MICROENCAPSULATION OF POLYPHENOLS FROM *MENTHA AQUATICA* LINN. VAR. *CRISPA* BY SPRAY-DRYING TECHNIQUE

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Abstract. *Mentha aquatica* Linn. var. *crispa* is a spice plant commonly used and found in traditional markets and supermarkets in Vietnam. *Mentha* species have been identified with antioxidant and antibacterial properties. Polyphenols are a class of compounds found in most plants. The practical application of polyphenols is highly dependent on maintaining biological activity and the unpleasant taste of phenolic compounds is also a limitation. To overcome these disadvantages, polyphenol preparations in the form of microencapsulation by spray drying is proposed. This study evaluated the effects of drying temperature and the concentration of gum Arabic (GA) on the encapsulation yield (EY), total polyphenol contents (TPC), and antioxidant capacity (AC) of products microencapsulated by a spray drying process. Results showed that spray drying at 150 °C and 15 % GA concentration gave the best microencapsulation efficiency with EY of 52.3 % and TPC of 74.53 mg GAE/g dw, IC_{50} value, according to 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, was 3.41 mg/mL. The minimum inhibitory concentration (MIC) value against *Escherichia coli, Salmonella enteritidis*, and *Staphylococcus aureus* was 6.25 mg/mL, while it was 3.13 mg/mL against *Bacillus subtilis*. The results show that the polyphenol microencapsulation from *Mentha aquatica* by spray drying can be applied in studies of food preservatives with antioxidant and anti-microbial properties.

Keywords: antioxidant capacity, *Mentha Aquatica,* microencapsulantion, polyphenols, spray drying

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Introduction

Polyphenols are a group of natural compounds found in most plants. The effective application of polyphenols relies on preserving their biological activity, while the unpleasant taste of phenolic compounds is a hindrance. Microencapsulation by spray drying is the best way to overcome these drawbacks to maintain polyphenol.

Microencapsulation is a technique of encapsulating solids, liquids, or gases (substrate) in an extremely thin envelope which will preserve and protect the substrate from being changed, reducing its quality, or minimizing damage. However, it only releases these substrates under certain special conditions, such as diffusion, biodegradation, pressure effect, pH change, temperature change, and osmosis. These diverse techniques include spray drying, coagulation, liposome entrapment, and extrusion, etc [1]. The advantages of spray drying are that it helps to stabilize polyphenols while reducing the unpleasant flavors of phenolic compounds. Microencapsulation by a spray drying method has the advantages of simplicity and low cost, and it can meet the requirements of polyphenol microencapsulation used in food production. Spray drying can protect biologically active ingredients and mask the unpleasant flavors of biologically active ingredients [2].

The genus *Mentha* includes eighteen species and eleven hybrids. Some species have high-value essential oils and good taste and have been used in traditional medicine and the food industry. Extracts of *Mentha* spices possess high TPC with values from 14.66 to 43.21 mg GAE/g dry weight (dw) and high AC according to DPPH with IC_{50} value from 7.50 to 44.66 µg/mL [3]. *Mentha aquatica* is a popular spice in Vietnam and is obviously found in traditional markets and supermarkets. It is also often added to dishes or eaten fresh and utilized in traditional medicine to treat headaches and diarrhea.

Nowadays, there are common studies on polyphenol microencapsulation; for instance, Quoc and Muoi [4] microencapsulated polyphenol from *Polygonum multiflorum* Thunb., Papoutsis et al. [5] also encapsulated citrus by-product extracts and determined TPC of products, etc. However, microencapsulation of polyphenols from *Mentha aquatica* has not been performed.

In this study, the production of microencapsulated polyphenol prepared from *Mentha aquatica* by spray drying, using GA as carriers, evaluated TPC and AC, and determined the antibacterial activity against four bacteria strains of *E. coli, S. enteritidis*, *B. subtilis*, and *S. aureus*. Scanning electron microscopy (SEM) and dynamic light scattering spectroscopy (DLS) were used to determine the particles' morphology and size. The study results will help use polyphenol extracts from *Mentha aquatica* more effectively and can be applied in food preservatives research with antioxidant and anti-microbial properties.

Materials and Methods

Chemicals

Gum Arabic (GA) was used as wall material and supplied by Tianjin Dengfeng (China). DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2- carboxylic acid) were purchased from Sigma-Aldrich (USA). Solvents acetone (99.5 %) and other chemicals were analytical standards from Xilong (China).

Media and Bacteria

Nutrient Broth (NB, Himedia, India) was used in antibacterial activity test. The microorganisms were four different strains: *Escherichia coli* – ATCC 25922, *Salmonella enteritidis* – ATCC 13076, *Staphylococcus aureus*– ATCC 25923, and *Bacillus subtilis* – ATCC 25924. They were obtained from Microbiologics (USA).

Preparation of Acetone Extract

M. aquatica used in the study was obtained from Long An province, Vietnam. Plants were harvested about 2 months old, chose pest-free plants, removed yellow leaves, and used both stems and leaves. Samples were washed and dried at 40 °C until the moisture content was 8%. They were then grounded and passed through a 60-mesh sieve. The prepared powder was added to solvents (acetone 50% in water) with a material to solvent ratio of 1/20 (w/v), extracted for 2 h at 40 °C, then filtered and vacuum evaporated at 60 °C.

Effect of Drying Temperature and GA Concentration on the Yield, Total Polyphenol Content (TPC), and Antioxidant Capacity (AC) of the Obtained Products

Dissolved acetone extract to distilled water with extract to water ratio of 6:100. Gum Arabic was dissolved in the prepared extract at ratios of 10, 15, and 20% (w/v) and homogenized by stirring at a high speed of 10,000 rpm for 10 min; and then spray dried in a Lab Plant SD-06 spray dryer at temperatures of 130, 150, and 170 °C, the outlet temperature ranged from 65 to 70 °C. The moisture content, EY, TPC, and AC were determined for the obtained products.

Evaluation of Antimicrobial Activity (AA), AC, SEM, and DLS of the Microencapsulated Productions

The best sample obtained from microencapsulation by spray drying using GA as a carrier was evaluated. IC_{50} was determined according to a DPPH assay, antibacterial activity (AA) was evaluated according to MIC on four bacterial strains *E.coli – ATCC 25922*, *Salmonella enteritidis – ATCC 13076*, *Staphylococcus aureus– ATCC 25923*, and *Bacillus subtilis – ATCC 25924*). In addition, the sample was analysed by size distribution and micromorphology.

Determination of Size Distribution and Micromorphology of Spray-dried Powder

DLS (dynamic light scattering) was used to analyze the size distribution of dried powder (using a Malvern instrument at 25 °C). The dried powder's morphology, size and microstructure were determined by scanning electron microscope (SEM, model FE-SEM S-4800, Hitachi, Japan). SEM was carried out at 5 kV and a pressure of 0.04 Pa.

Determination of Encapsulation Yield (EY) of Spray Dried Powder

The EY was calculated using the Equation (1), where MSA is the total dried mass of the microcapsules obtained and MSB is the total dried mass before encapsulation [4].

$$\%EY = \frac{MSA}{MSB} \times 100 \quad (1)$$

Minimum Inhibitory Concentration (MIC) of Spray Dried Powder

The MICs of samples (dried powders or extracts) were performed according to the method of Jiang et al. [6] with a few changes. Microorganisms were prepared using 0.5 McFarland standard (~10^8 CFU/mL) and diluted in sterilized water to 10^6 CFU/mL. Samples were dissolved and diluted in sterilized water to 250 mg/mL, 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.63 mg/mL and 7.81 mg/mL. The prepared samples solution (1 mL) and 1 mL of microorganisms (10^6 CFU/mL) were added to 8 mL of NB media solution and incubated for approximately 24 h. The negative control sample, including 8 mL of NB media solution, 1 mL sterilized water and 1 mL of microorganisms (10^6 CFU/mL) were mixed. The MIC of the samples was the lowest concentration which did not permit any turbidity of the tested microorganism, while the negative control sample was turbid.

Determination of Total Polyphenol Content (TPC)

The TPC was analyzed based on the method published by Laczkó-Zöld et al. [7] with some modifications. The reagent used was FC, with gallic acid as the standard. Amount 0.5 mL of sample was added 2.5 mL of FC, then shaken, and incubated in the dark for 5 min. Then, 2.5 mL of 7.5% Na_2CO_3, was added, followed by vortexing and incubation at room temperature for 30 min. The absorbance of the samples was measured at 760 nm. The results were expressed as gallic acid equivalents per gram of dry weight (mg GAE/g dw).

Determination of Antioxidant Capacity (AC) by DPPH Assay

The DPPH assay was evaluated by the procedure of Khamphasan et al. [8] with some minor changes. The sample (0.2 mL) was mixed with 4 mL of 0.1 mM DPPH in ethanol 99.5%, vortexed, and put in the dark for 30 min. Using a spectrophotometer (UVS-2800, Labomed USA), the absorption was measured at 517 nm. The results were expressed as the ability to scavenge 50% of free radical DPPH or μmol TE/g dry weight (μmol TE/g dw).

Data Analysis

All experiments were performed in triplicate, the results were expressed as the average of three samples ± standard deviation (mean ± SD). IBM SPSS statistics software, version 20 (IBM, USA), was used to compare results with a significance level of 95% ($p \leq 0.05$).

Results and Discussion

Moisture Content, TPC, and AC of Extract and GA

Extract and GA were stored at 4 °C until used. They were analyzed for moisture content, TPC and AC. The results were shown in Table 1.

Table 1: Moisture, TPC, and AC of acetone extract and GA

Sample	Moisture content (%)	TPC (mg GAE/g dw)	AC (µmol TE/g dw)
Extract	6.80±0.10	247.25±0.71	419.59±0.52
GA	10.07±0.15	-	-

The results showed that GA does not have TPC and AC, while the TPC of the extract was 247.25±0.71 mg GAE/g dw. This result is higher than that of Babbar et al. [9], who researched extracts from six important fruit residues (kinnow peel, kinnow seeds, litchi pericarp, litchi seeds, grape seeds, and banana peel); the TPC values ranged from 3.68 mg GAE/g dw to 37.4 mg GAE/g dw. The AC value of the extract (419.59±0.52 µmol TE/g dw) was higher than the results of Quoc and Muoi [10], who researched the extract from *Polygonum multiflorum* Thunb. root. The DPPH value was 334.07±3.04 µmol TE/g dw. In addition, the moisture content of the initial GA in this study was quite low and near 10.07%, which is an advantage for long-term storage and use.

Effect of Inlet Drying Temperature and GA Concentration on EY, TPC, and AC of the Products

After spray drying, the powder obtained was analyzed for EY, moisture content, TPC, and AC. The results are shown in Table 2. The moisture content of the samples was from 3.52 to 4.72%. It was found that all of the samples had very low moisture content, which is perfect for storage and use. The results showed that the moisture content of the dried powder was lower than the extract and the initial GA. These results are also similar to result Navarro-Flores et al. [11] when encapsulated *Crotalaria longirostrata* (chipilin) extract, the moisture content ranged from 3.15 to 4.31%.

Table 2 shows that the EY values of all samples were from 34.69 to 52.30%. The sample with the highest EY (52.30%) was dried at 150 °C and had a GA concentration of 15%. This EY was higher than the result of Bucurescu et al. [12]. When encapsulated curcumin using GA as the encapsulating agent, the EY was 42%. The 52.3% value is lower than the EY value (64.39%) Navarro-Flores et al. [11], who researched spray drying of *Crotalaria longirostrata* (chipilin) extract using GA and MD as the carrier. In samples with the same carrier concentration, the AC of samples dried at 150 °C was higher than that of other samples. This can be explained as follows. When drying at high temperatures, the Maillard reaction can occur and form some substances which possess AC, however, a high drying temperature will lead to the loss of polyphenols [2].

Table 2: Effect of inlet drying temperature and GA concentration on moisture content, EY, TPC, and AC of the obtained products

Drying temperature (°C)	GA 10%	GA 15%	GA 20%
		Moisture content (%)	
130	$4.25\pm0.01^{a,x}$	$4.45\pm0.03^{b,x}$	$4.72\pm0.03^{c,x}$
150	$3.84\pm0.05^{a,y}$	$4.01\pm0.04^{b,y}$	$4.14\pm0.04^{c,y}$
170	$3.52\pm0.03^{a,z}$	$3.67\pm0.02^{b,z}$	$3.73\pm0.01^{c,z}$
		EY (%)	
130	$34.69\pm1.83^{a,x}$	$41.19\pm1.08^{b,x}$	$37.68\pm0.50^{c,x}$
150	$35.52\pm1.53^{a,x}$	$52.30\pm1.75^{b,y}$	$39.63\pm1.12^{c,y}$
170	$44.89\pm1.26^{a,y}$	$50.47\pm1.61^{b,y}$	$48.11\pm0.99^{b,z}$
		TPC (mg GAE/g dw)	
130	$66.02\pm0.42^{a,xy}$	$74.88\pm0.10^{b,x}$	$64.23\pm0.21^{c,x}$
150	$65.56\pm0.10^{a,x}$	$74.53\pm0.52^{b,x}$	$64.38\pm0.27^{c,x}$
170	$66.17\pm0.31^{a,y}$	$66.75\pm0.45^{a,y}$	$67.75\pm0.30^{b,y}$
		AC (µmol TE/g dw)	
130	$80.17\pm0.30^{a,x}$	$84.47\pm0.44^{b,x}$	$89.90\pm0.36^{c,x}$
150	$84.45\pm0.40^{a,y}$	$111.52\pm0.10^{b,y}$	$96.60\pm0.17^{c,y}$
170	$92.24\pm0.20^{a,z}$	$93.13\pm0.10^{b,z}$	$81.24\pm0.36^{c,z}$

x, y, z in the same column and a, b, c in the same row indicate significant difference ($p \leq 0.05$)

All samples' TPC and AC values were lower than the extract. The best condition for the sample with 15% GA carrier was obtained at 150 °C inlet drying temperature and the TPC was 74.53 mg GAE/g dw. This value is higher than the results of Papoutsis et al. [5], who researched the encapsulation of citrus by-product extracts by spray-drying using maltodextrin as a carrier and obtained a TPC value of 1.26 mg GAE/g dw. It is lower than the results of Pratami et al. [13], who studied spray-dried microcapsules propolis from *Tetragonula species* and obtained a TPC of 237.33 mg GAE/g. The AC was 111.52 µmol TE/g dw. This value is lower than the result of Quoc and Muoi [4], who researched the encapsulation of *Polygonum multiflorum* Thunb. with GA as a carrier and obtained an AC value of 146.97 µmol TE/g dw.

The results in Table 2 show that using the GA carrier at a concentration of 15% and an inlet drying temperature of 150 °C gave the best results. This result also coincides with the results of Kalajahi and Ghandiha [14], who spray-dried Nettle (*Urtica dioica* L.) extract gave the best temperature of 150 °C.

Determination of AA, AC, Micromorphology, and Size Distribution of Dried Powder

The best sample from the experiments above and the extracts were assayed for antimicrobial activity (AA) and IC_{50}. In addition, the product was analyzed by SEM and DLS. The results are shown in Table 3, Figures 1 and 2.

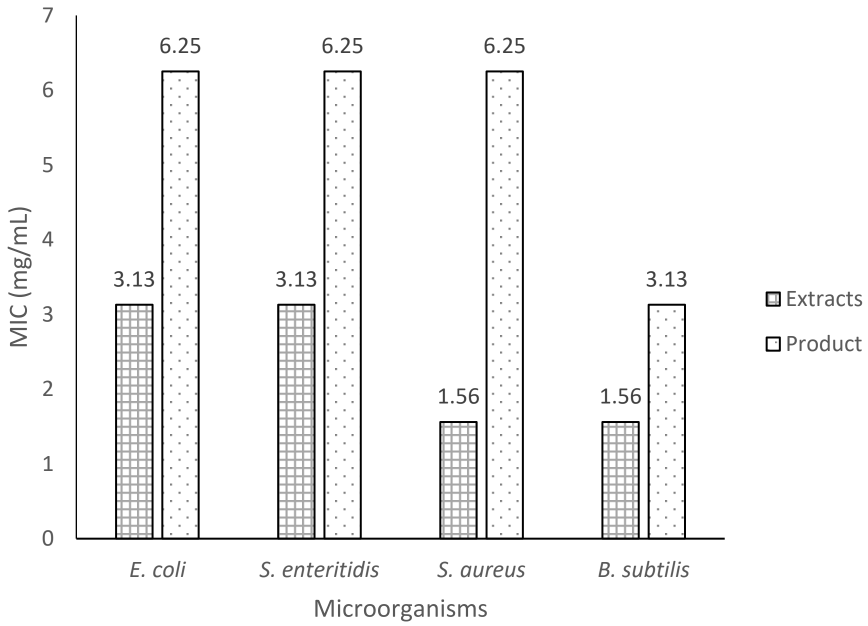

Figure 1: Antimicrobial activity of extracts and product

Table 3: Antioxidant capacity of product and extracts

Samples	**Trolox**	**Extract**	**Product**
IC_{50} (mg/mL)	0.42±0.00	1.24±0.01	3.41±0.02

Figure 1 shows that the AA of the product against *E.coli, S. enteritidis, S. aureus*, and *B. subtilis* were lower than the extract. This result was obtained because when spray drying, there was a combination of extract and carrier to form products. Therefore, the content of biologically active substances in the dried powder will be smaller than in the extract; so, the TPC, AC, and AA of dried powder are lower than the extract.

The AA of the product against *S. aureus* with MIC values of 6.25 mg/mL; it was lower than the result of Othman et al. [15], who researched water extracts from S. *persica* against *S. aureus* with MIC value of 2.49 mg/ mL. The AA of the product against *E. coli* with MIC values of 6.25 mg/mL, and this was higer than the result of Ma et al. [16], they studied the AA of anthocyanins with MIC value against *E.coli* was 10-400 mg/mL. The AA of the product against *S. enteritidis* with MIC values of 6.25 mg/mL was also higer than the result of Ma et al. [16] with MIC value of 10-400 mg/mL against *Salmonella*. These results can be explained by the composition of bioactive substances in different materials having different antibacterial activities.

Table 3 shows the results of IC_{50}, the AC of the product (IC_{50}=3.41±0.02 mg/mL) is lower than the extract (IC_{50}=1.24±0.01 mg/mL) and Trolox (IC_{50}= 0.42±0.00 mg/mL). The results can be attributed to the spray drying process at high temperatures affecting the AC of the polyphenols in the sample [2] and the spray drying process added carrier that reduced the concentration of biologically active substances in the product. The IC_{50} value of the product was 3.41 mg/mL. This value was lower than the result of Mohammed et al. [17], who researched microencapsulation of *Nigella sativa* oil and obtained IC_{50} values of 1.61 mg/g. This means that AC will depend on the chemical composition of the raw materials and the drying method. Hence, the results obtained are different.

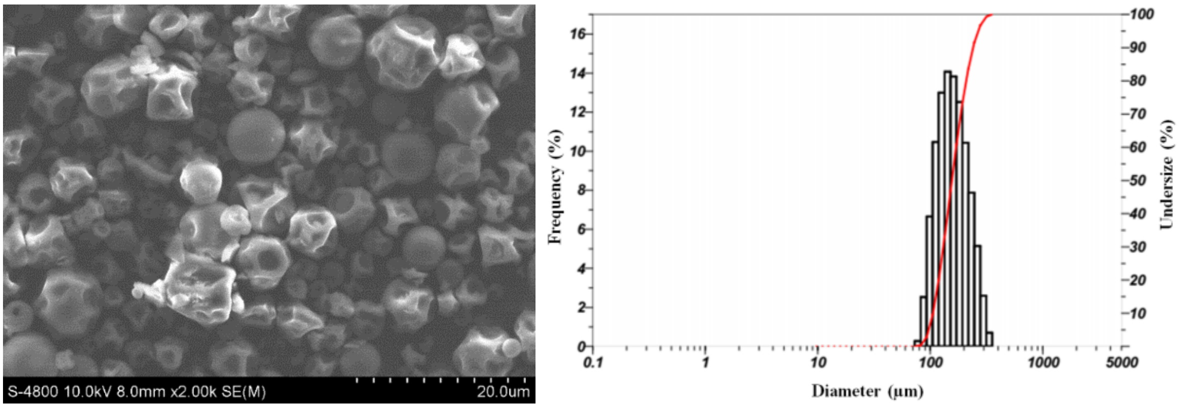

Figure 2: Micromorphology and size distribution of dried powder

The SEM images showed that the dried powder samples have small spheres with different sizes and discrete particles, and there was no adhesion phenomenon. This result differed from the study of Silva et al. [18], who researched microencapsulation of *Myrciaria jaboticaba*; the result was the adhesion of small particles to the surface of larger particles.

The particles produced have a similar shape, mainly spherical; this result was consistent with the study of Tonon et al. [19] when spray drying açai powder with different carrier agents, particles of similar shapes, mostly spherical, were obtained.

The GA carrier helped form more homogeneous particles. Some particles show a concave surface phenomenon. This phenomenon occurs because the particles are intensely dehydrated during the spray-drying process. In addition, the microstructure of the particles depends on the inlet temperature and the type of carrier. At a high drying temperature, water evaporates faster, which leads to the formation of a hard and smooth crust. At low temperatures, spray drying increases the number of particles shrunk on the surface [18-19]. This phenomenon is also consistent with the study of Sun-Waterhouse et al. [2], who researched spray-drying microencapsulation of bioactive compounds and obtained wrinkled surfaces when using sodium alginate as a carrier; however, the results are different from the study of Balasubramani et al. [20] who researched the microencapsulation of garlic oleoresin and obtained smooth spherical shapes. Therefore, perhaps the microencapsulation material itself has the strongest influence on the final product structure.

The products obtained with sizes less than 100 µm account for about 10%, while particles ranging from 100 to 200 µm make up the majority, more than 50%. The other particle size fluctuates from 250 to 350 µm. The presence of larger particles can be explained by the formation of irreversible link bridges in the early stages of agglomeration, which lead to the formation of larger particles [19]. The average size was 201.3 µm; this average particle size was larger than the results of Mohammed et al. [17], who researched the microencapsulation of *Nigella sativa* oil and observed particle sizes from 14.54 to 23.59 µm. The different size of the particles was due to the link formation between the carrier and the microencapsulated components. In addition, it also relates to the molecular size of the carrier.

Conclusions

The results showed that an inlet drying temperature of 150 °C and GA carrier concentration of 15 % gave the most relevant results with EY value was 52.30 % and the TPC of the obtained product was 74.53±0.52 mg GAE/g dw. The AC according to DPPH was 111.52±0.10 µmol TE/g dw and IC_{50} was 3.41 mg/mL. The AA of the product was effective on tested bacteria with MIC values of 6.25 mg/mL against *E. coli, S. enteritidis*, and *S. aureus*, while that of 3.13 mg/mL against *B. subtilis.* The moisture content of the obtained product is very low, which is convenient for the preservation and use of the product. Therefore, this study successfully microencapsulated polyphenols from *Mentha aquatica*. The dried powder obtained had relatively high antioxidant and antibacterial ability, which can be helpful for anti-microbial and antioxidant food preservation research.

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Author Contributions

All authors contributed to the data analysis, drafting, and critical revision of the paper, and they all agree to accept responsibility for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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