



Extraction, Characterization, and Encapsulation of Cinnamon Hydrosol Obtained via Microwave-Assisted Hydrodistillation: Analysis of Antioxidant and Antimicrobial Activities

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Abstract

Microwave-assisted hydrodistillation (MAHD) method was employed to extract cinnamon oil and hydrosol (a byproduct). The total polyphenol content (TPC) of the cinnamon hydrosol (CH) was determined using the Folin-Ciocalteu method, and its antioxidant power was assessed through the DPPH radical reduction method. Gas chromatography was utilized to quantify the main bioactive compound (cinnamaldehyde). The disc agar diffusion method was applied to evaluate the inhibition of pathogenic microorganisms. To protect the bioactive compound, an encapsulation method involving cross-linking with calcium alginate was utilized. The capsules were examined using environmental scanning electron microscopy (ESEM). The TPC content was found to be 15.63 ± 0.21 mg gallic acid/g dry matter, and the DPPH radical inhibition rate was $84.26 \pm 1.35\%$. CH exhibited a significant inhibitory effect against *Escherichia coli*, and a moderate inhibition effect against *Shigella flexneri*, *Salmonella* spp., *Salmonella typhimurium*, and *Escherichia coli* EPEC. Finally, successful encapsulation of CH was achieved using sodium alginate, resulting in bead sizes ranging between 1.75 and 2.75 mm.

Keywords: *Cinnamomum zeylanicum* hydrosol, microwave-assisted hydrodistillation, antioxidant power, microbiological characterization, encapsulation

1. INTRODUCTION

Interest in the use of natural sources of antioxidants has been increasing significantly in recent years. A current trend in modern society is green consumption, desiring fewer synthetic additives [1][2]. Cinnamon (*Cinnamomum zeylanicum*) is a rich source of antioxidants and it is extracted from the inner bark of various trees belonging to the *Cinnamomum* genus. It is commonly used as a food additive and has recently gained attention for its potential medicinal properties. Different parts of the cinnamon plant, such as the bark, leaves, and root, contain the same bioactive compounds but in varying proportions. The main components found in each part are cinnamaldehyde (in the bark), eugenol (in the

leaves), and camphor (in the root) [3].

Cinnamaldehyde and cinnamic acid offer a range of health benefits, including antioxidant and free radical scavenging properties, blood sugar reduction, anti-cholesterol effects, analgesic properties, antimicrobial activity, anti-inflammatory effects, anti-yeast properties, and anti-ulcer gastric effects. Additionally, the antibacterial function of cinnamaldehyde has been well-documented. This action occurs at the cell membrane level, leading to membrane disruption, increased permeability of the membrane to small ions, and destabilization of the membrane structure and lipid bilayer packing. Some of these effects can ultimately lead to microbial cell death [4]. One of the main products of cinnamon is its essential oil, typically obtained through the distillation process [5].

Conventional extraction techniques, such as solvent extraction (liquid-liquid or solid-liquid extraction) with the aid of external factors (e.g., mechanical agitation systems, pressing, or heating like Soxhlet extraction), involve extended processing times, low efficiency, and high solvent consumption [6]. As an alternative, new extraction techniques have been developed to meet environmental and health-friendly standards, yielding high-quality bioactive products from plant seeds and barks [7]. Microwave extraction is one

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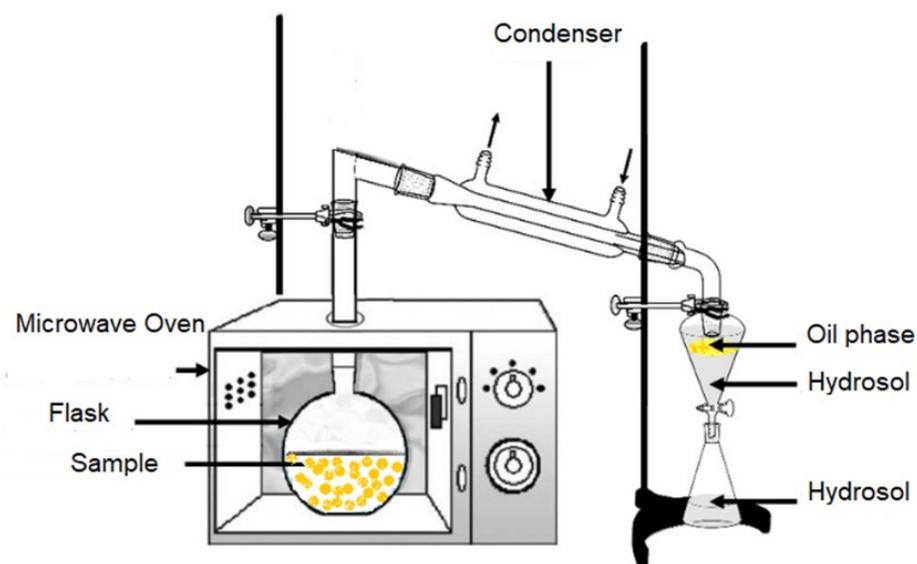


Figure 1. Scheme of microwave-assisted hydrodistillation system used to obtain cinnamon extract.

such environmentally friendly method, categorized as a green technology, which is nondestructive and ensures safe and high-quality extracts. Microwaves can be used as an assistance to conventional methods, such as hydrodistillation [8], or adapted to function as a stand-alone method, known as solvent-free microwave extraction [9]-[11]. In the microwave-assisted hydrodistillation (MAHD) method, microwave energy is employed to heat the solvent (typically water) and the sample mixture, accelerating the rate of solute mass transfer from the matrix to the solvent. This method offers the advantage of being efficient and fast, resulting in low energy consumption and yielding higher purity extracts compared to conventional techniques [7] [12]-[14].

It should be noted that the industry concentrates its efforts on obtaining essential oil. The hydrosol becomes a waste or a by-product with less added value. A significant quantity of hydrosol is produced when MAHD is applied to obtain essential oil from cinnamon, and it is usually discarded. To the best of our knowledge, there are no research works that have evaluated the properties of cinnamon hydrosol (CH) obtained by the MAHD method. According to the above, the objectives of this study were to obtain cinnamon hydrosol as a by-product of oil extraction from cinnamon bark using MAHD, to evaluate the potential of the hydrosol as a source of polyphenols

and antimicrobial agents, and to investigate encapsulation as a method for preserving and protecting the bioactive compounds present in the hydrosol.

2. MATERIALS AND METHODS

2.1. Microwave-assisted Hydrodistillation Method

Cinnamon sticks were purchased from a market in La Plata city, Buenos Aires, Argentina. The sticks were then crushed using an electric grinder (Peabody PE-MC9103, China). A total of 50 g of ground cinnamon was mixed with 500 mL of water in a flask [15]. This flask was placed in a MAHD equipment, which was custom-built in our laboratory (Fig. 1). The setup consists of a domestic microwave oven (Daewoo, China) connected to a Clevenger-type apparatus. During the process, the CH was collected from the condensed vapor in a 125 mL separating funnel attached to the bottom side of the apparatus. The first 100 mL of the CH was separated and reserved for further analysis.

2.1.1. Microwave Power

The microwave power was determined by a calorimetric method [16]. At each microwave program (minimum, defrost, medium, auto, and maximum power), the temperature increase of different water volumes was recorded. These values were used to estimate the absorbed power by the

following equation 1.

$$\text{Power} = \frac{m_{\text{H}_2\text{O}} \times C_{\text{pH}_2\text{O}} \times \Delta T}{\Delta t} \quad (1)$$

where Power is the microwave power (W), $m_{\text{H}_2\text{O}}$ is the water mass (kg), $C_{\text{pH}_2\text{O}}$ is the specific heat of water (4184 J/kg °C), ΔT is the difference between the initial and final temperatures (°C), and Δt is the heating time (s). The measurements were done in triplicate. Each of the curves of power vs volume was regressed using the following equation 2.

$$\text{Power} = A \times \text{Vol}^B \quad (2)$$

where Power represents the absorbed power (W) for each volume of water (Vol, m³), A and B correspond to regression parameters. The regression was done using Systat software (USA, 1970). The power level released by the magnetron in the cavity corresponds to the plateau of each curve [16].

2.2. Conventional Solvent Extraction Method

A conventional extraction method using conventional solvents (water and ethanol) was employed as the control process, following the procedure described [17]. For this purpose, extraction mixtures were prepared in Falcon tubes, containing 1 g of the sample along with 10 mL of water (called H₂O+maceration) or 96% ethanol (food grade) (called ETOH + maceration) as the solvent. Two batches of samples were prepared: one batch was left to macerate for 24 h at an average temperature of 25 °C, while another batch was subjected to extraction without maceration, with an

extraction time of 10 min. Once the extraction process was completed, centrifugation was carried out using a Sorvall ST 16R centrifuge (Canada) at 5500 rpm and 4°C. The resulting clear extracts were then used for further analysis.

2.3. Determination of Total Polyphenol Content

The determination of total polyphenol content (TPC) was carried out using the Folin-Ciocalteu method [18]. This assay is based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids at basic pH. When phosphomolybdotungstic acid (formed by the two salts in the acid medium), yellow in color, is reduced by the phenolic groups it forms a deep blue complex. The intensity of this complex was measured spectrophotometrically at 725–750 nm to determine the TPC [19]. For the analysis, 0.2 mL of the extract was taken and placed in a test tube containing 0.2 mL of Folin's reagent and allowed to stand for 2 min. Then, 2 mL of Na₂CO₃ 2% (w/v) in distilled water was added. After a reaction time of 30 min, the absorbance was measured in the spectrophotometer (model U-1900 Hitachi, Japan) at 725 nm. The calibration curve was constructed using a solution of gallic acid with a concentration of 0.2 mg gallic acid/mL, obtained from ICN Biomedicals Inc. (Ohio, USA). This solution was then subjected to dilutions to create different concentrations. The absorbance values obtained from the measurements were plotted as a function of the concentration of these gallic acid solutions. All measurements were performed in triplicate. The TPC was calculated from each measured absorbance value using the calibration curve for

Table 1. Strains used for bacterial inhibition assay.

Identification	Strains
LAMA 357	<i>Staphylococcus aureus</i>
LAMA 363	<i>Pseudomonas aeruginosa</i>
LAMA 392	<i>Shigella flexneri</i>
LAMA 1122	<i>Escherichia coli</i> EPEC
LAMA 1553	<i>Salmonella</i> spp.
LAMA 1570	<i>Salmonella typhimurium</i>
LAMA 1704	<i>Escherichia coli</i>



Figure 2. Capsules with cinnamon hydrosol. a) Colorless, b) stained with safranin.

gallic acid and considering the dilution performed (Eq. 3) [20].

$$\text{TPC} = \frac{\text{mg}_s \times 5.10^{-3} \text{ L} \times (A - \frac{b}{m})}{\text{mg}_{\text{ext}} \times \text{g}_{\text{MS}}} \quad (3)$$

where mg_s is the solvent mass used for the extraction (mg), A is the measured value of absorbance, m is the slope of the calibration curve, b is the intersection of the calibration curve, mg_{ext} is the extract aliquot (mg), g_{MS} is the sample mass on a dry basis (g) and TPC is expressed as gallic acid/g of dry matter (dry basis).

2.4 Determination of the Antioxidant Power

The determination of the antioxidant power of the extracts was carried out using the 2,2-diphenyl-1-picrylhydrazyl radical reduction method (DPPH[•]). DPPH[•] reduces its absorbance (violet solution in alcohol) when it is reduced by an antioxidant. A solution of 25 mg of DPPH[•] (Sigma) in 1 L of 96% ethanol was prepared [17]. The mixture was left to react at 25 °C in the dark for 90 min. Each measurement was made in triplicate in a spectrophotometer at 515 nm. The control was prepared by replacing the sample solution with 0.2 mL of DPPH[•] solution. The anti-radical activity was expressed as the percentage inhibition (% I) of the DPPH[•] free radical, calculated with the following equation 4.

$$\% \text{ Inhibition} = \frac{(\text{Abs}_b - \text{Abs}_m)}{\text{Abs}_m} \times 100\% \quad (4)$$

where Abs_b is the control absorbance value and Abs_m is the sample absorbance.

2.5. Gas Chromatography Analysis

Gas chromatography (GC) was used to determine the presence of cinnamaldehyde in cinnamon extracts obtained by MAHD (CH). For this purpose, the curves of a cinnamaldehyde standard were compared with the curves obtained by GC. The analysis was performed by GC with a flame ionization detector (GC-FID) using an Agilent Technologies 7890 (USA) with the following conditions: injector temperature at 200 °C with split ratio 150:1, injector flow of 3 mL/min, detector temperature 250 °C, HP-5 MS column (30 m × 0.32 mm × 0.25 mm). The temperature program was: 70 °C for 1 min, 6 °C/min up to 170 °C, and 3 mL/min flow through the column with a linear velocity of 45.4 cm/min. The standard used was cinnamaldehyde 2000 µg/mL in methanol (Sigma Aldrich S.A, Argentina). Samples were prepared by dissolution in acetonitrile, the same method was validated for use in HPLC [20]. The assays were done in duplicate.

2.6. Inhibition Tests on Pathogenic Microorganisms

The inhibition tests were conducted using seven bacterial strains from the Food Safety Laboratory collection, affiliated with the Faculty of Veterinary Sciences at the National University of La Plata (Table 1), following the method outlined [21][22]. The strains were grown in nutrient broth (Britania,

Argentina) for 18–24 hours at 37 °C, and their density was standardized using DEN-1B equipment (Biosan Biosystem, Argentina) to achieve an optical density equivalent to 10^8 CFU/mL. Each microorganism was inoculated onto nutrient agar plates (Britania, Argentina) in a streaked pattern using a Drigalsky spatula. For the diffusion tests, 5 mm diameter filter paper discs (Whatman® 91, England) were saturated with CH. Two discs per plate were placed on the agar, each containing their respective bacterial strain. The experiments were conducted in duplicate to ensure reliability. The antimicrobial activity was qualitatively assessed by observing the formation of inhibition zones around the discs and was expressed as inhibitory (+) or non-inhibitory (-). Besides the inhibition halos were measured using Image J software.

2.7. Encapsulation

First, a 2% (w/v) solution of sodium alginate (Protanal, Norway) was prepared in distilled water using a magnetic stirrer thermostated at 50 °C until complete dissolution. This solution was then stored in a refrigerator at 4 °C until it was ready for use. Next, a 0.05 M calcium chloride encapsulating solution (Cicarelli Laboratory, Argentina) was prepared with distilled water and stored in a glass bottle under refrigeration (4 °C). The capsules were created by slowly dripping the hydrocolloid solution (250 mL) mixed with the CH (250 mL) into the encapsulating solution at a rate of approximately 1 drop per second. The capsules were allowed to remain in this solution for 15 min with agitation. Afterward, the capsules were filtered and washed with acetic acid/sodium acetate buffer at pH 4.5, followed by a wash with distilled water [23]. The capsules were then dried using paper towels and stored at 4 °C until further analysis.

Additionally, in a separate test, safranin was added to the CH solution to verify the presence of CH inside the formed capsules (see Fig. 2).

2.8. Scanning Electron Microscopy

Scanning electron microscopy (SEM) analysis was performed using the SEM FEI Quanta 200 (USA) installed at the Laboratory of Physical Metallurgy Research (LIMF) in the Faculty of Engineering of the National University of La Plata (UNLP). The capsules obtained were dried in an oven at a constant temperature of 45 °C. Then, they were characterized using the ESEM technique [24].

2.9. Statistical Analysis

Statistical analysis was performed using STATGRAPHICS Plus 5.1 software. Results were analyzed using an analysis of variance (ANOVA) and Tukey's test was applied to compare differences among mean values. Significant differences were found when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Microwave Power

First, the microwave power acting on the extraction balloon was determined for each program of the microwave oven. From the temperature rise measurements, it was possible to calculate the power as a function of volume for each microwave program (Eq. 1). From the plateau of the curves, the absorbed power was measured: 569, 394, 384, 249, and 112 W for maximum, auto, medium, defrost, and minimum programs, respectively. In this work, the extraction was carried out at 569 W/500 mL. Other authors found that the use of power levels in the range of 300 to 600 W allows obtaining extracts with better quality of the volatile substances of

Table 2. Total polyphenol content (TPC) and %I of cinnamon extracts obtained by different methods.

Sample	TPC (mg gallic acid/g dry sample)	SD	%I	SD
Cinnamon hydrosol (MAHD)	15.63	0.21	84.26	0.22
Cinnamon extract (H ₂ O+maceration)	9.68	0.34	55.18	0.28
Cinnamon extract (ETOH+ maceration)	12.54	0.60	64.84	0.15
Cinnamon extract (H ₂ O)	4.74	0.26	25.64	0.12
Cinnamon extract (ETOH)	6.21	0.4	34.48	0.18

Table 3. Total polyphenol content (TPC) and %I of cinnamon extracts obtained by different methods.

Bacterial Strain	Inhibition
<i>Escherichia coli</i>	++++
<i>Salmonella</i> spp.	+++ -
<i>Escherichia coli</i> EPEC	++ - -
<i>Shigella flexneri</i>	++ - -
<i>Salmonella typhimurium</i>	+ - - -
<i>Staphylococcus aureus</i>	- - - -
<i>Pseudomonas aeruginosa</i>	- - - -

Note: Bacterial inhibition (+), No bacterial inhibition (-). The number of signs refers to the number of discs in the Petri dishes tested.



Figure 3. Capsules observed under the optical microscope.

interest [25]-[27]. In the previous study, Rezvanpanah et al. employed MAHD for the extraction of essential oils from two genotypes of an aromatic plant *Satureja hortensis* and *Satureja montana*. Three microwave power levels (220, 440, and 660 W) were selected for the extraction. Their findings revealed that extraction efficiency improved at higher power levels [28].

3.2. Determination of Total Polyphenol Content and Antioxidant Power

Table 2 summarizes the TPC results of the cinnamon extracts obtained through MAHD and conventional methods. The CH obtained by MAHD exhibited the highest TPC values [29]. Among the extracts obtained by the conventional method, the macerated samples yielded the best results, and ethanol was more effective than the aqueous solution for the extractions (Table 2). Comparing

the results with literature data, Khan et al. obtained a TPC of 14.82 ± 0.28 mg gallic acid/g dry sample for a cinnamon extract obtained with 50 mL of 80% methanol and a 15-h contact time [30][31]. In our study, a similar or slightly higher value was achieved using the MAHD method, which involved a much shorter processing time and used water as the solvent. Similarly, Dvorackova et al. investigated TPC in different cinnamon extracts subjected to various treatments such as agitation with ethanol, maceration with ethanol, and application of sonication. The results from those treatments were 0.6, 0.25, and 0.75 mg gallic acid/g dry samples, respectively [32]. Thus, the MAHD treatment was more effective in obtaining polyphenolic compounds compared to the treatments mentioned above.

Li et al. suggested that a higher microwave temperature and shorter extraction time are more effective in extracting antioxidant phenolic compounds [33]. However, extending the irradiation time with higher microwave temperatures may lead to the thermal degradation of polyphenols [34]. MAHD, by generating rapid heat and pressure within the biological system, allows compounds to be released from the biological matrix, obtaining good quality extracts with better recovery of the bioactive compound [35].

In addition, the results indicated that the cinnamon hydrosol obtained with MAHD exhibited the highest inhibition of the DPPH radical at $84.26 \pm 1.35\%$. Asimi et al. determined antioxidant activity through the DPPH radical inhibition method in extracts of cinnamon samples obtained with ethyl acetate solvent at 70 °C for 24 h, and they reported an inhibition percentage of $86.41 \pm 0.23\%$, which is similar to the value found in our study ($84.26 \pm 1.35\%$). Furthermore, a positive correlation between antioxidant activity and the content of phenolic compounds was observed in this study ($r^2 = 0.94$, $p < 0.05$), indicating that the content of these compounds significantly contributes to the antioxidant activity of the cinnamon extracts [36].

3.3. Gas Chromatography

The main component of interest in cinnamon is cinnamaldehyde [37][38]. In this study, we analyzed the presence of cinnamaldehyde in the CH

obtained through MAHD. By comparing the chromatograms of the cinnamaldehyde standard with that of the CH, we investigated the presence of cinnamaldehyde in the hydrosol. The chromatograms obtained for the cinnamaldehyde standard displayed two peaks with elution times of 15.85 and 19.12 min after the sample was injected into the equipment (data not shown). Vargas-Vega conducted a chromatographic analysis of cinnamon essential oil and identified components with the highest peaks as cis-cinnamaldehyde and trans-cinnamaldehyde, with retention times of 15.88 and 19.95 min, respectively [39].

From the measurement of the peak areas, the cinnamaldehyde standard presented a value of 71.7 mm² (30.37% of the whole area) and the second peak of 164.5 mm² (69.63% of the whole area). The CH showed an area of the first peak of 51.1 mm² (96.36% of the whole area) and the second peak of 1.9 mm² (3.64% of the whole area). From the area ratio, the first peak of the CH sample showed a reduction of 29% corresponding to cis-cinnamaldehyde, while the second peak showed a reduction of 99%, corresponding to trans-cinnamaldehyde, compared to the standard. Thus, the cinnamon hydrosol sample contains cinnamaldehyde with a lower content than the standard. The reduction was more significant for trans-cinnamaldehyde, which is visualized in the second peak with a longer retention time. Similar results were presented by Friedman et al., who found a transformation of pure cinnamaldehyde to benzaldehyde when heated at temperatures above 60 °C. In the present work, this reduction can be attributed to the MAHD thermal processing, in which 100 °C is reached during the extraction [40].

3.4. Inhibition Tests on Pathogenic Microorganisms

Hydrosols have recently gained attention due to their antimicrobial activity, both *in vitro* and in food matrices, especially against pathogenic and spoilage microorganisms, including bacteria and fungi [41]. In the current study, the CH exhibited inhibitory properties against certain bacterial strains tested, with *E. coli* being the most affected (Table 3). This inhibition was demonstrated by the presence of a transparent halo surrounding the discs soaked with CH (without dilution), indicating the absence of microorganism growth. CH showed moderate

inhibition for *S. flexneri*, *Salmonella* spp., *S. typhimurium*, and *E. coli* EPEC. The diameter of inhibition haloes was measured. The average values were 7.15 ± 1.24, 9.25 ± 1.05, 7.15 ± 0.60, 7.43 ± 0.15, and 5.89 ± 0.6 mm diameter for *E. coli*, *Salmonella* spp, *E. coli* EPEC, *S. flexneri* and *S. typhimurium*, respectively.

In contrast, in the *S. aureus* and *P. aeruginosa* strains no inhibition halos are observed, demonstrating that the cinnamon hydrosol could not inhibit the growth of these microorganisms. Herrera and García-Rico conducted an inhibition study of several microorganisms showing that strains of *E. coli* and *Salmonella* spp. were inhibited with aqueous extracts of cinnamon [42]. In our work, *Salmonella* spp. had a moderate inhibition. This could be attributed to the technique applied to obtain the extracts. The authors performed an aqueous extraction, while in the present work, the heat treatment was used (MAHD). As previously described, we reported a decrease in the concentration of cinnamaldehyde, the compound responsible for the inhibition of microbial growth [43]. In addition, the inhibitory power is specific to species variety, the ones used in this work being autochthonous strains. Fernández et al. found that cinnamon extracts did not show inhibitory power against *E. coli* and *Salmonella* spp. Strains [44], while in the present work, they were sensitive to CH. Besides, they indicated that cinnamon showed inhibition against *E. coli* O157:H7, enteropathogenic *E. coli* (EPEC), and *S. typhimurium*, in agreement with the present work.

3.5. Encapsulation of the Active Compound and Microstructural Analysis

The CH sample was encapsulated to maintain and preserve its antioxidant capacity and antimicrobial activity. The capsules obtained are shown in Fig. 3; they are colorless after washing with buffer (acetic acid/sodium acetate pH 4.5) (Fig. 3). In Fig. 3, the capsules can be seen stained with safranin, which is an indicator of the presence of the hydrosol inside the capsules. In addition, in this work the colorless CH capsules were observed by optical microscope (Arcano, ST302, USA) at a maximum magnification of 40× to determine their size and shape (Fig. 4). As seen in Fig. 4 the capsules have a hemispherical shape and a size of

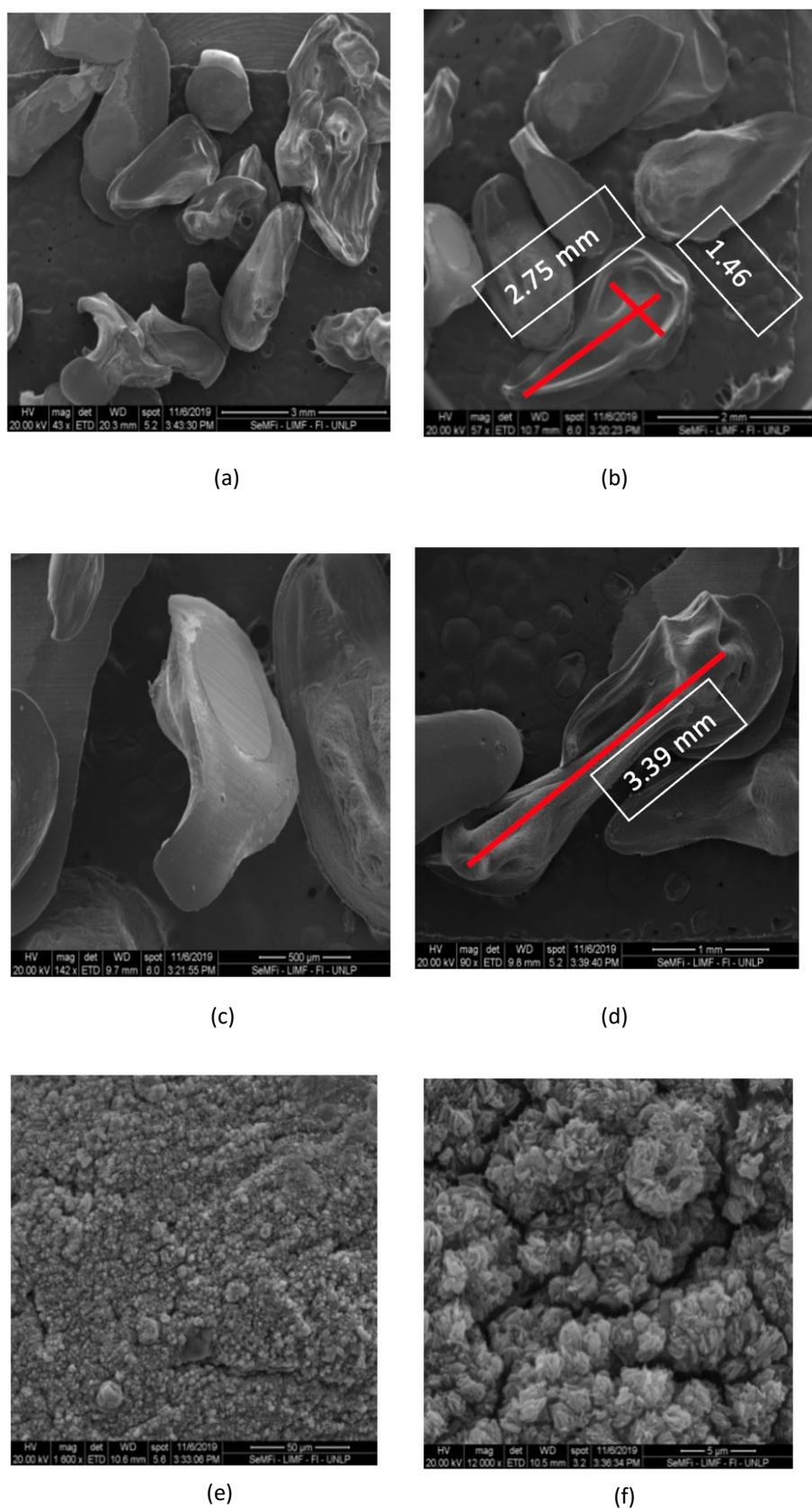


Figure 4. ESEM micrographs of cinnamon hydrosol capsules. a) Variety of capsules, b) cross-section of a capsule, c) oblique section of a closer capsule, d) several capsules with their measurements, e) and f) surface of the capsules.

about 4 mm. Micrographs showed the structure of the dried calcium alginate capsules (Fig. 4(a)). They presented a surface with pores and cracks (Fig. 4(e) and 4(f)). These pictures agree with those obtained by Fundueanu et al [45]. In Fig. 4(b) and 4(d), the size distributions of the capsules can be observed; they ranged between 1.75 and 2.75 mm. Noren et al. encapsulated ascorbic acid and phenolic compounds from the extract of tumbo serrano (*Passiflora mollissima* H.B.K.) in sodium alginate by ionic gelation [46]. The author obtained capsules with sizes ranging between 1.76 and 2.45 mm in diameter, which is in agreement with the results obtained in the present work.

4. CONCLUSIONS

The use of MAHD is appropriate since it reduces the extraction time, eliminates the use of organic solvents, and yields a higher content of polyphenols. Microwave treatments, called ecological processes, allow preserving the environment due to the low energy consumption and the elimination of the use of organic solvents. In this study, cinnamon hydrosol, which is typically considered a waste product of hydrodistillation, was found to contain the highest content of total polyphenols, exhibit the highest antioxidant capacity, and demonstrate the greatest inhibitory power against microorganisms of public health significance, compared to extracts obtained by conventional methods using organic solvents. Through gas chromatography analysis, it was confirmed that the cinnamon hydrosol sample contained cinnamaldehyde, a key component responsible for the antioxidant capacity and antimicrobial activity of the hydrosol, as verified by comparison with a pure standard. Moreover, the sodium alginate encapsulation of cinnamon hydrosol enables the preservation of its active principle, making it suitable for incorporation into food matrices. This encapsulation method holds great potential for utilizing the cinnamon hydrosol in various food applications. In the future, conducting further studies, particularly investigating the release of the compounds from the encapsulated matrices, will be essential. We are committed to actively working on this research to gain a deeper understanding of the behavior and

potential applications of the encapsulated cinnamon hydrosol.

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Author Contributions

B. E.: Methodology, Research, Writing - Original Draft; L. C.: Conceptualization, Supervision, Writing, Review and Edition. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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