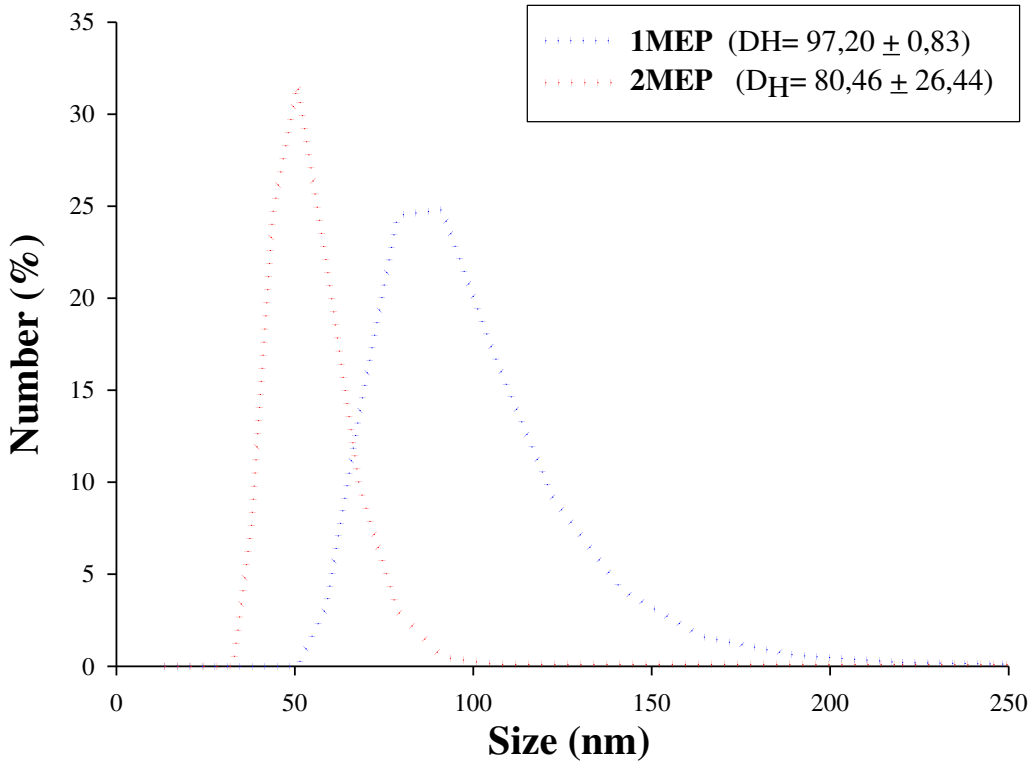


Figure 3. Droplet size distribution of selected Pequi oil microemulsions (1MEP and 2MEP)

Our results showed that formulation 1MEP presented 97.20 nm of diameter and standard deviation indicating small variation in the size of the droplets, besides a PDI value of 0.467 ± 0.0310, and formulation 2MEP, 80.46 nm of diameter with a PDI value of 0.450 ± 0.0219. Internal phase droplets with extremely small sizes are characteristic of thermodynamically stable systems if they are formed spontaneously, but the increase of the diameter of the internal phase usually leads to the modification of the internal microstructure of the system.[40]

3.6. Antioxidant activity

Results for the antioxidant activity of pequi oil, as well for the control ME and the pequi oil ME are shown in Figure 4. Formulations 1MEP and 2MEP presented a significantly superior capacity of radical sequestration when compared to the control.

The potential antioxidant activity by the DPPH method showed that formulations 1MEP and 2MEP presented 33.28 and 31.22%, respectively, of free radical sequestration, showing that the activities are distinct.

The oil incorporated in the ME presented higher potential than the pequi oil in natura, although it was a slight increase (3.84%) when compared to the base formulation. The activity of the base formulation is probably attributed to the fact that capric and caprylic acids have proven antioxidant activity,[45] therefore the CCT potentially carries along a part of this activity.

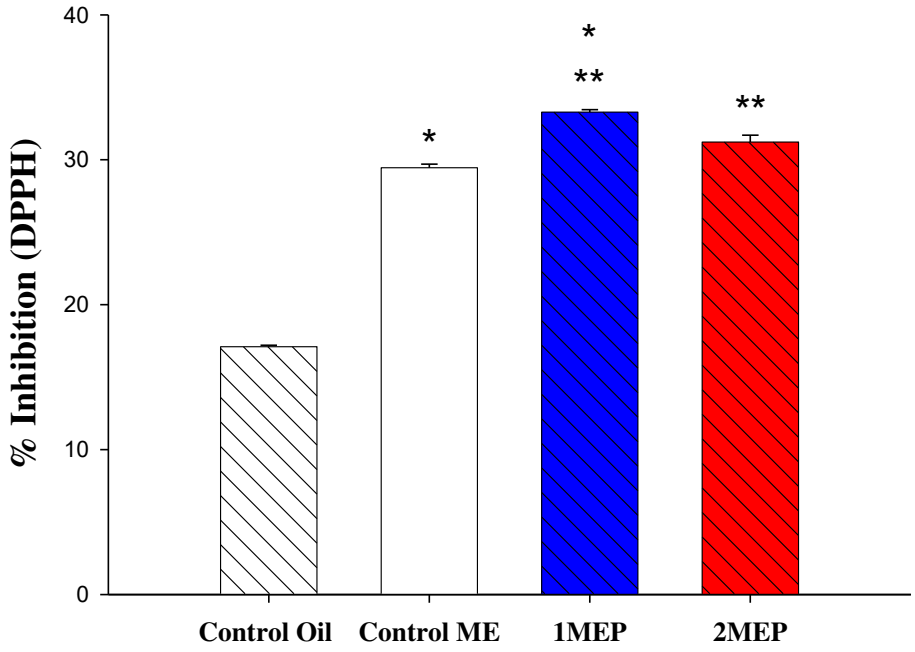


Figure 4. Potential antioxidant activity of pequi oil microemulsions (1MEP and 2MEP) compared to the control microemulsion and pequi oil, expressed as % of inhibition. Where: Values were expressed as the mean ± SD of triplicate. ANOVA: $p < 0.0001$ and $F = 2126.2344$

Several other oils extracted from seeds of fruits from cerrado showed potential antioxidant activity,[46] highlighting this biome as a potential source of natural products with interesting activities.

Hamed, Sadek and Edris [47] and Chaiyana, Phongpradist and Leelapornpisid [48] analyzed the antioxidant activity of crude oils and microemulsions containing these oils, coming to the conclusion that microemulsions presented increased activities, showing that the potential antioxidant activity of oil is enhanced when it is carried in a microemulsified formulation, corroborating our results. In contrast, Kim et al. in their [49] study exhibited a decrease on the antioxidant activity in microemulsions with *Melaleuca alternifolia* oil, when compared to the pure oil. The formulations had different components to those used in this study, what suggests the influence of the formulation composition on the activities.

The developed formulations showed significant antioxidant potential when compared to other emulsified formulations using isolated antioxidants,[50] and control formulations containing BHT.[51]

Although formulation 1MEP presented a larger droplet diameter, it displayed a lower standard deviation, which demonstrates a greater uniformity in the droplets of the system, in addition to presenting a potential antioxidant activity that was significantly higher than 2MEP, which would make it the formulation of choice for later studies.

The advantage of the developed formulations is the fact that the antioxidant activity observed for pequi oil is related to the presence of compounds that inhibit the formation of free radicals, such as carotenoids and phenols.[38] This fact associated with microemulsion systems make it an option for the development of modified release cosmetics and / or

pharmaceuticals,[4,39] since it collaborates on the bioavailability of active ingredients.

4. Conclusion

The development of ME associated with natural compounds with potential antioxidant activity as a vehicle for pharmaceuticals and/or cosmetics probably represents the greatest potentiality of these systems, since they may increase the bioavailability and / or activity of some drugs and / or active ingredients.

Acknowledgments

The financial support from the Brazilian agencies CNPq and FAPEMAT/MT, is gratefully acknowledged.

References

[1] Faraji, A. H.; Wipf, P. Nanoparticles in cellular drug delivery. *Bioorganic and Medicinal Chemistry* **2009**, *17*, 2950. [CrossRef] [PubMed]

[2] Bonifácio, B. V.; da Silva, P. B.; Ramos, M. A. dos S.; Negri, K. M. S.; Bauab, T. M.; Chorilli, M. Nanotechnology-based drug delivery systems and herbal medicines: A review. *Inernational Journal of Nanomedicine* **2014**, *9*, 1. [CrossRef] [PubMed]

[3] Lawrence, M. J.; Rees, G. D. Microemulsion-based media as novel drug delivery systems. *Advanced Drug Delivery Reviews* **2012**, *64*, 175. [CrossRef] [PubMed]

[4] de Oliveira, A. G.; Scarpa, M. V.; Correa, M. A.; Cera, L. F. R.; Formariz, T. P. Microemulsões: estrutura e aplicações como sistema de liberação de fármacos. *Química Nova* **2004**, *27*, 131. [CrossRef]

[5] Formariz, T. P.; Cristina, M.; Urban, C.; Antônio, A.; Palmira, M.; Gremião, D.; Oliveira, A. G. De Microemulsões e fases líquidas cristalinas como sistemas de liberação de fármacos. *Revista Brasileira de Ciências Farmacêuticas* **2005**, *41*, 301. [CrossRef]

[6] Fanun, M. Microemulsion as delivery systems. *Current Opinion in Colloid and Interface Science* **2012**, *17*, 306. [CrossRef]

[7] Lawrence, M. J. Microemulsions as drug delivery vehicles. *Current Opinion in Colloid & Interface Science* **1996**, *1*, 826. [CrossRef]

[8] Rane, S. S.; Anderson, B. D. What determines drug solubility in lipid vehicles: is it predictable? *Advanced Drug Delivery Reviews* **2008**, *60*, 638. [CrossRef] [PubMed]

[9] Callender, S. P.; Mathews, J. A.; Kobernyk, K.; Wettig, S. D. Microemulsion utility in pharmaceuticals: Implications for multi-drug delivery. *International Journal of Pharmaceutics* **2017**, *526*, 425. [CrossRef] [PubMed]

[10] Nornoo, A. O.; Zheng, H.; Lopes, L. B.; Johnson-Restrepo, B.; Kannan, K.; Reed, R. Oral microemulsions of paclitaxel: In situ and pharmacokinetic studies. *European Journal of Pharmaceutics and Biopharmaceutics* **2009**, *71*, 310. [CrossRef] [PubMed]

[11] Ghosh, P. K.; Majithiya, R. J.; Umrethia, M. L.; Murthy, R. S. R. Design and development of microemulsion drug delivery system of acyclovir for improvement of oral bioavailability. *AAPS PharmSciTech* **2006**, *7*, 77. [CrossRef]

[12] Ribeiro, E. B.; Kelly, P.; Lanes, D.; Galdeano, N.; Chaud, A.; Pessoa, R. S.; Honorio-França, A. C.; França, E. L. Microemulsions with Levamisole Delivery Systems as Novel Immunomodulating Agents with Potential for Amebiasis Therapies. *Science of Advanced Matererials* **2015**, *7*, 15. [CrossRef]

[13] Kumar, A.; Kushwaha, V.; Sharma, P. K. Pharmaceutical microemulsion: Formulation, characterization and drug deliveries across skin. *International Journal of Drug Development and Research* **2014**, *6*, 1. [Link]

[14] Kogan, A.; Garti, N. Microemulsions as transdermal drug delivery vehicles. *Advances in Colloid and Interface Science* **2006**, *123–126*, 369. [CrossRef] [PubMed]

[15] Peira, E.; Carlotti, M. E.; Trotta, C.; Cavalli, R.; Trotta, M. Positively charged microemulsions for topical application.

International Journal of Pharmaceutics **2008**, *346*, 119. [CrossRef] [PubMed]

[16] de Oliveira, M. L. M.; Nunes-Pinheiro, D. C. S.; Tomé, A. R.; Mota, E. F.; Lima-Verde, I. A.; Pinheiro, F. G. D. M.; Campello, C. C.; de Morais, S. M. In vivo topical anti-inflammatory and wound healing activities of the fixed oil of Caryocar coriaceum Wittm. seeds. *Journal of Ethnopharmacology* **2010**, *129*, 214. [CrossRef] [PubMed]

[17] Camargo, M. P. de; Estevam, A.; Feroldi, M.; Cremonez, P. A. A Cultura do Pequi (Caryocar Brasiliense Camb.) na recuperação de áreas degradas e como alternativa para a produção de biodiesel no Brasil. *Journal of Agronomic Sciences* **2014**, *13*, 180. [Link]

[18] Alves, A. M.; Fernandes, D. C.; Souza, A. G. de O.; Naves, R. V.; Naves, M. M. Características físicas e nutricionais de pequis oriundos dos estados de Tocantins , Goiás e Minas Gerais. *Brazilian Journal of Food Technology* **2014**, *17*, 198. [CrossRef]

[19] Barra, P. M. de C.; Oliveira, M. A. L. de; Nery-enes, B.; Cardoso, L. D. M.; Cesário, C. C.; Moreira, A. V. B.; Ana, H. M. P.; Peluzio, M. C. G. Simultaneous analysis of saturated and unsaturated fatty acids present in pequi fruits. *Quimica Nova* **2013**, *36*, 1430. [CrossRef]

[20] Lima, A. D. E.; Mara, A. N. A.; Silva, D. E. O. E.; Trindade, R. A.; Torres, R. P.; Mancini-filho, J. Composição química e compostos bioativos presentes na polpa e na amêndoas do pequi (Caryocar brasiliense , Camb.). *Revista Brasileira de Fruticultura* **2007**, *29*, 695. [CrossRef]

[21] Faria, W. C. S.; Damasceno, G. A. de B.; Ferrari, M. Moisturizing effect of a cosmetic formulation containing pequi oil (Caryocar brasiliense) from the Brazilian cerrado biome. *Brazilian Journal of Pharmaceutical Sciences* **2014**, *50*, 131. [CrossRef]

[22] Bailão, E. F. L. C.; Devilla, I. A.; Conceição, E. C. da; Borges, L. L. Bioactive Compounds Found in Brazilian Cerrado Fruits. *International Journal of Molecular Sciences* **2015**, *16*, 23760. [CrossRef] [PubMed]

[23] Rocha, L. B.; Melo, A. M. .; Paula, S. L. A.; Nobre, S. A. M.; Abreu, I. N. Gallic acid as the major antioxidant in pequi (*Caryocar brasiliense* Camb.) fruit peel. *Revista Brasileira de Plantas Medicinais* **2015**, *17*, 592. [CrossRef]

[24] Roesler, R.; Catharino, R. R.; Eberlin, M. N.; Pastore, G. Antioxidant activity of Caryocar brasiliense (pequi) and characterization of components by electrospray ionization mass spectrometry. *Food Chemistry* **2008**, *110*, 711. [CrossRef]

[25] Colombo, N. B. R.; Rangel, M. P.; Martins, V.; Hage, M.; Gelain, D. P.; Barbeiro, D. F.; Grisolia, C. K. Caryocar brasiliense camb protects against genomic and oxidative damage in urethane-induced lung carcinogenesis. *Brazilian Journal of Medical and Biological Research* **2015**, *48*, 852. [CrossRef] [PubMed]

[26] Boonme, P.; Krauel, K.; Graf, A.; Rades, T.; Junyaprasert, V. B. Characterization of microemulsion structures in the pseudoternary phase diagram of isopropyl palmitate/water/Brij 97:1-butanol. *AAPS PharmSciTech* **2006**, *7*, 1. [CrossRef] [PubMed]

[27] Wilk, K. a; Zielińska, K.; Hamerska-Dudra, A.; Jezierski, A. Biocompatible microemulsions of dicephalic aldonamide-type surfactants: formulation, structure and temperature influence. *Journal of Colloid and Interface Science* **2009**, *334*, 87. [CrossRef] [PubMed]

[28] USP *United States Pharmacopeial USP30/NF25*, 30 th Rockville, MD, 2007.

[29] França, E. L.; Ribeiro, E. B.; Scherer, E. F.; Cantarini, D. G.; Pessôa, R. S.; França, F. L.; Honorio-França, A. C. Effects of Momordica charantia L. on the Blood Rheological Properties in Diabetic Patients. *BioMed Research International* **2014**, *2014*, 1. [CrossRef]

[30] ANVISA, A. N. de V. S. *Guia de estabilidade de produtos cosméticos*, 1a. ed, Brasília, 2004. [Link]

[31] BRASIL *Farmacopeia Brasileira*, 1a. ed, Brasília, 2010. [Link]

[32] Elmasta, M.; Gulçin, I.; Isildak, O.; Küfrevio, Ö.; Ibaoglu, K.; Aboul-enein, H. Y. Iranian Chemical Society Radical Scavenging Activity and Antioxidant Capacity of Bay Leaf Extracts.

Journal of the Irananian Chemical Society **2006**, *3*, 258. [CrossRef]

[33] Lange, M. K.; Heberlé, G.; Milão, D. Avaliação da estabilidade e atividade antioxidante de uma emulsão base não-iônica contendo resveratrol. *Brazilian Journal of Pharmaceutical Sciences* **2009**, *45*, 145. [CrossRef]

[34] Li, W. J.; Cheng, X. L.; Liu, J.; Lin, R. C.; Wang, G. L.; Du, S. S.; Liu, Z. L. Phenolic compounds and antioxidant activities of Liriope muscari. *Molecules* **2012**, *17*, 1797. [CrossRef] [PubMed]

[35] Prajapati, H. N.; Dalrymple, D. M.; Serajuddin, A. T. M. A Comparative Evaluation of Mono- , Di- and Triglyceride of Medium Chain Fatty Acids by Lipid / Surfactant / Water Phase Diagram , Solubility Determination and Dispersion Testing for Application in Pharmaceutical Dosage Form Development. *Pharmaceutical Research* **2012**, *29*, 285. [CrossRef] [PubMed]

[36] Pessoa, R. S.; França, E. L.; Ribeiro, E. B.; Abud, N. G.; Honorio-, A. C. Microemulsion of babassu oil as a natural product to improve human immune system function. *Drug Design Development and Therapy* **2015**, *9*, 21. [CrossRef]

[37] Lanes, P. K. D.; Ribeiro, E. B.; Chaud, N. G. A.; Pessoa, R. S.; França, E. L.; Honorio-França, A. C. Effects of microemulsion incorporated with Orbignya martiana Rodr on the functional activity of blood phagocytes. *Wulfenia Journal* **2016**, *1*, 214. [CrossRef]

[38] Pianovski, A. R.; Lima, C. G.; Silva, K. K.; Franco, V.; Carvalho, M.; Musis, C. R. De; Regina, S.; Machado, P. Desenvolvimento e avaliação da estabilidade de emulsões múltiplas O/A/O com óleo de pequi (Caryocar brasilienses). *Revista Brasileira de Farmácia* **2008**, *89*, 155. [Link]

[39] Ribeiro, E. B.; Honório-França, A. C.; França, E. L.; Soler, M. A. G. Design and Development of Nanoemulsion Systems Containing Interferon Gamma. *Protein and Peptide Letters* **2016**, *23*, 626. [CrossRef] [PubMed]

[40] Constantinides, P. P.; Yiv, S. H. Particle size determination of phase-inverted water-in-oil microemulsions under different dilution and storage conditions. *International Journal of Pharmaceutics* **1995**, *115*, 225. [CrossRef]

[41] Ferreira, E. de S.; Silveira, C. da S.; Lucien, V. G.; Amaral, A. S. Caracterização físico-química da amêndoa, torta e composição dos ácidos graxos majoritários do óleo bruto da castanha-do-Brasil (Bertholletia excelsa H.B.K). *Alimentos e Nutrição* **2006**, *17*, 203. [Link]

[42] Cotrim, A. C. de M.; Honorio-França, A. C.; França, E. L. Rheology analysis can be added in thermal stability test for design microemulsion materials. *Biointerface Research in Applied Chemistry* **2016**, *6*, 1128. [Link]

[43] Jiao, J.; Burgess, D. J. Rheology and stability of water-in-oil-in-water multiple emulsions containing Span 83 and Tween 80. *AAPS PharmSciTech* **2003**, *5*, 1. [CrossRef] [PubMed]

[44] Fryd, M. M.; Mason, T. G. Advanced Nanoemulsions. *Annual Review of Physical Chemistry* **2012**, *63*, 493. [CrossRef] [PubMed]

[45] Sengupta, A.; Ghosh, M.; Bhattacharyya, D. K. In vitro antioxidant assay of medium chain fatty acid rich rice bran oil in comparison to native rice bran oil. *Journal of Food, Science and Technology* **2015**, *52*, 5188. [CrossRef] [PubMed]

[46] LUZIA, D. M. M. Propriedades funcionais de óleos extraídos de sementes de frutos do cerrado brasileiro. 2012. 234p. Tese (Doutorado em engenharia e ciência de alimentos) – UNESP, São José do Rio Preto. 2012. [Link]

[47] Hamed, S. F.; Sadek, Z.; Edris, A. Antioxidant and Antimicrobial Activities of Clove Bud Essential Oil and Eugenol Nanoparticles in alcohol-free Microemulsion. *Journal of Oleo Science* **2012**, *648*, 641. [CrossRef] [PubMed]

[48] Chaiyana, W.; Phongpradist, R.; Leelapornpisid, P. Characterization of hydrodistillated pomelo peel oil and the enhancement of biological activities using microemulsion formulations. *International Journal of Pharmacy and Pharmeutical Sciences* **2014**, *6*, 596. [Link]

[49] Kim, S.; Kiong, W.; Shen, S.; Dong, Y.; Tan, R. B. H. Phase behavior, microstructure transition , and antiradical activity of sucrose laurate / propylene glycol / the essential oil of Melaleuca alternifolia / water microemulsions. *Colloids Surfaces A: Physicochemical and Engineering Aspects* **2009**, *348*, 289. [CrossRef]

[50] Cefali, L. C.; Souza-moreira, T. M.; Corrêa, M. A.; Regina, H.; Salgado, N.; Lucia, V.; Isaac, B. Development and evaluation of an emulsion containing lycopene for combating acceleration of skin aging. *Brazilian Journal of Pharmaceutical Sciences* **2015**, *51*, 579. [CrossRef]

[51] Tozetto, J. T.; Tozetto, A. T.; Hoshino, B. T.; Andrighetti, C. R.; Ribeiro, E. B.; Cavalheiro, L.; Ferrarinia, S. R. Extract of Punica granatum L.: an alternative to BHT as an Antioxidant in semissolid emulsifild systems. *Química Nova* **2017**, *40*, 97. [CrossRef]