Multidisciplinary Sciences Reports
Original article

PHYSICAL-CHEMICAL ANALYSIS OF POLYMER NANOCAPSULES CONTAINING COPAÍBA (*Copaifera* spp.) AND ANDIROBA (*Carapa guianensis* Aubl.) VEGETABLE OILS

ANÁLISE FÍSICO-QUÍMICA DE NANOCÁPSULAS POLIMÉRICAS CONTENDO ÓLEOS VEGETAIS DE COPAÍBA (*Copaifera spp*.) E ANDIROBA (*Carapa guianensis* Aubl.)

1 William Ferreira Alves william.alves@ufac.br

2 Marcelo Castanheira da Silva marcelo.silva@ufac.br

3 Rogério de Freitas Lacerda rogerio.lacerda@ufac.br

4 Anselmo Fortunato Ruiz Rodriguez anselmo.rodriguez@ufac.br

5 Bráulio Soares Archanjo bsarchanjo@inmetro.gov.br

1 Nanobiotechnology Laboratory, Center for Biological and Natural Sciences, Federal University of Acre

2-3 Center for Biological and Natural Sciences, Federal University of Acre

4 Biotechnology and Biodiversity Postgraduate Program, Federal University of Acre

5 Materials Metrology Division, National Institute of Metrology, Quality and Technology (Inmetro)

Mult. Sci. Rep. 2023; v. 3 n. 4 / ISSN: 2764-0388
DOI: https://doi.org/10.54038/ms.v3i4.51
Subject: 06/26/2023 – Accepted: 08/12/2023

ABSTRACT

Vegetable oils are natural products that are in liquid form, volatile, and aromatic, extracted from different parts of plants. In the Amazon region, vegetable oils are traded and sold by the community to increase their family income. Polymeric nanocapsules are nanoparticles composed of an encapsulating agent (biodegradable polymers) such as polycaprolactone, poly(methyl methacrylate), and creatine, which enclose lipophilic substances (active core). The main technological characteristics of these nanostructured systems are the controlled release of active ingredients, biodisponibility, and chemical and physical stability. In this study, nanocapsules containing copaiba and andiroba vegetable oils, as well as their mixtures, were synthesized. The nanocapsules showed particle sizes, Zeta potentials, and polydispersity indices (PDI) ranging from 117 to 218 nm, 28.5 to 35 mV, and 0.130 to 0.251, respectively. The 30/70 ratio (Copaiba/Andiroba) exhibited the most stable mixture among them.

KEYWORDS: Bionanotechnology. Natural Products. Amazônia Ocidental.

RESUMO

Os óleos vegetais são produtos naturais que se apresentam na forma líquida, volátil e aromática, extraídos de diversas partes das plantas. Na região amazônica, os óleos vegetais são comercializados e vendidos pela comunidade para aumentar a renda familiar. Nanocápsulas poliméricas são nanopartículas compostas por um agente encapsulante (polímeros biodegradáveis) como policaprolactona, poli(metacrilato de metila) e creatina, que encerram substâncias lipofílicas (núcleo ativo). As principais características tecnológicas desses sistemas nanoestruturados são a liberação controlada de princípios ativos, a biodisponibilidade e a estabilidade química e física. Neste estudo foram sintetizadas nanocápsulas contendo óleos vegetais de copaíba e andiroba, bem como suas misturas. As nanocápsulas apresentaram tamanhos de partícula, potenciais Zeta e índices de polidispersidade (PDI) variando de 117 a 218 nm, 28,5 a 35 mV e 0,130 a 0,251, respectivamente. A relação 30/70 (Copaíba/Andiroba) apresentou a mistura mais estável entre elas.

PALAVRAS-CHAVE: Bionanotecnologia. Produtos Naturais. Amazônia Ocidental.

INTRODUCTION

During sociocultural development, the use of medicinal plants for therapeutic purposes has overcome all barriers and obstacles. These medicinal plants are widely used by the population with the aim of healing or relieving many diseases (1-2). Currently, there is a significant increase in the prescription of medicinal plants and guidance provided by healthcare professionals. This increase can be attributed to various factors, such as the encouragement of government policies, the influence of social media, and aesthetic motivations (3-5).

Medicinal plants are known to be natural sources of numerous substances, which are typically produced by the plant as a defense mechanism against pathogens. Often, they are used as complementary therapy to established treatments, either influenced by ancient practices or recommended by family members and/or close associates (5-7).

Thus, medicinal plants containing essential oils and vegetable oils (known as fixed oils) have attracted attention of the people due to their promising biological activity. Vegetable oils contain bioactive compounds that have potential therapeutic properties. Their antimicrobial, anti-inflammatory, antifungal, and wound-healing properties have sparked the interest of researchers and healthcare professionals (8). These oils have been highlighted as highly promising active raw materials since they can be rapidly degraded (degradation by light radiation, temperature, heat, oxidation, etc.) and practically do not present toxicity and/or environmental problems (8-9). These characteristics have driven studies to further explore the potential of medicinal plants and their vegetable oils as natural and safe alternatives in the field of health and well-being (10-11).

Polymeric nanocapsules with vegetable oils are tiny structures made of polymers that surround vegetable oils and contain active substances. These nanocapsules have generated significant interest in the field of materials science and nanotechnology due to their special properties and potential applications in various segments, such as cosmetics, food, and medicine. The manufacturing of these nanocapsules involves the use of specific techniques such as complex coacervation,

Mult. Sci. Rep. 2023; v. 3 n. 4 / ISSN: 2764-0388
DOI: https://doi.org/10.54038/ms.v3i4.51
Subject: 06/26/2023 – Accepted: 08/12/2023

emulsification, or miniemulsion polymerization. The goal is to obtain extremely small particles on the nanometer scale, with diameters ranging from 10 to 500 nanometers, that are stable and efficient in the controlled release of the encapsulated vegetable oils (14-16).

Andiroba oil, from Carapa guianensis seeds, is rich in fatty acids and limonoids. Used in skincare for anti-inflammatory and wound-healing properties (16-17). Copaiba oil, from Copaifera tree resin, is high in beta-caryophyllene, an anti-inflammatory compound. It helps with pain, inflammation, immune response, and respiratory health. Both oils are potent natural remedies for well-being.

There are several studies that have developed nanocapsules containing essential oils, synthesized with various concentrations, and characterized by size and chemical and physical stability. However, there are few studies that have used essential oils from parts of plants from the Western Amazon in nanostructured systems (nanocapsules). Therefore, this present study is important for the Amazon region since it is related to technological processing studies and the development of nanocapsules using nanotechnology based on raw materials from the Amazon Rainforest.

Mult. Sci. Rep. 2023; v. 3 n. 4 / ISSN: 2764-0388
DOI: https://doi.org/10.54038/ms.v3i4.51
Subject: 06/26/2023 – Accepted: 08/12/2023

MATERIAL AND METHODS

The vegetable oils of copaiba and andiroba were extracted and acquired from a cooperative in Mâncio Lima, Acre, Brazil. Subsequently, they were stored in a sealed container and kept in a regular refrigerator to prevent oxidation in the presence of oxygen and degradation due to light radiation. Once the samples were obtained, the quality was assessed by analyzing the acidity index and density for all the oils.

Analysis of Physicochemical Parameters

The vegetable oils obtained from parts of plants from the Western Amazon were stored in sealed containers and kept in a regular refrigerator (temperature of 5,0 °C for 45 days maximum) to prevent oxidation in the presence of oxygen and degradation caused by light radiation. After obtaining the samples, the quality of the oils was verified through the analysis of acidity index and density.

Density determination

The determination of oil density was carried out according to the standards of the *Instituto Adolfo Lutz* (2008), using the 5 mL pipette method. 3.00 mL of oil were added to the pipette, and after 10 minutes at a temperature of 20 °C, the density of each oil was evaluated on a semi-analytical balance. The density of the vegetable oils was evaluated in triplicate, obtaining the average density value and its respective standard deviation (σ) (14).

Refractive index determination

The benchtop ABBE refractometer was used to determine the refractive index of the oil samples. Approximately four drops of each oil were added to the sample holder of the refractometer. After the temperature reached 20 °C, the refractive index value of each sample was recorded. The refractive index was evaluated in triplicate, obtaining the average value and its respective standard deviation (σ) (14).

Mult. Sci. Rep. 2023; v. 3 n. 4 / ISSN: 2764-0388
DOI: https://doi.org/10.54038/ms.v3i4.51
Subject: 06/26/2023 – Accepted: 08/12/2023

Acidity determination

2 g of oil were placed in a 125.00 mL Erlenmeyer flask, and then 25.00 mL of a neutral ether-alcohol solution (2:1) previously prepared was added. Two drops of phenolphthalein indicator were added to this solution. After homogenization, the solution was titrated with a 0.1 M sodium hydroxide solution until a color change to pink occurred. The acidity index was evaluated in triplicate, obtaining the average value and its respective standard deviation (σ) (14).

pH Determination

The pH analysis of the samples was performed using the QUIMIS® pH meter with the universal pH electrode from the same brand. The pH was evaluated in triplicate, obtaining the average value and its respective standard deviation (σ) (14).

Nanocapsules

To obtain nanocapsules containing vegetable oils from the Western Amazon, the technique of preformed polymer interfacial deposition described by Fessi (1989) (15) was used. In the organic phase, 1 mL of the mentioned oils was added along with 250.0 mg of poly(ε-caprolactone) (PCL) polymer, 130.00 mL of acetone, and 191.5 mg of sorbitan monostearate. In the aqueous phase, a mixture was prepared containing 0.33 mL of caprylic/capric acid triglycerides with 191.5 mg of polysorbate 80. The organic phase was added to the aqueous phase and magnetically stirred for 15 minutes at 45 °C. The suspension was concentrated to 2/3 of the total volume in a rotary evaporator to remove the organic solvent. All suspensions were prepared in triplicate.

Statistical

The data were subjected to analysis of variance (ANOVA) and regression analysis. Differences between the means of the treatments were tested using the Tukey's test at a 5% significance level. Analyses were carried out using the RStudio Core Team software, version 3.6.3, with the ExpDes.pt, car, stats, and fBasics packages.

Mult. Sci. Rep. 2023; v. 3 n. 4 / ISSN: 2764-0388
DOI: https://doi.org/10.54038/ms.v3i4.51
Subject: 06/26/2023 – Accepted: 08/12/2023

RESULTS AND DISCUSSIONS

According to Table 1, differences can be observed in the acidity index analysis of copaiba and andiroba essential oils. Copaiba oil showed a value of (2.53 ± 0.02) mg KOH/g, while andiroba oil showed a value of (3.50 ± 0.04) mg KOH/g, indicating a low acidity level. In line with ANVISA (2005) (16), the maximum acidity index value is 4.0 mg KOH/g, and oils above this value are not acceptable for human consumption.

Table 1 - Analysis of acidity index and density of oils. Source: the authors.

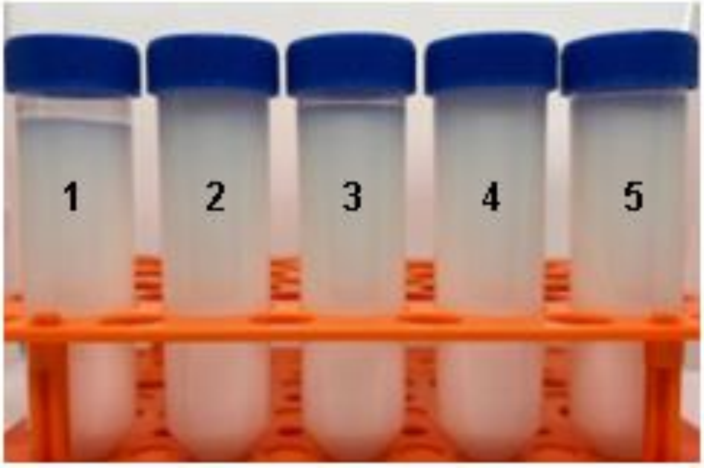
Oil	Acidity Index (mg KOH/g)	Density (mg/mL)
Copaiba	2.53 ± 0.02	0.94 ± 0.02
Andiroba	3.50 ± 0.04	0.87 ± 0.01

The density values of the oils also showed differences, with (0.94 ± 0.02) mg/mL for copaiba oil and (0.87 ± 0.01) mg/mL for andiroba oil. This indicates that andiroba oil has a higher amount of unsaturation in its carbon chain (Table 1). As stated by Ribeiro and Seravalli I (2004) (18), the density measurement of the oil is related to the number of double or triple bonds. In other words, the lower the molecular weight of the oil, the higher the degree of unsaturation present.

The nanocapsules of copaiba, andiroba, and their mixtures were synthesized, and their physical and chemical properties were studied (Figure 1).

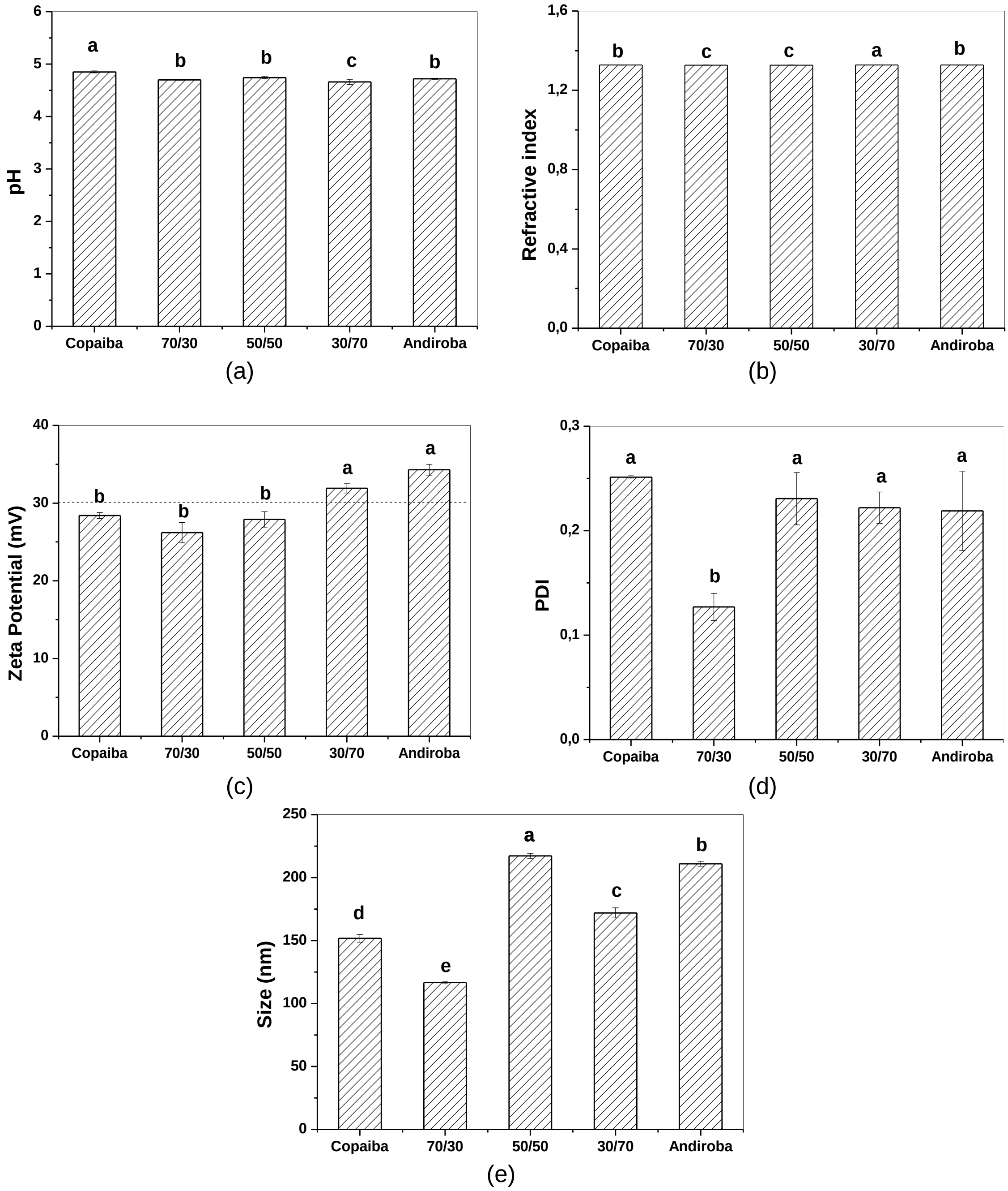
Mult. Sci. Rep. 2023; v. 3 n. 4 / ISSN: 2764-0388
DOI: https://doi.org/10.54038/ms.v3i4.51
Subject: 06/26/2023 – Accepted: 08/12/2023

Figure 1 - Image of the nanocapsule synthesized using the low-energy method: (1) pure copaiba, (2) 70% copaiba and 30% andiroba mixture, (3) 50% copaiba and 50% andiroba mixture, (4) 30% copaiba and 70% andiroba mixture, and (5) pure andiroba.



In Figure 2, the results of the nanocapsules analyses (pH, Refractive Index, Zeta Potential, PDI, and particle size) and the outcome of the Tukey test are observed. The use of different letters (a, b, c) suggests that there were statistically significant differences between some groups, with groups marked by the same letter not differing significantly from each other.

Figure 2 - Graphs of the analyses of the nanocapsules: (a) Zeta Potential, (b) PDI, (c) size, (d) pH, and (e) refractive index. a,b,d – For each nanocapsule, means of oil doses followed by the same lowercase letter do not significantly differ from each other, as determined by the Tukey's test (α = 0.05).



The Zeta potential of the nanocapsules (a characteristic that assesses the intensity of electrostatic repulsion or attraction between the nanocapsules) can be influenced by pH through the ionization of functional groups present in the oil molecules or capsule components. The equipment used to measure Zeta potential is from the brand Zetasizer, Nano series, Nano - ZS90, Malvern Panalytical. If the surface of the nanocapsules has charged groups, changes in pH can alter the ionization of these groups and, consequently, affect the Zeta potential. Additionally, pH can affect the polydispersity of the nanocapsules, as some oil molecules or capsule components may be sensitive to pH changes.

The pH of the nanocapsules was slightly below 5 - figure 2(a), while the ideal would be 7, compatible with the physiological value. A pH of this order can cause biocompatibility problems, affect the controlled release of drugs and the therapeutic effect of nanoparticles. This indicates that it will be necessary to adjust the pH of the nanocapsules to ensure more reliable results.

Polymeric materials used in the synthesis of the capsules can exhibit greater stability within certain pH ranges, resulting in a more uniform distribution of particle sizes. The particle size of the oil-containing nanocapsules can also be influenced by pH, as changes in pH affect the interaction forces between particles and can lead to alterations in the average size. At certain pH levels, certain electrostatic interactions may become stronger, leading to increased particle aggregation and consequently an increase in the average particle size (18-20).

In Figure 2, the results of the nanocapsules' analyses (pH, Refractive Index, Zeta Potential, PDI, and particle size) and the outcome of the Tukey test are displayed. The use of different letters (a, b, c) suggests that there were statistically significant differences between some groups, with groups marked by the same letter not differing significantly from each other. In Figure 2(a), it is evident that the copaiba/andiroba oil mixture in a 30/70 ratio had the lowest pH value among the mixtures, followed by the 70/30 and 50/50 mixtures, respectively.

In the refractive index analysis, Figure 2(b), the results were similar to those of the pH, showing significant differences between the nanocapsules, as determined by the Tukey test. However, the Zeta Potential analysis, presented in Figure 2(c), of the 30/70 nanocapsule was (31.9 ± 0.6) mV, and the PDI was 0.222 ± 0.015, as shown in

Figure 2(d). For the nanocapsule containing only andiroba, the Zeta Potential was (35.10 ± 0.7) mV and the PDI was 0.219 ± 0.038, as shown in Figures 2(d) and 2(d), respectively. Meanwhile, the nanocapsules containing only copaiba and the 70/30 and 50/50 mixtures showed lower Zeta Potential values. According to Mohanraj and Chen (20), nanoparticles with a Zeta Potential higher than ± 30 mV are considered stable in suspension, that is, the charge on the surface prevents particle aggregation and, consequently, phase separation.

Particle size analyses of the nanocapsules, shown in Figure 2(e), revealed significant differences. In Figure 2(e), the largest particle size was from the 50/50 mixture, with a value of (218 ± 2) nm and a PDI of (0.231 ± 0.025). The lowest value was from the 70/30 nanocapsule mixture, with a size of (117 ± 1) nm. De Conto et al. (2021) (22) studied ten nanoprecipitation process formulations by varying the concentrations of Span 60, ethanol, Tween, while keeping the concentrations of PCL, acetone, and distilled water constant. The authors obtained a particle size variation ranging from 151.1 nm to 256.9 nm and a Zeta potential ranging from -21 mV to -37.6 mV, resulting in four formulations with Zeta potential above -30 mV. In another study, Christofoli et al. (2015) (23) developed and characterized biodegradable nanocapsules containing essential oils from Zanthoxylum rhoifolium leaves, with a particle diameter below 500 nm and a Zeta potential around -20 mV. Bazylinska et al. (2013) (24) produced nanocapsules containing coconut oil using polymers such as PLA (polylactide) and PCL (polycaprolactone), with nanosphere sizes ranging from 127 to 276 nm for PLA and 162 to 287 nm for PCL. By varying the ratio of organic to aqueous phase, they obtained sizes ranging from 87 to 214 nm for PLA and 108 to 257 nm for PCL, but with Zeta potential values below 11 mV.

CONCLUSION

The nanocapsules containing vegetable oils were developed, and their physicochemical parameters were studied, confirming that it is possible to synthesize them in different proportions. Copaiba and andiroba nanocapsules showed differences in Zeta Potential and Polydispersity values. The 70/30 mixture exhibited the smallest size and polydispersity values among the blends, with a Zeta Potential above 30 mV.

REFERENCES

1. Fernandes TM. Plantas medicinais: memória da ciência no Brasil [online]. SciELO Books, 2004. 260 p.
2. Pedroso RS, Andrade G, Pires RH. Plantas medicinais: uma abordagem sobre o uso seguro e racional. Physis: Revista de Saúde Coletiva. 2021; 31:2-19.
3. Junior RA, Petry K. Viabilidade de implantação de fitoterápicos e plantas medicinais no Sistema Único de Saúde (SUS) do município de Três Passos/RS. Rev Bras Farm. 2012;93(1):60-67.
4. Sousa IO, de Araújo S, Negreiros PS, França ARS, Rosa GS, Negreiro FS, et al. A diversidade da flora brasileira no desenvolvimento de recursos de saúde. Rev UNINGÁ Review. 2017; 31:35-39.
5. Fernandes TM. Plantas medicinais: memória da ciência no Brasil. Editora FIOCRUZ; 2004. 260 p.
6. Mulat M, Pandita A, Klan F. Medicinal Plant Compounds for Combating the Multi-drug Resistant Pathogenic Bacteria: A Review. Curr Pharm Biotechnol. 2019;20(3):183-196.
7. Akram M, Hamid A, Khalil A, Ghaffar A, Tayyaba N, Saeed A, et al. Review on medicinal uses, pharmacological, phytochemistry and immunomodulatory activity of plants. Int J Immunopathol Pharmacol. 2014;27(3):313-319.
8. Moretti MDL, et al. Essential oil formulations useful as a new tool for insect pest control. AAPS Pharm Sci Tech. 2002;3(3):64-74.

Mult. Sci. Rep. 2023; v. 3 n. 4 / ISSN: 2764-0388
DOI: https://doi.org/10.54038/ms.v3i4.51
Subject: 06/26/2023 – Accepted: 08/12/2023

9. Simões CMO, Spitzer V. Óleos voláteis. In: Simões CMO, et al., editors. Farmacognosia: da planta ao medicamento. Porto Alegre: Ed. Universidade-UFRGS; Florianópolis: Ed. da UFSC; 1999. p. 387-415.
10. Pirbalouti AG, Hamedi B, Abdizadeh R, Malekpoor F. Antifungal activity of the essential oil of Iranian medicinal plants. J Med Plants Res. 2011;5(21):5089-5093.
11. Kumar M, Prakash S, Radha, Kumari N, Pundir A, Punia S, et al. Beneficial Role of Antioxidant Secondary Metabolites from Medicinal Plants in Maintaining Oral Health. Antioxidants. 2021;10(2):10.
12. Ishizuka F, Stenzel MH, Zetterlund PB. Microcapsule synthesis via RAFT photopolymerization in vegetable oil as a green solvent. Green Chem. 2018;20(3):831-839.
13. Pina-Barrera AM, Perez MSR, Roman RA, Gonzalez JGB, Guerra CAA, Rodriguez SAG. Alginate-based edible coating in combination with nanoencapsulated eugenol and its preservative effect on the shelf life of tomato (Solanum lycopersicum). Biotecnia. 2021; 23:133-141.
14. Instituto Adolfo Lutz. Métodos físico-químicos para análise de alimentos/coordenadores Odair Zenebon, Neus Sadocco Pascuet e Paulo Tiglea. São Paulo: Instituto Adolfo Lutz, 2008.
15. Fessi H, et al. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm. 1989;55(1): R1-R4.
16. Amazon Oil. Andiroba. Available from: https://amazonoil.com.br/rainforest-products/andiroba-en. Accessed on: September 6, 2023.
17. Amazon Oil. Andiroba. Available from: https://amazonoil.com.br/rainforest-products/copaibaoilresin-en/. Accessed on: September 6, 2023.
18. Ribeiro EP, Seravalli EAG. Química de Alimentos. 2004. p. 194.
19. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surf B Biointerfaces. 2010;75(1):1-18.
20. Joye IJ, McClements DJ. Biopolymer-based nanoparticles and microparticles: Fabrication, characterization, and application. Curr Opin Colloid Interface Sci. 2014;19(5):417-427.

Mult. Sci. Rep. 2023; v. 3 n. 4 / ISSN: 2764-0388
DOI: https://doi.org/10.54038/ms.v3i4.51
Subject: 06/26/2023 – Accepted: 08/12/2023

21. Mohanraj VJ, Chen Y. Nanoparticles - A review. Trop J Pharm Res. 2006;5(1):561-573.
22. Joye IJ, McClements DJ. Biopolymer-based nanoparticles and microparticles: Fabrication, characterization, and application. Curr Opin Colloid Interface Sci. 2014;19(5):417-427.
23. De Conto D, dos Santos V, Zattera AJ, Santana RMC. Swelling of biodegradable polymers for the production of nanocapsules and films with the incorporation of essential oils. Polym Bull. 2021;78(12):7261-7278.
24. Christofoli Bazylińska U, et al. Polymeric nanocapsules and nanospheres for encapsulation and long sustained release of hydrophobic cyanine-type photosensitizer. Colloids Surf A Physicochem Eng Asp. 2014;442: 42-49.
25. Bazylińska U, Lewińska A, Lamch Ł, Wilk KA. Polymeric nanocapsules and nanospheres for encapsulation and long sustained release of hydrophobic cyanine-type photosensitizer. Colloids Surf A Physicochem Eng Asp. 2014;442: 42-49.

Mult. Sci. Rep. 2023; v. 3 n. 4 / ISSN: 2764-0388
DOI: https://doi.org/10.54038/ms.v3i4.51
Subject: 06/26/2023 – Accepted: 08/12/2023