https://doi.org/10.3311/PPch.20698

Periodica Polytechnica Chemical Engineering, 67(1), pp. 161–171, 2023

Antioxidant and Antimicrobial Agents from Avocado (*Persea americana*) Seed Extract Encapsulated in Gum Arabic through Spray Drying Method

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Received: 22 June 2022, Accepted: 24 October 2022, Published online: 11 January 2023

Abstract

In this work, the objective is to utilize avocado seed extract as a cheap alternative source of active compounds and was successfully encapsulated using gum arabic by spray drying as an antioxidant and antimicrobial agent. First, the active compounds were extracted from avocado seed using solvent n-hexane and followed by solvent separation using rotary vacuum evaporator. Next, gum arabic as the encapsulation agent was added to avocado seed extract to form an emulsion, then spray-dried with drying air at a temperature of 140 °C. The mass ratio of avocado seed was varied at extract: gum arabic of 5:5, 5:10, 5:15, and 5:20. Then, the particle morphology, yield, moisture content, encapsulation efficiency, loading capacity, chemical groups, antioxidant activity, and antibacterial ability were analyzed to investigate the performance of the encapsulation particles. Among the ranges studied, the extract–gum arabic with a mass ratio of 5:10 exhibited the best properties of yield powder, loading capacity, and encapsulation efficiency at 49.78%, 3.43%, and 7.33%, respectively. The encapsulated particles have smooth surface, crackless, lower indentation, and a single-core encapsulation structure with an average diameter of 4.60 µm. Besides, the stability of antioxidant activity decreased by 2.36%, from 94.82% to 92.46% for four weeks of storage. They also performed antimicrobial activity against *E. coli* and *S. aureus*, which were maintained after seven days. Meanwhile for avocado seed extract only, the unencapsulated stability of antioxidant activity has continually decrease from 91.58% to 84.05% and performed no antimicrobial activity against *E. coli* after seven days.

Keywords

avocado seed, gum arabic, microencapsulation, spray drying, prolonged shelf-life

1 Introduction

The free radicals have been attracting interest to result in a health product that promises degenerative disease prevention and management, which could come from initiation or propagation of oxidative chain reaction that causes oxidation of lipid and proteins in cells of the human body [1–3]. Antioxidants, as free radical scavengers, take essential roles in this case as one of the promising antioxidant raw materials. Avocado is rich in active compounds. The main part of this fruit is pulp (65%), followed by seeds (20%) and peel (15%). The pulp is the part that is often used, while the seeds and peel become waste. Meanwhile, avocado seeds are one of the best sources of dietary fiber and contain valuable bioactive compounds [4, 5]. In addition, avocado seed can produce many beneficial compounds such as phenolic, polyphenol, and tocopherols with the ability to use as antioxidants and antimicrobial [6, 7]. From an economic point of view, this predominance leads avocado seeds to be more interested in the application as a cheap alternative source that provides antioxidant and antimicrobial activity. However, the high sensitivity characteristic of these active compounds to unfavorable environmental conditions such as light, air, and temperature limits the commercial application of active compounds in avocado seed extract [8].

A compatible method is needed to protect the health benefits of the active compounds from degradation. Entrapping the active compounds into edible stable coating through microencapsulation is one of the most effective strategies to overcome these problems. By doing so, the active compounds would be isolated from exposure to potential damage conditions, increasing their stability

Cite this article as: Kautsar, D. B., Rois, M. F., Faizah, N., Widiyastuti, W., Nurtono, T., Setyawan, H. "Antioxidant and Antimicrobial Agents from Avocado (*Persea americana*) Seed Extract Encapsulated in Gum Arabic through Spray Drying Method", Periodica Polytechnica Chemical Engineering, 67(1), pp. 161–171, 2023. https://doi.org/10.3311/PPch.20698

and prolonging active compound shelf life [9]. The most common method for microcapsule preparation is spray drying due to the predominance of high-speed particle production, low production cost, simple continuous process, and handily scale up to an industrial scale for mass production [8, 10–12]. With this drying method, the feed, consisting of active compounds dispersed in the selected encapsulation agent as an emulsion that would rapidly be converted into a stable microcapsule, provides a low temperature around the core [13]. Regarding the fulfilled these purposes, one of the essential factors in microencapsulation is selecting a suitable encapsulation agent. To obtain the emulsion feed of spray drying, the encapsulation agent must have excellent emulsifier properties and low viscosity [14]. Gum arabic has been considered a promising candidate due to the many advantages properties of excellent emulsifier, high solubility, low viscosity, good retention, non-toxic, environmentally friendly, not having a prominent unpleasant taste, and good properties film-formation. These characteristics are suitable for the encapsulating agent of essential oil [13, 15, 16].

The active compounds from avocado seed have been extensively studied and shown good potential antioxidant and antimicrobial activity performance. However, there is no effort yet reported to increase the stability of the active compound of avocado seed extract, which has inhibited the expansion of avocado seed application in the food industry. Therefore, further attention was paid to investigating the stability performance and the properties of avocado seed extract in gum arabic microcapsule. Here, the effect of various concentrations of gum arabic is investigated by spray-drying to increase the commercial value of the avocado seed utilization as a health product.

2 Materials and methods

2.1 Materials

Avocado (*Persea americana*) seed was obtained from a local collector in a traditional market in Surabaya, Indonesia. As an encapsulation agent, gum arabic was purchased from Indo Food Chem (Jakarta, Indonesia). N-hexane (C_6H_{14}; 99%, p.a.) and methanol (CH_4O; 99.9%, p.a) were purchased from Merck. DPPH (2,2-diphenyl-1-picrylhydrazyl; $C_{18}H_{12}N_5O_6$, reagent grade) was purchased from SMART LAB (Tangerang, Indonesia). Demineralized water was used for emulsion preparation processes. All chemicals were used as received without any further purification.

2.2 Extraction of avocado seed

Avocado seed with 0.5–1 mm thick slices was dried to constant weight in an oven at 80 °C, followed by milling and sieving to obtain a powder with approximately particle size 500 μm. 25 g of the powder was then extracted with n-hexane (200 mL) at 68 °C in Soxhlet for 60 min. The mixture solution extract was concentrated using a rotary vacuum evaporator (Büchi 461, Switzerland), 11 mL for every 500 mL solution at a 45 °C water bath, and 154 mbar to remove n-hexane. The avocado seed extract was kept at −20 °C and isolated from light and air before use [17, 18].

2.3 Microencapsulation

Gum arabic was hydrated with demineralized water under stirring at 200 rpm and room temperature (29 °C) overnight. The avocado extract was then mixed with the hydrated gum arabic to obtain a total of 50 ml of emulsion, so the observed samples were avocado extract only, gum arabic only, and the extract-gum arabic with a mass ratio of 5:5, 5:10, 5:15, and 5:20. The emulsions were prepared by blending the mixture at 13,500 rpm for 1 min with the shearing machine (Ultra-Turrax, T18, IKA, Germany). The fresh emulsion was fed immediately into a spray dryer (TFS-2L, China) using a peristaltic pump at 3.7 mL/min while the emulsion was kept stirred with a magnetic stirrer and sprayed through a two-fluid nozzle [19]. The inlet drying air temperatures at 140 °C. The particles that passed the spray chamber were collected, as illustrated in the spray drying system in Fig. 1. The powder product called extract-gum arabic particles was then isolated from air and placed in a dark room and room temperature before further characterization. The spray dried gum arabic solution from hydrated gum arabic was also analyzed for comparison to encapsulated particles.

2.4 Characterization

The particle morphology was observed using scanning electron microscopy (SEM; Hitachi, FlexSEM 1000, Japan). ImageJ software examined the particle size distribution and average particle size by measuring at least 200 particles in SEM images. Further morphology observations of the microcapsule structure were carried out using optical microscopy (Nikon, E100LED, Japan). Previously, the sample was prepared into methanol solution to make the non-encapsulated oil can be eliminated from optical microscopy observation. Furthermore, the chemical structures and bonding interaction between gum arabic and avocado

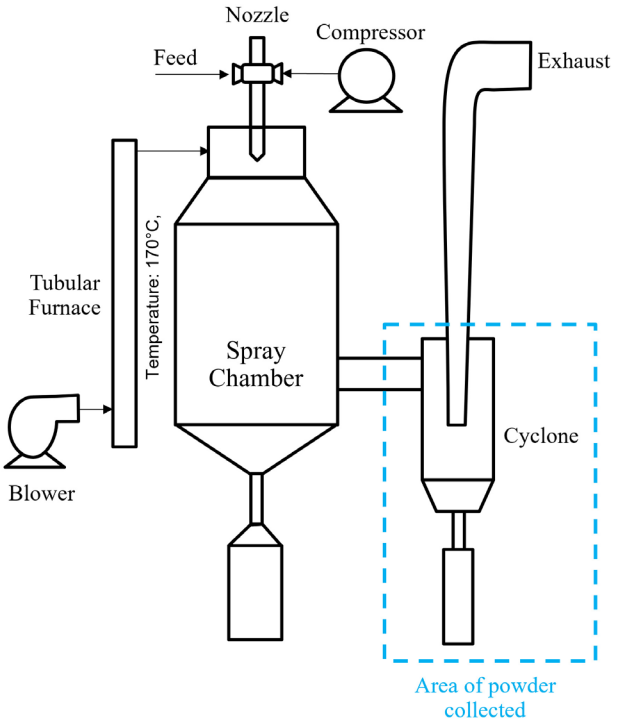


Fig. 1 Spray drying system

seed extract in microcapsules powders were investigated by Fourier transform infrared spectroscopy (FTIR; Thermo Scientific Nicolet, iS10, Massachusetts USA) at wavenumber ranging from 500 to 4,000 cm^{-1}.

The yield of dried microcapsules was determined gravimetrically as the weight of microcapsules collected divided by the total weight of gum arabic and avocado extract in the emulsion feed. It follows Eq. (1) [20]:

$$\text{Powder yield } (\%) = \frac{W_{\text{powder}}}{W_{\text{avocado seed extract}} + W_{\text{gum arabic}}} \times 100\%. \quad (1)$$

Conformably, for determination of moisture content, 1 gram of fresh microcapsule product as weight before oven (WBO) was further dried at 80 °C for 48 h. It was then placed in a desiccator containing silica for an hour, and the mass measurement was considered as weight after the oven (WAO). The moisture content is examined using Eq. (2):

$$\text{Moisture content } (\%) = \frac{\text{WBO} - \text{WAO}}{\text{WBO}} \times 100\%. \quad (2)$$

One gram analysis sample was mixed with 100 mL of demineralized water at 150 rpm for 30 min to determine the stability of the microcapsule. The mixture is then centrifuged for 10 min. Afterward, 25 mL of supernatant were collected in the preweighed flask and dried at 105 °C for five hours. After an hour in desiccators containing silica, the different weight of the flask was measured as solute weight. Equation (3) was used to calculate the capsule stability [21]:

$$\text{Capsule stability } (\%) = 100\% - \frac{\text{solute weight}}{\text{analysis sample weight}} \times \frac{100 \text{ mL}}{25 \text{ mL}} \times 100\%. \quad (3)$$

Besides, the determination of encapsulation efficiency, loading capacity, and free avocado seed extract were prepared by sonication of 1 gram analysis sample of a microcapsule in 10 mL n-hexane for two hours to dissolve the microcapsule. Afterward, the mixture was poured through the filter paper and rinsed with n-hexane until the filtrate looked clear. The filtrate collected in a preweighed flask was then heated to remove n-hexane. The difference weight of the flask was expressed as total extract microcapsule (W_{EM}). On the other hand, one gram of microcapsule sample was stirred with 10 ml n-hexane at 150 rpm for 2 minutes to dissolve the unencapsulated avocado extract. The mixture was then filtered, and the solid sample was finally dried to evaporate the n-hexane left. The difference weight of the flask of these steps was expressed as an extract on surface microcapsule (W_{ES}). As a result, free avocado seed extract, loading capacity, and encapsulation efficiency were represented by Eqs. (4), (5) and (7), respectively [22]:

$$\text{The free avocado seed extract } (\%) = \frac{W_{ES}}{W_{\text{analysis sample of microcapsule}}} \times 100\%, \quad (4)$$

$$\text{Loading capacity } (\%) = \frac{W_{EM} - W_{ES}}{W_{\text{analysis sample of microcapsule}}} \times 100\%, \quad (5)$$

$$\text{Encapsulation efficiency } (\%) = \frac{(W_{EM} - W_{ES}) \times W_{\text{powder}}}{W_{\text{avocado seed extract}}} \times 100\%. \quad (6)$$

The stability of antioxidant activity of avocado seed extract with and without encapsulation was evaluated by DPPH assay. For avocado seed extract encapsulation, the microcapsules powders were extracted using 10 mL methanol for each 0.3 g sample in a sonication water bath for two hours to break down the encapsulating agent. This mixture was centrifuged for 10 minutes to separate encapsulating agent as sediment, and then the supernatant was collected. Next, 3 mL of 0.1 mM DPPH was added to 0.5 mL of supernatant, followed by shaking for 30 minutes

under light and air insulation. The absorbance of the solution was measured at a wavenumber of 517 nm as absorbance of the sample (Abs_{sample}) by spectrophotometer (West Tune, N2S). At the same time, the determination of the antioxidant activity of avocado seed extract was prepared by dissolving 6 mg avocado seed extract into 10 mL methanol, followed by DPPH addition, shaking, and air insulation. The observation was carried out weekly for four weeks of storage to evaluate antioxidant activity stability. The antioxidant activity was expressed as DPPH* inhibition percentage using Eq. (7) [19]:

$$\text{Antioxidant activity by DPPH}^{*}\text{ assay (\%)} = \frac{Abs_{\text{DPPH}^{*}} - Abs_{\text{sample}}}{Abs_{\text{DPPH}^{*}}} \times 100\%. \quad (7)$$

Escherichia coli and *Staphylococcus aureus* were used as representatives for pathogenic microbial to investigate the antimicrobial activity. Each bacterial suspension was diluted into 10 mL of demineralized water and then transported for 4 µL to a nutrient agar petri dish. The bacteria dilution was inoculated on the surface nutrient using a cell spreader. A small amount of each sample was placed in the canter of the plate and then covered by circle filter paper for gum arabic and avocado seed extract encapsulated sample. The inhibition zone indicating the antimicrobial activity was observed on the first and the seventh days after production [23].

3 Results and discussion

The morphology of the extract-gum arabic particles observed by SEM, then compared with only the gum arabic particles, is shown in Fig. 2 at 1000× magnification. The gum arabic particles in Fig. 2 (a)–(d) are gum arabic only with 5–20% concentrations by an interval of 5%. Qualitatively, the particle of gum arabic reveals a concave shape, regardless of the concentration, and has an average particle diameter from 4 to 6 µm. Different to Fig. 2 (e)–(h), after the avocado seed extract was introduced at the mass ratios of avocado seed extract: gum arabic are 5:5, 5:10, 5:15, and 5:20, respectively, they exhibit less concave particles compared to the particle of gum arabic only. Even for the smaller particles, the shape is nearly spherical. Besides, it can be seen that after the presence of avocado seed extract, the increasing gum arabic concentration increased particle size, from 3 to 6.4 µm. Without avocado seed extract, the emulsion consists of only gum arabic and water. After the spray dried, the water content evaporated, leaving a space inside the gum arabic particle. Since the solidification speed of gum arabic is lower than the speed of vapor coming out from the particle, the particle shell got wrinkled and made a deep concave shape. The deformation shape of a particle usually depends on the material stiffness. For stiff materials such as silica, this phenomenon usually makes the particle in doughnut-like form. However, since the character of gum arabic is quite ductile, it only becomes a concave particle [12, 24]. On the other hand, after the presence of avocado seed extract, the dopped liquid consists of not only gum arabic and water but also the extract that has a higher boiling point [6, 7, 25]. Therefore, after being contacted with hot air at the spray dryer, the avocado seed extract is still in the liquid phase, making a less concave shape. Especially in Fig. 2 (e), for microcapsule with avocado seed extract-gum arabic at a mass ratio of 5:5, it is clear that there is agglomeration among the particles. The content of gum arabic in that concentration is too few, so not all the avocado seed extract could be well encapsulated. Some of them stick to the surface of the particle. Carried by the airflow, the sticky particles were then agglomerated with others. Contrary to Fig. 2 (h), the microcapsule with avocado extract-gum arabic at a mass ratio of 5:20, the particle could stand independently but have a rough and concave surface caused by the thick shell of the microcapsule. The wall material is already dry on the outer surface, while the inner surface is still wet. Therefore, when the water content inside the particle evaporates, the surface cannot maintain a spherical surface, so it is roughly concave. However, microcapsules with avocado seed extract-gum arabic at the mass ratios of 5:10 and 5:15 exhibited in Fig. 2 (f) and (g) show less agglomeration. At the same time, the particles have a better spherical shape, indicating that the amount of gum arabic can fully encapsulate the whole avocado seed extract. Besides, the thin wall material caused the particle to have an equal solidification rate between the inner and outer surface. Therefore, the particle obtained can be maintained to a spherical shape. From a particle size point of view, it can be seen that by increasing gum arabic concentration, the particle size increases from 3.31 to 6.41 µm, but their distribution is heterogeneous. From Table 1, the amount of gum arabic plays a significant role in the viscosity and density of emulsion. Even more, the high amount of gum arabic led to a non-extract atomized droplet, which, after drying, the particle with space inside got shrinkage. Therefore, we can see heterogeneous particle sizes.

The sample was then analyzed using the microscope to investigate the morphology of particle and microcapsule obtained, and the results were presented in Fig. 3.

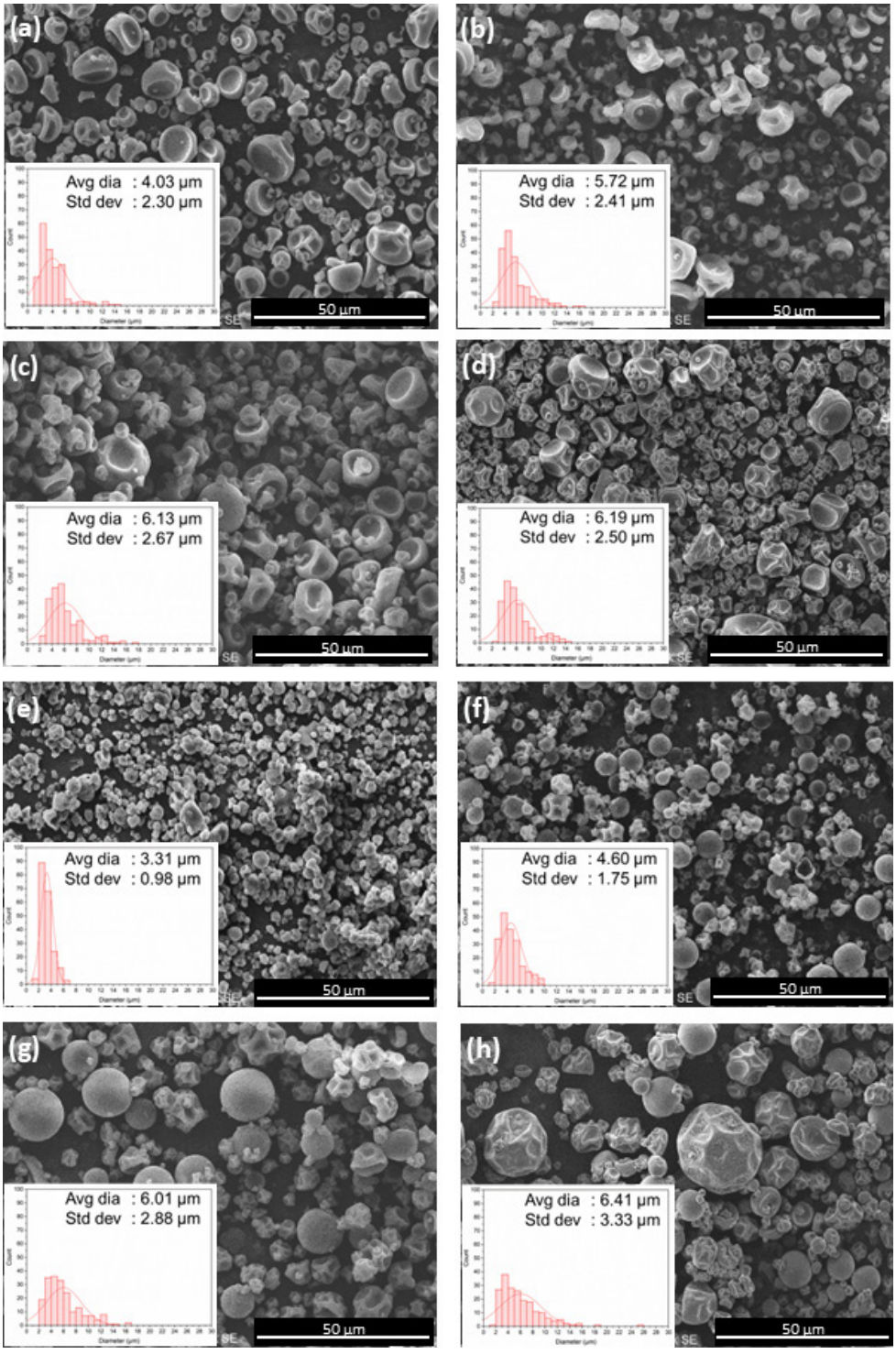


Fig. 2 SEM images at magnification of 1000× of gum arabic particles with concentration of (a) 5%; (b) 10%; (c) 15%; (d) 20%; and extract-gum arabic particles with mass ratio of (e) 5:5; (f) 5:10; (g) 5:15; (h) 5:20

The gum arabic particles are shown in Fig. 3 (a)–(d) with concentrations of 5%, 10%, 15%, and 20%, respectively. It can be clearly seen that in Fig. 3, some gum arabic particles are shrinkage, and the other particles form a circle without any core material. It also can be seen that as the concentration of gum arabic increases, led to an increase in particle size. Then Fig. 3 (e)–(h) shows the morphology of the microencapsulate particles of avocado seed extract with gum arabic as the wall material at the mass ratio of avocado seed extract-gum arabic of 5:5, 5:10, 5:15, and 5:20, respectively. Opposite to the non-extract particle, it is seen that the morphology formed is the core material

Table 1 Density and viscosity of avocado extract, gum arabic, and mixture in various concentrations

The concentration of avocado seed extract: gum arabic (w:w)	Density (g/mL)	Viscosity (mPa·s)
100:0	0.92	-
0:100	1.35	-
5:5	1.06	3.37
5:10	1.09	9.48
5:15	1.12	29.20
5:20	1.14	57.00

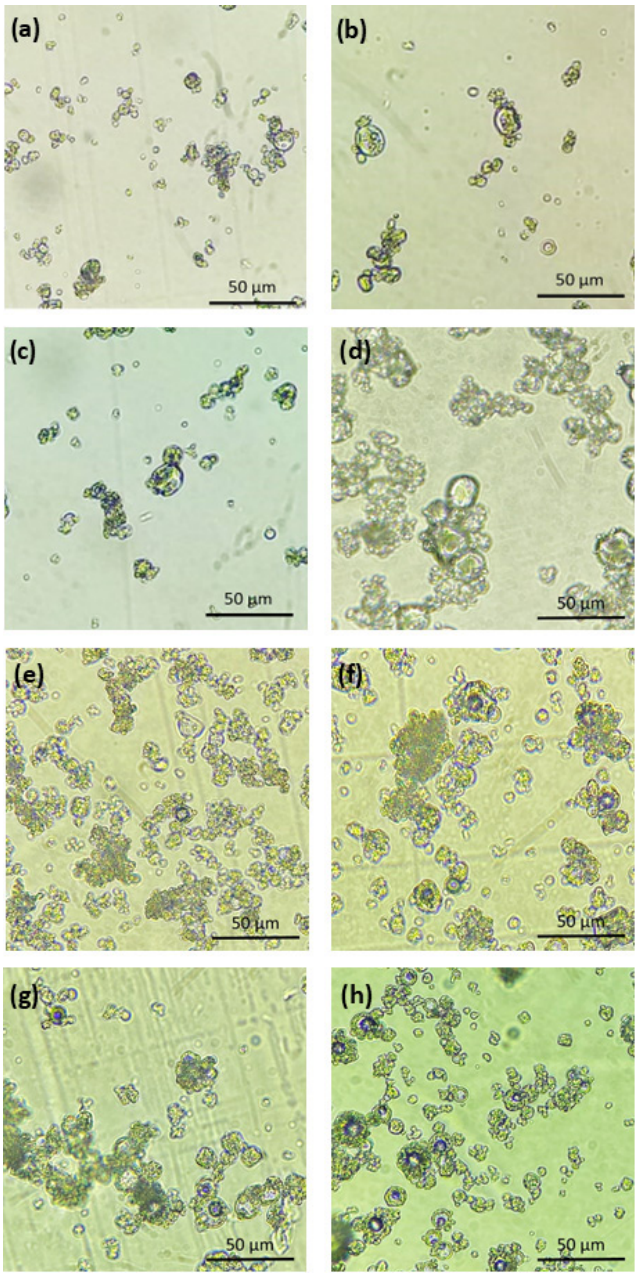
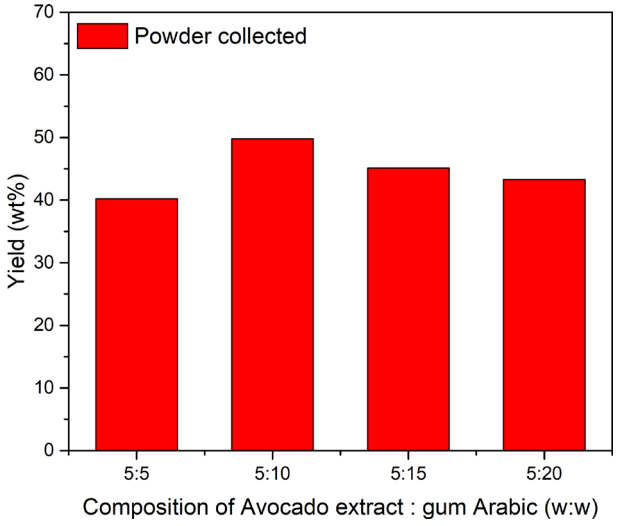


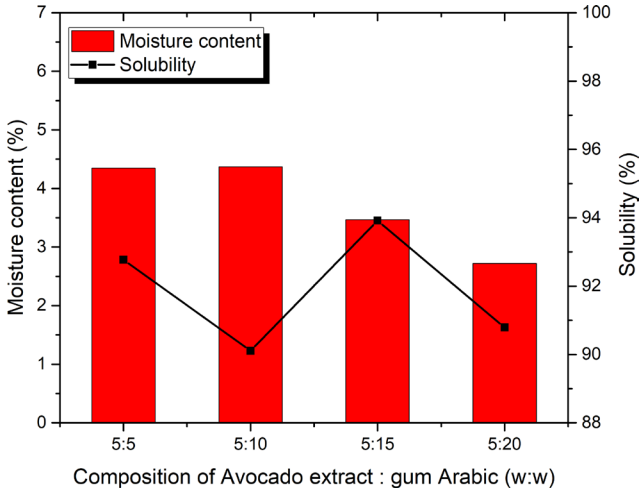
Fig. 3 Light microscope images at 400× magnification of gum arabic with concentration (a) 5%; (b) 10%; (c) 15%; (d) 20%; and extract-gum arabic with mass ratio of (e) 5:5; (f) 5:10; (g) 5:15; (h) 5:20

surrounded by wall material. A black core inside a circle indicates the core material, and the transparent part surrounding the core is gum arabic as the wall material. It shows that some avocado seed extract was successfully encapsulated inside the gum arabic. However, there are also some hollow particles due to the increasing concentration of gum arabic, while the concentration of avocado seed extract was constant.

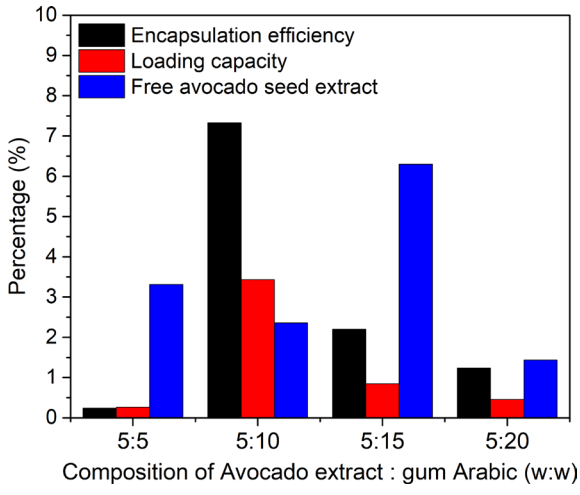
Furthermore, the microcapsule collected was gravimetrically analyzed, as presented in Fig. 4. The yield of particles collected at various avocado seed extract-gum arabic mass ratios are expressed in Fig. 4 (a). The yield of avocado extract-gum arabic at the mass ratio of 5:5 to 5:10 reveal an increase from 40 wt% to 50 wt%. However, after reaching mass ratios 5:15 and 5:20, the yield decreased to 45 wt% and 43 wt%, respectively. The phenomenon of yield increase indicates that there is still unencapsulated avocado extract for avocado extract-gum arabic at a mass ratio of 5:5. Since gum arabic as a wall material increased to 5:10, the yield increased, indicating more avocado seed extract could be well encapsulated. However, the subsequent addition of gum arabic drastically increased the viscosity, as presented in Table 1. It leads to the particle agglomeration trapped in the chamber or dropped to the bottom, which may cause the yield to decrease. Furthermore, in Fig. 4 (b), the moisture content of microcapsules tends to decrease as the gum arabic increases. The composition of emulsion feed may influence these phenomena. The emulsion feed with less gum arabic content will have more water content. Therefore, more water content should be evaporated under the same operating condition. Nevertheless, all the obtained microcapsule moisture content is less than 5%. On the other hand, the solubility of microcapsules fluctuated in, ranging from 90% to 94% with the increase of gum arabic concentration. As depicted in Fig. 4 (c), the loading capacity and encapsulation efficiency are similar to powder yield. In this case, the concentration of the encapsulation agent was affected significantly. The most negligible result was found in the extract-gum arabic with a mass ratio of 5:5 due to the insufficient encapsulation agent to form microcapsule properly. An increase in the extract-gum arabic with a mass ratio of 5:10 of emulsion led to a sharp increase in loading capacity, and encapsulation efficiency reached 3.43% and 7.33%, respectively. The extract-gum arabic with a mass ratio of 5:15 and 5:20 have a decreasing loading capacity and encapsulation efficiency. These



(a)



(b)



(c)

Fig. 4 Chart of product obtained (a) yield of powder; (b) solubility and moisture content of encapsulated avocado seed extract; (c) loading capacity, encapsulation efficiency, and free avocado seed extract

facts indicated that the extract-gum arabic with a mass ratio of 5:10 is sufficient to form a good microcapsule. Several studies on seed extract microencapsulation also stated that encapsulated particles using 10% mass of wall material showed the highest encapsulation efficiency because increasing the concentration beyond the optimum state significantly reduced the mechanical strength of the wall material film [26]. The extract-to-wall material mass ratio significantly affected the encapsulation efficiency. The chia seed extract encapsulation reached the optimum extract-to-wall material mass ratio of 1:2 [27]. Therefore, the same result was found in this study. The extract to gum arabic mass ratio was optimum at 5:10. However, when the gum arabic ratio increased to 5:15 and 5:20, the solid shell crust formation can be hindered due to the higher viscosity at the same operating condition affected to poor entrapped avocado seed extract. Therefore, loading capacity is inversely proportional to free avocado seed extract.

The FTIR (Fig. 5) analysis to observe the interaction between avocado seed extract and gum arabic as wall material was conducted for the avocado seed extract only, gum arabic particles, and microcapsule of avocado extract-gum arabic particles with the mass ratio of 5:10, as the highest yield among the variable observed. For avocado seed extract, the significant peaks identified at a wavenumber of 3389 cm^{-1}, 2922 cm^{-1}, and 2853 cm^{-1} indicate the presence of –OH and two aliphatic –CH_2 groups, respectively [28]. Besides, the peaks at 884 cm^{-1} indicate the =CH functional group [29]. The significant peak of

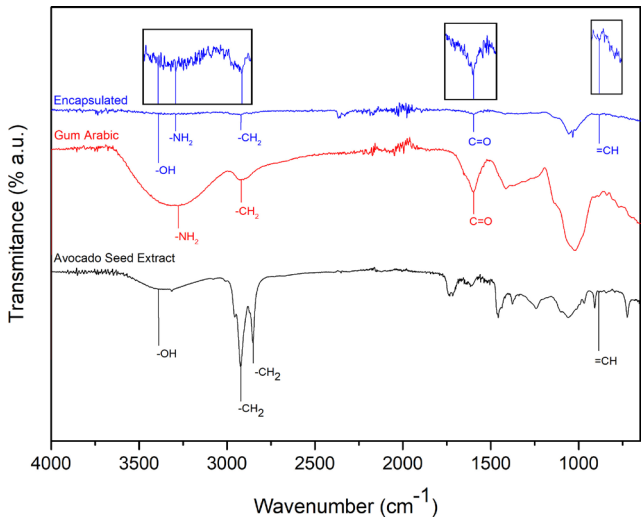


Fig. 5 FTIR spectra of avocado seed extract only, gum arabic particles, and avocado seed extract encapsulated with the extract-gum arabic particle with a mass ratio of 5:10

–CH_2 indicates the bioactive compound of avocado seed extract, which consists of antioxidant and antimicrobial power such as polyphenols, condensed tannins, phenolic acids, and flavonoids. Most of them have aromatic groups [30–33]. The double bond of =CH broke to react with free radicals by delaying the oxidation reaction of other molecules and inhibiting the initial reaction of the oxidizing chain reaction free radicals [34]. For gum arabic particles, the peaks at 3278 cm^{-1}, 2919 cm^{-1}, and 1598 cm^{-1} in gum arabic spectra contributed to the –NH_2, –CH_2, and C=O bands, respectively, are related to the characteristics of hetero-polysaccharide compounds in gum arabic itself [15]. Interestingly, both spectra peaks of avocado seed extract and gum arabic could also be seen in the spectra of the encapsulated sample. Regarding weak band intensity in encapsulated spectra, it probably relates to the concentration of incorporation between avocado seed extract and gum arabic. The encapsulated product came from an emulsion feed consisting of extract-gum arabic with a mass ratio of 5:10. It causes the peak's intensity to appear lower than the raw materials. Nevertheless, both peaks appear in encapsulated samples with no significant wavenumber difference, reassuring that there was no chemical bonding between the core and encapsulating agent. Therefore, the interaction of avocado seed extract and gum arabic was only physical bonding. However, the bands appear in the exact wavenumber without any significant difference. It can be interpreted that the interaction between the core material of avocado seed extract and the encapsulation agent of gum arabic is only a physical bonding that does not change the functional group [35].

2,2-Diphenyl–1–picrylhydrazyl DPPH assay was used for the antioxidant observation activity and the stabilization of microcapsules. As presented in Fig. 6, the percentage of antioxidant activity by DPPH assay with storage time is tested periodically every week. In Fig. 6, samples with avocado seed extract only, particles with avocado seed extract: gum arabic at 5:5, 5:10, 5:15, and 5:20 have been tested for their antioxidant activity abilities. From the view of a percentage of antioxidant activity at week 0, it can be seen that avocado seed extract only indicated the lowest antioxidant activity. It is followed by avocado extract-gum arabic at mass ratios of 5:20, 5:15, and 5:10. The highest antioxidant activity is revealed in the avocado seed extract-gum arabic at a mass ratio of 5:5. By increasing gum arabic concentration, the antioxidant activity decreased. It is correlated to the encapsulation efficiency as shown in Fig. 4 (c), in which some

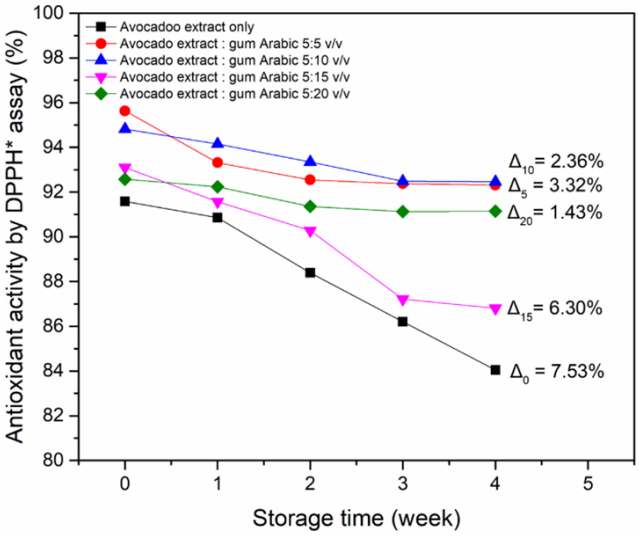


Fig. 6 The stability of antioxidant activity for four weeks storage

samples taken are hollow particle–gum arabic without extract inside. However, after four weeks of storage, the antioxidant activity decreased significantly in the avocado extract only by 7.53%, from 91.58% to 84.05%. In comparison, the encapsulated samples could effectively maintain the antioxidant activity, as shown by the lower percentage decrease of antioxidant activity due to the protective layer of the encapsulation agent. This phenomenon is related to the degradation of the active compound as the effect of direct interaction against unsupportive environment conditions for unencapsulated avocado extract [36, 37]. In addition, the decrease of antioxidant activity in the encapsulated samples at weeks 0 to 3 is attributed to the degradation potential of some free avocado seed extract on the surface microcapsules. These phenomena fit the free avocado seed extract value in Fig. 4 (c). The encapsulated particles with the extract-gum arabic at a mass ratio of 5:20 had the most stable antioxidant stability. After four weeks of storage, the antioxidant stability decreased by 1.43%, from 92.58% to 91.14%. Then it is followed by the encapsulated particles with the extract-gum arabic at mass ratio 5:10, 5:5, and 5:15 with decreases of 2.36%, 3.32%, and 6.30%, respectively. These results indicated that the active compounds encapsulated successfully were protected from degradation and exhibited stability of antioxidant activity. The encapsulated samples could effectively reduce the decrease of antioxidant activity due to the protective layer of the encapsulation agent. This phenomenon is related to the degradation of the active compound as the effect of direct interaction against unsupportive environment conditions for unencapsulated avocado extract. Finally, selecting the best mass ratio between the avocado

seed extract and gum arabic requires not only a high antioxidant activity but also high stability in antioxidant activity. Therefore, the best concentration chosen is avocado seed extract: gum arabic with a mass ratio of 5:10 with a decrease of 2.36% from 94.82% to 92.46%.

The antimicrobial activity of avocado seed extract only, gum arabic particles, and microcapsule particles of avocado seed extract-gum arabic with the mass ratio of 5:5, 5:10, 5:15, and 5:20 against *E. coli* and *S. aureus* are shown in Fig. 7 and Fig. 8, they were resumed in Table 2. On the first day of observation, all samples performed inhibition zones against both microbial. These phenomena relate to the active compounds in avocado seed extract, such as polyphenols, condensed tannins, phenolic acids, and flavonoids which make an inhibition zone around the observing sample [25]. Besides, the antimicrobial activity comes from the existence of saponins and alkaloids in

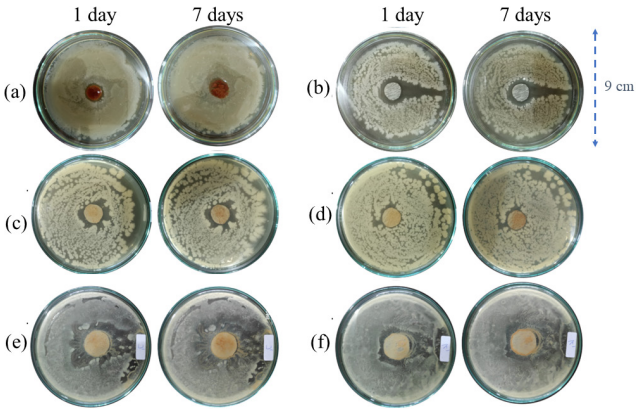


Fig. 7 Antimicrobial activity of (a) avocado seed extract only; (b) gum arabic particles; and the extract-gum arabic particles with the mass ratio of (c) 5:5; (d) 5:10; (e) 5:15; and (f) 5:20 against *E. coli* for the first and seventh days

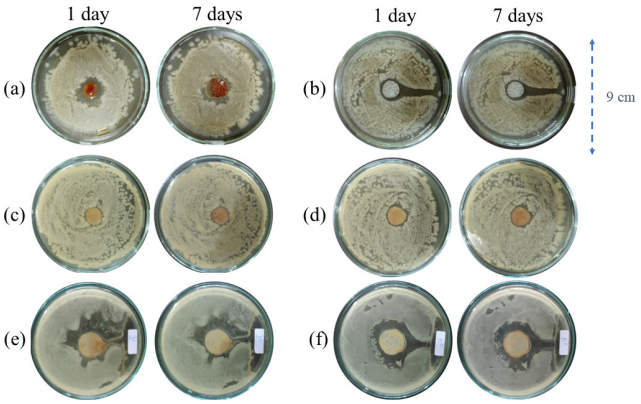


Fig. 8 Antimicrobial activity of (a) avocado seed extract only; (b) gum arabic particles; and the extract-gum arabic with the mass ratio of (c) 5:5; (d) 5:10; (e) 5:15; and (f) 5:20 against *S. aureus* for the first and seventh days

Table 2 Antimicrobial activity against *E. coli* and *S. aureus*

The concentration of avocado seed extract: gum arabic (w:w)	Antimicrobial activity			
	E. coli 1 day	*E. coli* 7 days	*S. aureus* 1 day	*S. aureus* 7 days
100:0	√	×	√	√
0:100	√	√	√	√
5:5	√	√	√	√
5:10	√	√	√	√
5:15	√	√	√	√
5:20	√	√	√	√

gum arabic [38]. However, the observation against *E. coli* after seven days shows that avocado seed extract unencapsulated has become covered with the microbial. Therefore, contrary to antimicrobial activity against *S. aureus* has an inhibition zone maintained after seven days. It may be attributed to the wall structure of bacteria *E. coli* is gram-negative bacteria composed of several layers coated with an outer membrane that reduces the interaction between bacteria and extract. Therefore, the degradation of active compounds causes unstable protection over time. While, *S. aureus*, as gram-positive bacteria, provides higher interaction because there is no outer membrane, resulting in a longer maintained inhibition zone [39, 40]. Nevertheless, all encapsulated samples perform antimicrobial activity against both bacteria, maintained after seven days. This phenomenon related to enhanced stability of active compound after encapsulation promotes controlled release through microcapsule shell and prolonged antimicrobial effect [41].

4 Conclusions

In this work, an encapsulation of avocado seed extract by gum arabic through spray drying is successfully conducted to make antioxidant and antibacterial compounds that remain stable for longer. The increasing concentration of gum arabic as wall material enlarged the microcapsule diameter. However, the increase in particle size was not followed by encapsulation efficiency. The highest yield and encapsulation efficiency were shown in the avocado seed extract-gum arabic microcapsule with a mass ratio of 5:10. The antioxidant activity reduced by 2.36% after five weeks of encapsulation, from 94.82% to 92.46%. In addition, after seven days of observation, the sample performed antimicrobial activity against *E. coli* and *S. aureus* bacteria. Thus, the obtained microcapsules can be used commercially for cheap, stable nutraceutical

products, promoting long-term storage. However, further studies are necessary to investigate the effect of drying air temperature on microcapsule properties and their stability of active compound performance before it can be applied as powdered herbal cosmetics or medicine.

Acknowledgment

We thank the Directorate of Research and Community Service, Institut Teknologi Sepuluh Nopember, for supporting this research through the Postgraduate Research ITS Grant (Contract Number: 933/PKS/ITS/2020).

References

[1] Lobo, V., Patil, A., Phatak, A., Chandra, N. "Free Radicals, Antioxidants and Functional Foods: Impact on Human Health", Pharmacognosy Reviews, 4(8), pp. 118–126, 2010. https://doi.org/10.4103/0973-7847.70902

[2] Ramana, K. V., Reddy, A. B. M., Majeti, N. V. R. K., Singhal, S. S. "Therapeutic Potential of Natural Antioxidants", Oxidative Medicine and Cellular Longevity, 2018, 9471051, 2018. https://doi.org/10.1155/2018/9471051

[3] Xu, D.-P. Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J.-J., Li, H.-B. "Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources", International Journal of Molecular Sciences, 18(1), 96, 2017. https://doi.org/10.3390/ijms18010096

[4] Satriana, S., Supardan, M. D., Arpi, N., Wan Mustapha, W. A. "Development of Methods Used in the Extraction of Avocado Oil", European Journal of Lipid Science and Technology, 121(1), 1800210, 2019. https://doi.org/10.1002/ejlt.201800210

[5] Vinha, A. F., Moreira, J., Barreira, S. V. P. "Physicochemical Parameters, Phytochemical Composition and Antioxidant Activity of the Algarvian Avocado (*Persea americana* Mill.)", Journal of Agricultural Science, 5(12), pp. 100–109, 2013. https://doi.org/10.5539/jas.v5n12p100

[6] Jimenez, P., Garcia, P., Quitral, V., Vasquez, K., Parra-Ruiz, C., Reyes-Farias, M., Garcia-Diaz, D. F., Robert, P., Encina, C., Soto-Covasich, J. "Pulp, Leaf, Peel and Seed of Avocado Fruit: A Review of Bioactive Compounds and Healthy Benefits", Food Reviews International, 37(6), pp. 619–655, 2021. https://doi.org/10.1080/87559129.2020.1717520

[7] Salazar-López, N. J., Domínguez-Avila, J. A., Yahia, E. M., Belmonte-Herrera, B. H., Wall-Medrano, A., Montalvo-González, E., González-Aguilar, G. A. "Avocado fruit and by-products as potential sources of bioactive compounds", Food Research International, 138, 109774, 2020. https://doi.org/10.1016/j.foodres.2020.109774

[8] Mehran, M., Masoum, S., Memarzadeh, M. "Microencapsulation of *Mentha spicata* essential oil by spray drying: Optimization, characterization, release kinetics of essential oil from microcapsules in food models", Industrial Crops and Products, 154, 112694, 2020. https://doi.org/10.1016/j.indcrop.2020.112694

[9] Corrêa-Filho, L. C., Lourenço, M. M., Moldão-Martins, M., Alves, V. D. "Microencapsulation of β-Carotene by Spray Drying: Effect of Wall Material Concentration and Drying Inlet Temperature", International Journal of Food Science, 2019, 8914852, 2019. https://doi.org/10.1155/2019/8914852

[10] Liang, R., Huang, Q., Ma, J., Shoemaker, C. F., Zhong, F. "Effect of relative humidity on the store stability of spray-dried beta-carotene nanoemulsions", Food Hydrocolloids, 33(2), pp. 225–233, 2013. https://doi.org/10.1016/j.foodhyd.2013.03.015

[11] Chaabane, D., Yakdhane, A., Vatai, G., Koris, A., Nath, A. "Microencapsulation of Olive Oil: A Comprehensive Review", Periodica Polytechnica Chemical Engineering, 66(3), pp. 354–366, 2022. https://doi.org/10.3311/PPch.19587

[12] Ajiz, H. A., Albar, A. A., Widiyastuti, W., Nurtono, T., Setyawan, H., Joni, I. M. "High Surface Area Silica Particles Using Anionic Surfactant Template Prepared by One-step Spray Drying System for Dye Removal Application", Periodica Polytechnica Chemical Engineering, 66(4), pp. 585–595, 2022. https://doi.org/10.3311/PPch.19730

[13] Hu, Y., Li, Y., Zhang, W., Kou, G., Zhou, Z. "Physical stability and antioxidant activity of citrus flavonoids in arabic gum-stabilized microcapsules: Modulation of whey protein concentrate", Food Hydrocolloids, 77, pp. 588–597, 2018. https://doi.org/10.1016/j.foodhyd.2017.10.037

[14] Vladić, J., Nastić, N., Janković, T., Šavikin, K., Menković, N., Lončarević, I., Vidović, S. "Microencapsulation of *Sideritis raeseri* Boiss. & Heldr. subsp. *raeseri* Extract Using Spray Drying with Maltodextrin and Whey Protein", Periodica Polytechnica Chemical Engineering, 66(2), pp. 229–238, 2022. https://doi.org/10.3311/PPch.19060

[15] Shaddel, R., Hesari, J., Azadmard-Damirchi, S., Hamishehkar, H., Fathi-Achachlouei, B., Huang, Q. "Use of gelatin and gum Arabic for encapsulation of black raspberry anthocyanins by complex coacervation", International Journal of Biological Macromolecules, 107, pp. 1800–1810, 2018. https://doi.org/10.1016/j.ijbiomac.2017.10.044

[16] He, R., Ye, J., Wang, L., Sun, P. "Preparation and Evaluation of Microcapsules Encapsulating Royal Jelly Sieve Residue: Flavor and Release Profile", Applied Sciences, 10(22), 8126, 2020. https://doi.org/10.3390/app10228126

[17] Evwierhoma, E. T., Ekop, I. E. "Extraction and Characterization of Oils from Some Local Seeds", 7(5), pp. 1280–1294, 2016.

[18] Pokorný, J., Yanishlieva, N., Gordon, M. "Antioxidants in food: Practical applications", Woodhead, 2001. ISBN 9781855734630

[19] Mousavi Kalajahi, S. E., Ghandiha, S. "Optimization of spray drying parameters for encapsulation of Nettle (*Urtica dioica* L.) extract", LWT, 158, 113149, 2022. https://doi.org/10.1016/j.lwt.2022.113149

[20] Nizori, A., Bui, L. T. T., Jie, F., Small, D. M. "Spray-drying microencapsulation of ascorbic acid: impact of varying loading content on physicochemical properties of microencapsulated powders", Journal of the Science of Food and Agriculture, 100(11), pp. 4165–4171, 2020. https://doi.org/10.1002/jsfa.10455

[21] Rigon, R. T., Zapata Noreña, C. P. "Microencapsulation by spray-drying of bioactive compounds extracted from blackberry (*rubus fruticosus*)", Journal of Food Science and Technology, 53(3), pp. 1515–1524, 2016. https://doi.org/10.1007/s13197-015-2111-x

[22] Anandharamakrishnan, C., Ishwarya, S. P. "Spray Drying Techniques for Food Ingredient Encapsulation", Wiley-Blackwell, 2015. ISBN 9781118864197

[23] Martí, M., Frígols, B., Serrano-Aroca, A. "Antimicrobial Characterization of Advanced Materials for Bioengineering Applications", Journal of Visualized Experiments, 138, e57710, 2018. https://doi.org/10.3791/57710

[24] Albar, A., Widiyastuti, W., Setyawan, H. "Controlled Morphology of Silica Particle by Spray Drying Method", IPTEK Journal of Proceedings Series, (6), pp. 330–335, 2020. https://doi.org/10.12962/j23546026.y2020i6.11118

[25] Egbuonu, A. C. C., Opara, I. C., Onyeabo, C., Uchenna, N. O. "Proximate, functional, anti nutrient and antimicrobial properties of avocado pear (*Persea americana*) seeds", Journal of Nutritional Health & Food Engineering, 8(1), pp. 78–82, 2018. https://doi.org/10.15406/jnhfe.2018.08.00260

[26] Zhu, J., Li, X., Liu, L., Li, Y., Qi, B., Jiang, L. "Preparation of spray-dried soybean oil body microcapsules using maltodextrin: Effects of dextrose equivalence", LWT, 154, 112874, 2022. https://doi.org/10.1016/j.lwt.2021.112874

[27] Timilsena, Y. P., Adhikari, R., Barrow, C. J., Adhikari, B. "Microencapsulation of chia seed oil using chia seed protein isolate-chia seed gum complex coacervates", International Journal of Biological Macromolecules, 91, pp. 347–357, 2016. https://doi.org/10.1016/j.ijbiomac.2016.05.058

[28] Larkin, P. J. "Infrared and Raman Spectroscopy: Principles and Spectral Interpretation", Elsevier, 2018. ISBN 978-0-12-804162-8 https://doi.org/10.1016/C2015-0-00806-1

[29] Lóránd, T., Deli, J., Molnár, P., Tóth, G. "FT-IR Study of Some Carotenoids", Helvetica Chimica Acta, 85(6), pp. 1691–1697, 2002. https://doi.org/10.1002/1522-2675(200206)85:6<1691::AID-HLCA1691>3.0.CO;2-G

[30] Bahru, T. B., Tadele, Z. H., Ajebe, E. G. "A Review on Avocado Seed: Functionality, Composition, Antioxidant and A Review on Avocado Seed: Functionality, Composition, Antioxidant and Antimicrobial Properties", Chemical Science International Journal, 27(2), pp. 1–10, 2019. https://doi.org/10.9734/CSJI/2019/v27i230112

[31] Melgar, B., Dias, M. I., Ciric, A., Sokovic, M., Garcia-Castello, E. M., Rodriguez-Lopez, A. D., Barros, L., Ferreira, I. C. R. F. "Bioactive characterization of *Persea americana* Mill. by-products: A rich source of inherent antioxidants", Industrial Crops and Products, 111, pp. 212–218, 2018. https://doi.org/10.1016/j.indcrop.2017.10.024

[32] da Silva, G. G., Pimenta, L. P. S., Melo, J. O. F., Mendonça, H. d. O. P., Augusti, R., Takahashi, J. A. "Phytochemicals of Avocado Residues as Potential Acetylcholinesterase Inhibitors, Antioxidants, and Neuroprotective Agents", Molecules, 27(6), 1892, 2022. https://doi.org/10.3390/molecules27061892

[33] Rodríguez-Sánchez, D. G., Pacheco, A., García-Cruz, M. I., Gutiérrez-Uribe, J. A., Benavides-Lozano, J. A., Hernández-Brenes, C. "Isolation and Structure Elucidation of Avocado Seed (*Persea americana*) Lipid Derivatives That Inhibit Clostridium sporogenes Endospore Germination", Journal of Agricultural and Food Chemistry, 61(30), pp. 7403–7411, 2013. https://doi.org/10.1021/jf401407s

[34] Rodríguez-Carpena, J.-G., Morcuende, D.,Andrade, M.-J., Kylli, P., Estévez, M. "Avocado (*Persea americana* Mill.) Phenolics, In Vitro Antioxidant and Antimicrobial Activities, and Inhibition of Lipid and Protein Oxidation in Porcine Patties", Journal of Agricultural and Food Chemistry, 59(10), pp. 5625–5635, 2011. https://doi.org/10.1021/jf1048832

[35] Hu, Q., Li, X., Chen, F., Wan, R., Yu, C.-W., Li, J., McClements, D. J., Deng, Z. "Microencapsulation of an essential oil (cinnamon oil) by spray drying: Effects of wall materials and storage conditions on microcapsule properties", Journal of Food Processing and Preservation, 44(11), e14805, 2020. https://doi.org/10.1111/jfpp.14805

[36] Mohammed, N. K., Tan, C. P., Manap, Y. A., Muhialdin, B. J., Hussin, A. S. M. "Spray Drying for the Encapsulation of Oils—A Review", Molecules, 25(17), 3873, 2020. https://doi.org/10.3390/molecules25173873

[37] Oxley, J. "Chapter 4 - Overview of Microencapsulation Process Technologies", In: Gaonkar, A. G., Vasisht, N., Khare, A. R., Sobel, R. (eds.) Microencapsulation in the Food Industry: A Practical Implementation Guide, Academic Press, 2014, pp. 35–46. ISBN 978-0-12-404568-2 https://doi.org/10.1016/B978-0-12-404568-2.00004-2

[38] Baien, S. H., Seele, J., Henneck, T., Freibrodt, C., Szura, G., Moubasher, H., Nau, Brogden, G., Mörgelin, M., Singh, M., Kietzmann, M., von Köckritz-Blickwede, M., de Buhr, N. "Antimicrobial and Immunomodulatory Effect of Gum Arabic on Human and Bovine Granulocytes Against *Staphylococcus aureus* and *Escherichia coli*", Frontiers in Immunology, 10, 3119, 2020. https://doi.org/10.3389/fimmu.2019.03119

[39] Choudhury, N., Meghwal, M., Das, K. "Microencapsulation: An overview on concepts, methods, properties and applications in foods", Food Frontiers, 2(4), pp. 426–442, 2021. https://doi.org/10.1002/fft2.94

[40] Amado, D. A. V., Helmann, G. A. B., Detoni, A. M., Carvalho, S. L. C., Aguiar, C. M., Martin, C. A., Tiuman, T. S., Cottica, S. M. "Antioxidant and antibacterial activity and preliminary toxicity analysis of four varieties of avocado (*Persea americana* Mill.)", Brazilian Journal of Food Technology, 22, e2018044, 2019. https://doi.org/10.1590/1981-6723.04418

[41] Sharkawy, A., Fernandes, I. P., Barreiro, M. F., Rodrigues, A. E., Shoeib, T. "Aroma-Loaded Microcapsules with Antibacterial Activity for Eco-Friendly Textile Application: Synthesis, Characterization, Release, and Green Grafting", Industrial & Engineering Chemistry Research, 56(19), pp. 5516–5526, 2017. https://doi.org/10.1021/acs.iecr.7b00741