BIOPOLYMERIC FILMS FOR ENCAPSULATION OF BIOACTIVE COMPOUNDS FROM *Rosmarinus officinalis L.*

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ABSTRACT

Edible films emerge as an innovation in replacing synthetic products and can be enhanced by incorporating bioactive compounds from plants, which exhibit antibacterial, antioxidant, and anti-inflammatory actions. This approach offers a sustainable alternative to conventional synthetic films and extends the shelf life of packaged foods. *Rosmarinus officinalis L.*, known as rosemary, contains bioactive compounds that offer various functionalities. Through hydrodistillation, essential oil is obtained, widely used in various industrial sectors, along with a residual water from the process, known as aqueous extract, rich in phenolic compounds. In this context, these bioactive compounds can be encapsulated in biopolymeric films in order to deliver the functionalities and preserve food. The technique used was casting where carboxymethyl cellulose (CMC) was the biopolymer. The characterization of the essential oil was conducted resulting in 52.8%(w/w) 1,8-cineol, being the major compound of it. Total phenolic compounds were measure in the residual water being 4.21±0.05 mg AGE/mL. The films produced with essential oil and aqueous extract present water vapor permeability (WVP) of $2.1 \pm 0.4 \times 10^{-10}$ g/m.s.Pa, consistent with CMC film studies. The water solubility was 53.3±3.5%, higher than standard films, making it more interesting in terms of food preservation. Furthermore, the scanning electronic microscopy was made to understand the characterization of the physical structure of the film.

Keywords: *Rosmarinus*. Rosemary. Eucalyptol. Phenolic compounds. Edible films.

1 INTRODUCTION

Rosmarinus officinalis L., widely known as rosemary, offers numerous health benefits, being used as an antifungal, antimicrobial, anti-inflammatory, and antibacterial agent. These actions come from the plant's bioactive compounds, generally phenolic compounds, which are phytochemicals responsible for their defense against external factors[1]. To extract rosemary essential oil from the plant, the leaves were submitted to hydrodistillation resulting in two distinct fractions: rosemary essential oil (lipophilic fraction) and as residue, aqueous extract (hydrophilic fraction) rich in phenolic compounds. The essential oil is widely used in the market as an additive in the food and cosmetic industries due to its potential treatment effects for various diseases[2]. However, the residual water, rich in phenolic compounds, is not reused and is discarded as waste in this process. As a result of its characteristics, the incorporation and availability of these bioactive in encapsulating systems become a promising strategy for new products, especially given the demand for applications in the food, pharmaceutical, agricultural, and other sectors. Edible films belong to encapsulating systems, and have the function to protect and deliver active substances in a controlled and targeted manner, significantly improving product stability, efficacy, and safety. Besides that, edible films serve as an alternative to conventional packaging as they protect the food and can be consumed with it, as they are composed of natural biopolymers, providing mechanical, barrier, and biodegradable properties[3]. It is possible to formulate films with numerous biopolymers and plasticizers available on the market.

This study investigated the effects of co-encapsulation of rosemary essential oil and phenolic compounds present in the residual water from the hydrodistillation process. Encapsulating matrices based on edible films produced from CMC and glycerol were used, and the main objective was to develop an encapsulating system with the potential to extend the shelf life of foods, taking advantage of the antioxidant and antimicrobial properties associated with rosemary's bioactive compounds.

2 MATERIAL & METHODS

2.1 Extraction and characterization of Rosemary bioactive compounds

The extraction of rosemary essential oil was carried out by hydrodistillation using a heating mantle with a volumetric flask attached to glassware of the Clevenger type. The residual water from the process, referred to as aqueous extract, was retained in the flask, and the essential oil was recovered in the recovery section of the system used. For the characterization of the volatile compounds in the essential oil, a gas chromatograph with a mass spectrometer detector and an HP-5MS column with predefined configurations were used. The total phenolic content of the aqueous extract was determined using the Folin-Ciocalteu colorimetric method with some modifications. In 300 µL of diluted sample, 300 µL of the Folin-Ciocalteu reagent and 2400 µL of sodium carbonate (5% w/v) were added. After mixing and incubation, the solution was kept in the dark for 20 minutes, and then its absorbance was measured at a wavelength of 760 nm.

2.2 Casting method

All film formulation had CMC as biopolymer that was previous hydrated 24 hours before the beginning of the process. Then was added extract and glycerol. This solution was submitted at constant stirring and heat at 80°C. Then, the solutions were casted. For the films that were added essential oil, the solution had to be cooled to 50°C and then added the oil and Tween 80. After casted, the plate was allocated in oven at 38°C during 24 hours and then were unmolded.

2.3 Edible films properties

This study used the methodologies described by Zhou et al.[4] as a reference for the characterization of film properties, with some modifications. For water vapor permeability, anhydrous calcium chloride was placed in sectioned cap vials, which were then sealed with the different film formulations. By measuring the change in mass over time, the average water vapor transmission was obtained, and permeability was calculated. Water solubility was determined by cutting film samples into 2x2 cm pieces, previously dried at 105°C for 24 hours, and then subjecting them to constant agitation at room temperature for 1 hour. The solution was filtered, and the residue was dried again under the same conditions. The difference in initial and final mass was used to calculate solubility. For scanning electron microscopy (SEM) analysis, the films were subjected to cryogenic temperatures to create fragments. The samples were coated with a gold/palladium mixture for better visualization.

3 RESULTS & DISCUSSION

The characterization of the essential oil volatile compounds showed a huge diversity of compounds that contribute for the therapeutical and sensorial properties. The major compounds were 1,8-ceneole, camphor, borneol, α-terpineol, β-pinene, cimene, limonene, linalool and caryophyllene. The chromatography analysis showed a ratio of 58.2g/100g of the 1,8-cineole on the essential oil proving that it is the majority compound. Those compounds are responsible for respiratory treatment, analgesic, antimicrobial, antioxidant and antifungal[5]. For the aqueous extract the content of total phenolic compounds was 4.21±0.05 mg AGE/mL. The quantification of the phenolic compounds is important due to the antioxidant potential and benefic effects for human health[6].

The different film forming formulation resulted in different coloration of films as demonstrate in Figure 1 (A-D).

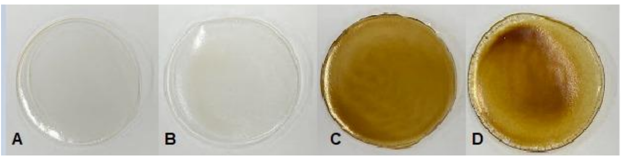

Figure 1 Films obtained by the casting method with different formulations: A) CMC, B) CMC/EO, C) CMC/AE, D) CMC/EO/AE.

The water vapor permeability of the films were similar within CMC and CMC/EO/AE being around 2.0±0.2 $\times 10^{-10}$ g/m.s.Pa, for the CMC/AE was 2.9±0.1 and for CMC/EO was 4.0±0.5, being the major result. Edible films for food applications must have a minor WVP for larger shelf-life. Due to the hydrophilic nature of aqueous extract, it was expected that the WVP were lower, and for the CMC/EO the result was explained because the negative impact of essential oil in the microstructure of the films forming holes or micropores resulting the diffusion of water vapor molecules.

For the solubility, it was expected lower values due to the food application where it is necessary the lower solubility. The polysaccharides used in the film formulation were mostly hydrophilic, resulting major solubility. But in order to reduce it, glycerol was added in the formulation with hydrophobic compounds, such as essential oil and phenolic compounds. Due to this fact, this study found that films with aqueous extract had minor solubility differing of the CMC and CMC/EO that were 100% soluble in water. CMC/AE was only 61% and CMC/EO/AE was 53.3%.

Scanning electronic microscopy (SEM) was used to visualize the microstructure of the films as size and distribution of holes, cracks, pores and other superficial characteristics, and it can be used to support the other results obtained like WVP. The Figure 2 present the results of SEM within the cross-section and surface area respectably.

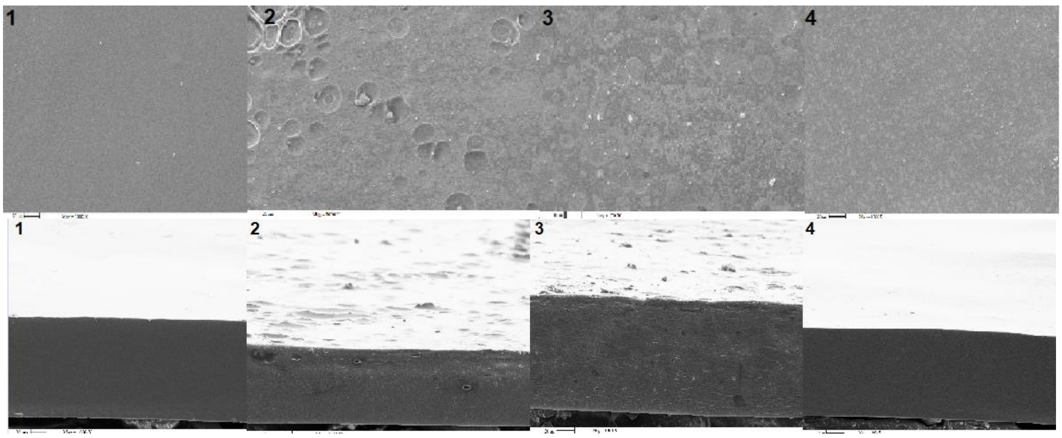

Figure 2 SEM for cross-section and surface area: 1) CMC, 2) CMC/EO, 3) CMC/AE; 4) CMC/EO/AE

Figure 2 showed that CMC was more homogeneous within minor deformities and blank spaces. The CMC/AE film presented a heterogeneous surface with bubbles and possible crystals or microparticles, along with voids in the cross-section. Nevertheless, it maintained plastic characteristics with small deformities. The CMC/OE film exhibited fewer deformities due to the elimination of solid impurities during the hydrodistillation process. However, larger voids were observed due to the coalescence, emulsion, and flocculation of the essential oil during drying, making the matrix slightly more fragile. The CMC/EO/AE film showed intermediate characteristics between the CMC/AE and CMC/EO films. Overall, all films presented a consistent matrix with plastic properties, albeit with some low-amplitude heterogeneities, indicating the success of the casting technique and film formulation.

4 CONCLUSION

Rosemary (Rosmarinus officinalis L.) is well-regarded for its diverse health benefits, primarily due to its phenolic compounds, which serve as natural defense mechanisms for the plant. This study investigates the potential of utilizing by-products from rosemary hydrodistillation—namely, essential oil and residual aqueous extract—in the creation of innovative edible films for food packaging applications. Gas chromatography analysis identified 1,8-cineole, comprising 58.2% (w/w) of the essential oil, recognized for its antimicrobial, antifungal, and analgesic properties, as well as its use in treating respiratory conditions. The total phenolic content in the aqueous extract was quantified at 4.21±0.05 mg GAE/mL, highlighting its antioxidant potential.The casting technique was successfully employed to produce four types of films: CMC, CMC/EO, CMC/AE, and CMC/EO/AE. Water vapor permeability (WVP) measurements revealed that CMC and CMC/EO/AE films exhibited a WVP of 2.0±0.2 ×10-10 g/m.s.Pa, while CMC/EA and CMC/EO films demonstrated WVP values 1.5 and 2 times higher, respectively. Contrary to expectations, the CMC/EO film exhibited the highest permeability, attributed to a fragile matrix with numerous bubbles and holes observed via SEM, caused by essential oil evaporation. In contrast, CMC/AE films contained some heterogeneities from filtration residues, while CMC/EO/AE films exhibited intermediate characteristics. The CMC film showed a homogeneous and continuous matrix without heterogeneities or voids. Film solubility was 100% for CMC and CMC/EO films, but 61% and 53.3% for CMC/AE and CMC/EO/AE films, respectively, due to the presence of hydrophobic agents that hindered solubilization. Lower solubility is advantageous for food packaging to prevent film deterioration. Hence, this study successfully produced and characterized rosemary essential oil and aqueous extract-based films, which exhibited desirable low water vapor permeability and solubility, alongside imparting antioxidant, antimicrobial, and antifungal properties to food products and, ultimately, to consumers.

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