DOI: https://doi.org/10.31351/vol31iss1pp20-31

Microencapsulation of Green Coffee Beans (*Coffea canephora*) Extract using Whey Protein Concentrate

Muhammad Ali Husni[***], Akhmad Kharis Nugroho[*], Teuku Nanda Saifullah Sulaiman[*] and Nanang Fakhrudin[1]**

[*]Department of Pharmaceutics, Faculty of Pharmacy, Universitas Gadjah Mada, ,Indonesia.
[**]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia.
[***]Department of pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, , Indonesia.
[***]Doctorate program, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia.

Abstract

Coffee bean contains bioactive compounds including caffeine and chlorogenic acid (CGA) that have a stimulant effect and are used for combating fatigue and drowsiness, and enhancing alertness. However, when the coffee bean was processed in the form of green coffee bean (GCB) extract, it has an unpleasant flavour and limitations instability, activity, and bioavailability. This study aimed to produce microcapsules of the GCB (*Coffea canephora*) ethanolic extract containing considerable amounts of the bioactive compounds for nutraceutical supplements. The GCB ethanolic extract was microencapsulated by spray drying using a whey protein concentrate (WPC) biopolimer. The particle size (PSA), morphology (SEM), and physicochemical characteristics (UV and LC-MS/MS), as well as radical scavenging activity (DPPH) of the microcapsule were determined. We found that the microencapsulation yield was 95.85% of the extract, with the particle mean of volume diameter was 1.312 µm (span value: 1.285 µm). The morphology of the microcapsule particles was microspheres with wrinkle and shrivel surface. The microcapsule demonstrated the caffeine content of 15.25%, the CGA content of 8.52%, the total phenolic content of 1.8 % (1794.7 mg/100g of the gallic acid equivalent (GAE)), and the radical scavenging activity of 179.23 µg/mL. The WPC can be used to encapsulate the GCB extract by using spray drying microencapsulation to produce a high yield microcapsule with a smaller and narrower particle diameter. This microencapsulation was able to engulf and package unpleasant flavor and aroma, and to preserve considerable amounts of the bioactive compounds.

Keywords: Spray drying, Coffee, Microcapsule; Caffeine, Chlorogenic acid.

Introduction

Coffee beans are the second-largest most traded commodity[1,2] and one of the most popular beverages in the world[3,4]. The top five coffee-producing countries are Brazil, Vietnam, Indonesia, Columbia, and Ethiopia, which account for almost 65% of the total global coffee production[5]. The coffee plant (Genus-*Coffea*; family-*Rubiaceae*) consists of more than 70 species. Among them, *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) commonly consumed by human beings as beverages and they account for almost 75% and 25% of the total coffee beans produced in the world, respectively[5]. Coffee contains various bioactive compounds, such as cellulose, soluble carbohydrates, insoluble polysaccharides, non-volatile and volatile compounds, aliphatic acids, nitrogenous compounds, polyphenols, proteins, lipids, amino acids, vitamins, and minerals. Among those bioactive natural compounds, chlorogenic acid (CGA) and caffeine are the two most important compounds from a nutraceutical perspective[5]. By drinking coffee, people expect to gain stimulating effect, get better taste and aroma sensation[6], reduce fatigue and drowsiness[7], and enhance alertness[8]. These effects are associated with the presence of its main phytochemical constituent, caffeine, and CGA. The effects of coffee consumption on human metabolism and psychological aspect have been reviewed by de Melo Pereira et al[9]. Coffee consumption is benefiting human health. It reduces the risk of various disorders such as type 2 diabetes, cardiovascular disease, neurological diseases (Parkinson's, Alzheimer's, cognitive impairment, and dementia), suicidal behaviour, cancer, hepatic injury and cirrhosis, human gut microbiota symbiosis, and gastrointestinal problems [9].

Coffee is lauded for its aroma and flavor, which are imparted by certain constituents such as alkaloids, trigonelline, proteins, amino acids, phenolics, and carbohydrates. On the other hand, the composition of these constituents is also responsible for the unpleasant odour and taste such as bit, sour, pungent, rancid, and repulsive that can cause nausea and vomiting[5].

[1]Corresponding author E-mail: nanangf@ugm.ac.id
Received:28 /4/2021
Accepted: 4/7 /2021

DOI: https://doi.org/10.31351/vol31iss1pp20-31

Therefore, before serving for consumption, the GCB is processed through fermentation, soaking, drying, defatting, decaffeinating, and blending or roasting to reduce or eliminate unpleasant odour and taste. to the formation of new compounds. Likewise, caffeine and CGA have been limited by their stability against oxidative degradation during process and storage, and bio-accessibility from the matrix during gastrointestinal digestion[11].

The alternative methods that can be done are extracting, fractionating, or isolating the phyto-constituents, but the extracts and fractions still leaving an unpleasant odour and taste of solvents, while the isolates are derived in a complicated way. Though, each process has its advantages and disadvantages related to stability, time, storage, and cost.

A promising approach to overcome those problems that are related to the bioactive constituents in GCB is by spray drying microencapsulation using whey protein concentrate (WPC) biopolymer. WPC is one of the polymeric shell materials that has been applied in gelation technology, thermal stabilization, and emulsification. Whey is the liquid remaining after the precipitation and curd removal during cheese production[12]. The important components of whey are soluble proteins, such as β-lactoglobulin (β-Lg), α-lactalbumin (α-La), immunoglobulin (Ig), bovine serum albumin (BSA), lactoferrin and lactoperoxidase enzymes, and caseinomacropeptide; lactose; lipids; and mineral salts. WPC provides a positive effect for the body against many diseases including cardiovascular diseases, nerve disorders, digestive problems, cancer, diabetes, osteoporosis, stress, and obesity. It can also enhance immune systems and demonstrates antimicrobial properties benefiting human health[13]. Whey proteins might increase the overall nutritional value of GCB and preserve the quality and property of the bioactive components in GCB. Besides, it also serves as a proper vehicle for bioactive compounds. The gelation property of the globular proteins (β-Lg) in WPC allows the development of micro- and nano structures-based formulations such as microgels, nanohydrogels, nanofibrils, and nanotubes.

Several studies have reported the use of WPC as wall material to microencapsulate various plant-based products, such as peanut[14], grape[15], vanillin[16], drumstick[17], apple jus[18], and phenolic extract[19]. The use of WPC as wall material to microencapsulate the extract of GCB (as a core material) using spray drying has not been reported. The present study aimed to prepare the formula and to produce microencapsulation of the extract of GCB using WPC. The product yield, particle size distribution, particle morphology, radical scavenging activity, total phenolic content, caffeine content, and CGA content were investigated.

However, the processing of GCB causes changes in chemical and physical parameters[10]
which alter the concentration, composition, structure, and activity of phyto-constituents and lead

Materials and Methods

Chemicals and plant material

The analytical grade of n-hexane, ethyl acetate, and ethanol was purchased from Merck (USA). The instant WPC (IWPC 80) was purchased from Milkspecialties (USA). The chromatography grade of caffeine and CGA were purchased from Sigma-Aldrich (St. Louis, USA). The coffee bean of the *Coffea canephora* was collected from the local plantation in Suka Makmur Timur, Bener Meriah district, Gayo highland, Aceh, Indonesia. The red cherry of the coffee bean was harvested from 10 years old plant.

Coffee beans preparation

The coffee bean (5 kg) was pulped and dried under indirect sunlight for 7 days. The parchment of the coffee bean was pulped and the bean was dried again for additional 7 days. The silver skin of the coffee bean was pulped and the beans were dried for 3 days to obtain dried GCB. The dried GCB was ground by blender and sifted (sieve number 40). The coarse GCB was collected in the vessel and stored in a dark and dry place before the extraction process (Figure 1).

Extractions procedure

The extraction procedure was performed by using maceration according to Khoddami et al[20] procedure with some modifications (Figure 1). 200 g of coarse GCB was macerated with 1 L of n-hexane at room temperature for 24 hours and then filtered by a vacuum-assisted filter. The filtrate was collected and then n-hexane residue was dried and then re-macerated with 1 L of ethyl acetate at room temperature for 24 hours and then filtered. The filtrate was collected and then the ethyl acetate residue was dried and then macerated with 1 L of ethanol (70%) at room temperature for 24 hours and filtered. Taken together, three filtrates were collected from this maceration process and all filtrates were evaporated by using a rotary evaporator (Buchi). The concentrated filtrates were collected in the dark bottles, weighed, and stored in a refrigerator. The maceration process was done in triplicate.

Microencapsulation process

The microencapsulation was processed by using a spray dryer (GEA Niro) according to the Abrahão et al[21] procedure (Figure 1). The extract (100 mg) was dispersed in WPC solution (0.1%) and diluted with water to obtain 200 µg/mL of feed solution. The feed solution was homogenized by using an Ultra-Turrax (IKA T18) at 40 °C, 1,000 rpm for 45 minutes, and then stored in a refrigerator overnight to allow the complete formation of

DOI: https://doi.org/10.31351/vol31iss1pp20-31

monomer dispersion. The spray drying was performed at an inlet temperature of 180 °C, outlet air temperature of 70 °C, and feed flow rate of 5 mL/min. The microcapsule was collected in an airtight plastic bag, weighed, and stored at a desiccator for analysis. The spray drying microencapsulation was performed in triplicate.

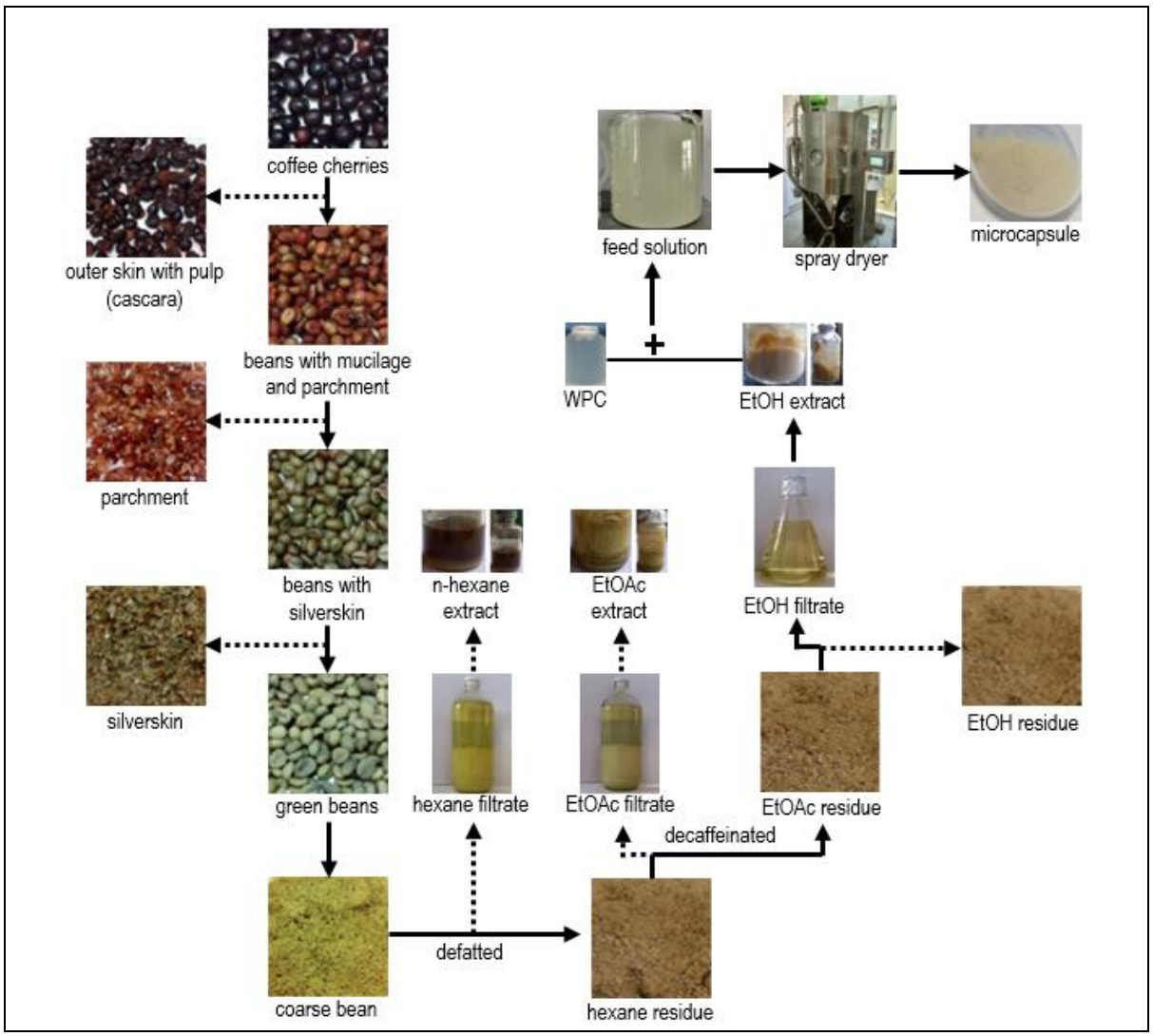

Figure 1. Scheme of GCB preparation, extraction, and microencapsulation process.
The extraction was macerated gradually using n-hexane (defatted), ethyl acetate (decaffeinated), and ethanol solvents (analytical grade). Microencapsulation performed by processing the feed solution (200 µg/mL) containing extract (100 mg) and WPC (0.1%) using a spray dryer (180 °C inlet temperature, 70 °C outlet temperature, and 5 mL/min feed flow rate). EtOAc: ethyl acetate; EtOH: ethanol; WPC: whey protein concentrate.

Extracts and microparticles yield

The extracts yield was calculated according to the Fikry et al[22]. Extracts yield (%) was expressed as a ratio of the weight of the extracted solids and the initial sample weight. The physical properties of the extract were determined visually and by tactile.

The microcapsule yield was calculated according to the Gonçalves et al[23]. Microcapsule yield (%) was expressed as the ratio of the mass of microcapsule obtained in the spray dryer output and the solid content of the initial feed solution. The physical microcapsule was determined visually and by tactile.

Particle size distribution and morphology

The particle size was analyzed by a particle size analyzer (PSA) (Beckman Coulter LS 13 320) according to the Kusmayadi et al[24] procedure. The sample was dripped on the instrument, and the particle size of the sample was recorded by using a zeta potential analyzer (Beckman Coulter Delsa TM). Then, the span was calculated using an equation:

$span = [(d_{90} - d_{10})/d_{50}]$

where d_{90}: the portion of particles with diameters below this value is 90%; d_{50}: The portions of particles with diameters smaller and larger than this

DOI: https://doi.org/10.31351/vol31iss1pp20-31

value are 50%; d_{10}: the portion of particles with diameters smaller than this value is 10%.

Particle morphology was examined by using a scanning electron microscope (SEM) (Leo 40 XVP) equipped with ray-X microanalysis systems (Quantax EDS and Espirit Software) according to Kuck & Noreña[25] procedure. The sample was adhered to a double-sided adhesive tape, nailed to a stub, and then coated with vacuum gold. The SEM conditions were operated at 15 kV using 1.0 to 7.0k magnification.

Caffeine content

The caffeine content was determined using LC-MS/MS according to the previous methods described by Tine et al[26] and Vinson et al[27]. The LC instrument was a Flexar LC Perkin-Elmer with Flexar LC PE200 column oven (LUNA 3U C18 column). The MS/MS conditions were carried on an AB Sciex 3200 QTRAP fitted with an APCI ion source. The APCI was operated in the positive mode. The 5 mg of sample and caffeine standard were dissolved in acetonitrile/H_2O (1:1 v/v) to obtain the final concentration solution of 50 µg/mL and filtered using polytetrafluoroethylene (0.2 µm). Caffeine standard solutions were diluted with acetonitrile/H_2O (1:1 v/v) to obtain calibration curves with 5 points concentration range of 2-10. The caffeine (2 µg/mL) standard solutions were directly infused at the flow rate of 10 µL/min in the MS/MS apparatus. Multiple EPI mass spectra of caffeine were recorded of m/z 195 at 138 Da. The software for data acquisition and data analysis was Analyst 1.5.2. Each experiment was done in triplicate.

Chlorogenic acids content

The CGA content was determined according to Tine et al[26] and Vinson et al[27] procedures by using LC-MS/MS with some modifications. The LC instrument was a Flexar LC Perkin-Elmer with Flexar LC PE200 column oven (LUNA 3U C18 column). The MS/MS conditions were carried on an AB Sciex 3200 QTRAP fitted with an APCI ion source. The APCI was operated in the negative mode. The 5 mg of sample and standard were dissolved in acetonitrile/H_2O (1:1 v/v) to obtain the final concentration solution of 50 µg/mL and filtered using polytetrafluoroethylene (0.2 µm). The CGA standard solutions were diluted in acetonitrile/H_2O (1:1 v/v) to obtain calibration curves with 5 points concentration range of 10-50 µg/mL. The CGA (20 µg/mL) standard solutions were directly infused at the flow rate of 10 µL/min in the MS/MS apparatus. Multiple EPI mass spectra of CGA were recorded of m/z 359 at 191 Da. The software for data acquisition and data analysis was Analyst 1.5.2. Each experiment was done in triplicate.

Total phenolic assay

A total phenolic content (TPC) was determined according to the Abrahão et al[21] procedure by using a spectrophotometer (Shimadzu UV-1601). Shortly, 0.07 g of the samples were diluted in 25 mL of distilled water at 25 °C and stirred by a vortex. 200 µL of the sample solution was transferred to tubes containing 200 µL of Folin-Ciocalteu's reagent. Then, 200 µL of saturated calcium carbonate solution (10% w/v) was added together with 2 mL of distilled water and incubated in the dark at room temperature for 60 min. The absorbance value was measured using a spectrophotometer at 765 nm. The TPC was calculated using linear regression obtained from the standard curve of gallic acid (0–80 µg/mL). The TPC value was expressed in milligram of gallic acid equivalent (GAE) per gram of dry matter.

Radical scavenging activity assay

Radical scavenging activity was determined according to the Abrahão et al[21] procedure by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method in a spectrophotometer (Shimadzu UV-1601). Shortly, 0.07 g of the samples were diluted in 25 mL of distilled water at 25 °C and stirred by a vortex. 2.5 mL of the sample solution was pipetted into tubes containing 1 mL of DPPH solution (0.3 mM) reagent followed by stirring. After 30 min, the absorbance was recorded in the spectrophotometer at 518 nm. Ethanol (1.0 mL) containing a sample solution (2.5 mL) was used as a blank. DPPH solution (1.0 ml; 0.3 mM) in ethanol (2.5 mL) was used as a negative control. The absorbance values were converted to radical scavenging activity percentage using the following equation:

$$\%RSA=100 - \{[(Abs\ S - Abs\ B)] \times 100/Abs\ C\}$$

where %RSA: radical scavenging activity percentage; Abs S: sample absorbance; Abs B; blank absorbance; Abs C; control absorbance.

Results and Discussion

Extracts and microparticles yield

Prior to extraction using ethanol, the GCB was extracted with n-hexane and ethyl acetate to remove the nonpolar components such as lipids, fats, and oils. We found that the extraction yield was 2.38%. This extraction yield was lower that the previous studies[21,28–30] because we performed a pre-extraction process using relatively nonpolar solvents to obtain an enriched extract with fewer nonpolar constituents. Additionally, the difference in the type of coffee bean and the extraction method (solvent, duration, temperature, solvent-sample ratios) might also affected the extraction yield.

DOI: https://doi.org/10.31351/vol31iss1pp20-31

Table 1. The yield and physical characteristics of the GCB extract obtained from the extraction, and the microcapsule product.

Samples	Yields (%)	Physical Characteristics (visual and tactile)
GCB extract	2.38 ± 1.15	Semi-solid formed, yellowish-green color, smooth sandy texture bitter flavor, coffee distinctive odor, and stung ethanol aroma
Microcapsule	95.85 ± 3.60	Fine powder formed, smooth, greenish-yellow color, bitter flavorless, savory flavor, roasted coffee, and milk distinctive odor, and fragrant aroma

Data are expresed as mean ± SD (n = 3). GCB: green coffee bean

The extract was microencapsulated using a spray drying method and yielded a 95.85% microcapsule. The yield and characteristics of the extract and microcapsule product were showed in Table 1. Interestingly, the yield of microcapsule of the GCB extract in this study was an abundant. The other plant-based products reported previously, the microencapsulation yield of vanilla extract, peanut sprout extract, red grape juice were reported 94.4, 89.01, and 39.00%, respectively[14–16]. Also, the utilization of WPC in GCB extract microencapsulation to the other material using maltodextrin (40-84%)[28–31] and arabic gum (60%)[32]. This indicated that the type of wall material affected microencapsulation yield. Other factors that might affect microencapsulation efficiency are the inlet temperature[33–35] and cyclone efficiency[23,33,36] in the spray drying process.

Microcapsule size

In this study, it was found that the mean volume diameter and span of the microcapsule were 1.312 and 1.285 µm, respectively (Figure 2). These data demonstrated that the microcapsule particle has a homogeneous and uniform relatively, as well as narrower of size. The reported publications previously, the mean value of volume diameter was variated. Calva-Estrada et al[16] reported the Feret diameter of 16.25 µm for the microcapsule of natural vanilla extract using WPC; Oliveira et al[15] reported the De Brouckere mean diameter was ranging from 4.77 to 13.56 µm for microcapsule of red grape juice using WPC; Abrahão et al[21] reported the volume diameter of 15.78 µm for microcapsule of spent espresso coffee grounds using whey protein isolate (WPI); whereas Desai et al[28] reported the particle size of 2.3 µm for microcapsule of green coffee extract using maltodextrin. A study by Desai et al[31] has reported that a particle size of 0.08 µm for the extract of green coffee using maltodextrin with a nanospray dryer. These indicated that our data showed a typical microcapsule particle size. The particle size is determined by several processes including spray mesh size, solid particle concentration (viscosity), inlet temperature, spray rate, pump circulation rate, type of surfactant, and solvent[33].

Microcapsule morphology

The microcapsule morphology was observed with scanning electron microscopy (SEM). The result showed that the particle was a spherical dense, smooth, wrinkle, shrivel, homogenize, compact, and porous. We found that the particles of microcapsule were dispersed throughout the particles or molecules matrix (Figure 3). The particle morphology was similar to the other studies[16,19,21,28,31]. Indeed, the particle morphology in this study was in line with Arpagaus et al[33] showing that microcapsule particle has the morphology of dense, hollow, porous, and encapsulated structures with spherical, wrinkled, shriveled, or doughnut-like shapes[33]. Clearly, the microcapsule morphology of the microcapsule particle obtained in this study was microspherical. The spherical geometry of the microcapsule is determined by the wall material[23], feed property (material type, solid concentration, solvent, and surfactant), and drying temperature[33]. Generally, the slow-drying process results in more compact particles, while the fast-drying process yields hollow particles[33].

DOI: https://doi.org/10.31351/vol31iss1pp20-31

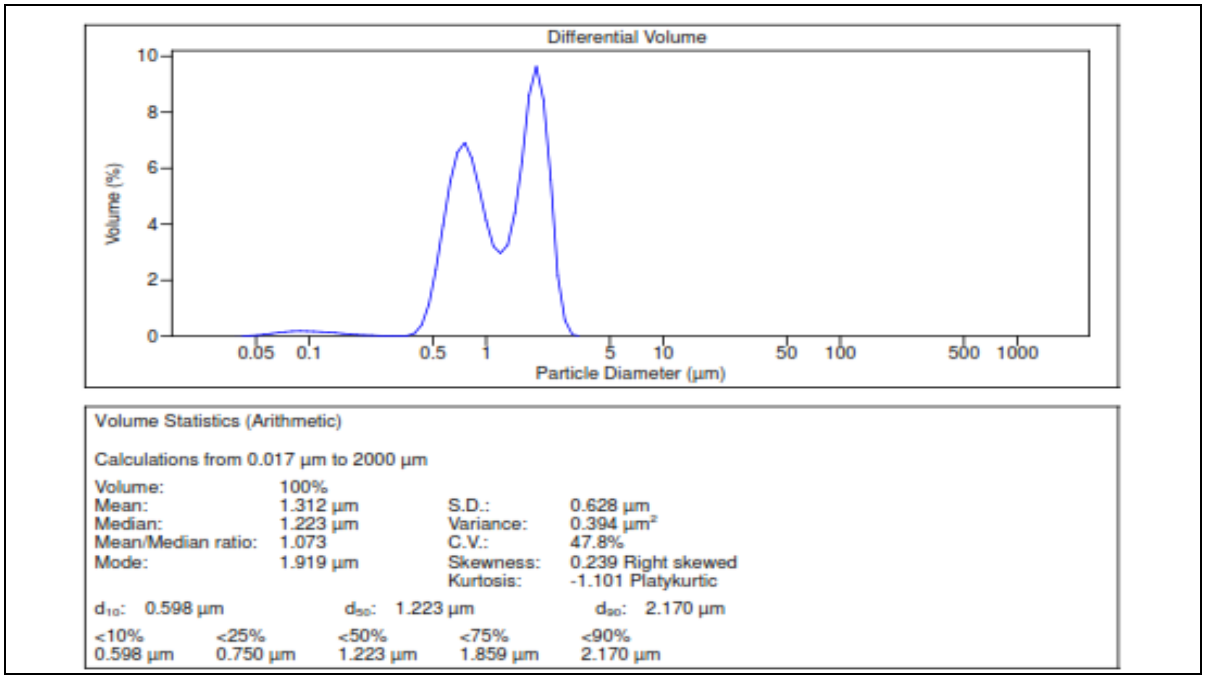

Figure 2. The particle diameter of the microcapsule measured with PSA (LS 13 320; Optical model: Fraunhofer.rf780d; fluid: H_2O).

Data are expresed as mean ± SD (n = 3). d_{90}: the portion of particles with diameters below this value is 90%; d_{50}: The portions of particles with diameters smaller and larger than this value are 50%; d_{10}: the portion of particles with diameters smaller than this value is 10%. Span = $(d_{90} – d_{10})/d_{50}$

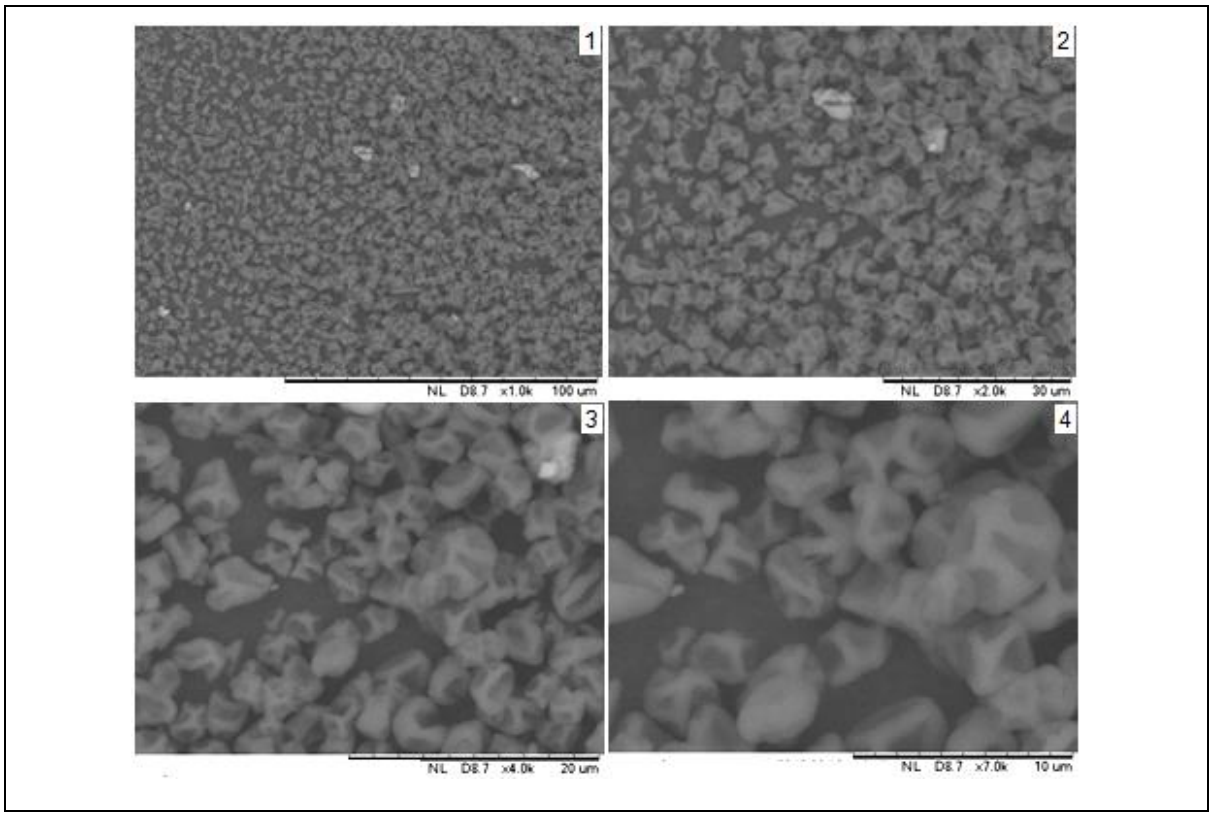

Figure 3. The SEM analysis results of microcapsule (Condition=Vacc=15.0kV). (1) 1.0k magnifications, 100 µm; (2) 2.0k magnifications, 50 µm; (3) 4.0k magnifications, 20 µm; and (4) 7.0k magnifications, 10 µm.

DOI: https://doi.org/10.31351/vol31iss1pp20-31

Caffeine content

In the LC-MS/MS analysis, the retention time of caffeine appeared at 4.22 min (Figure 4a) with the molecular ion m/z 195.081 in the positive ion mode (Figure 5). This was in line with Perrone et al[37] and Davies & Wasan[38] reported that the precursor ion (m/z) of caffeine was 195 with the retention time 4-5 min. The linear regression curve of the caffeine (2-10 µg/mL) was y = 1536.1x + 315.3 (R^2 = 0.998). By using this linear regression curve, the caffeine content of the microcapsule and extract was determined. We found that the caffeine content of the extract and the microcapsule was 18.23 and 15.25%, respectively (Table 2). The caffeine content of the microcapsule was lower than the extract (0.2 times) with an encapsulation efficiency was 80%. The result was in accordance with the previous studies showing the decrease of caffeine content after encapsulation. This occurred not only in the microencapsulation process with WPC as wall material but also with the use of other wall materials. In addition, the difference in the type of coffee extract also contributed to the variability of caffein content. Desai et al[28] reported the caffeine content of GCB and its microcapsule (using maltodextrin) were 12.78 and 11.98 mg/g, respectively; whereas Abrahão et al[21] showed that the caffeine content of spent espresso coffee ground extract and its microcapsule (using WPC) was 6.12 and 2.54 mg/g, respectively. In line with these data, our result also demonstrated that part of the caffeine was lost during the microencapsulation process. Caffeine is a thermo-stabile alkaloid. Caffeine loss might be due to the water-soluble properties of caffeine whereby partially carried by the water vapor eliminated[5].

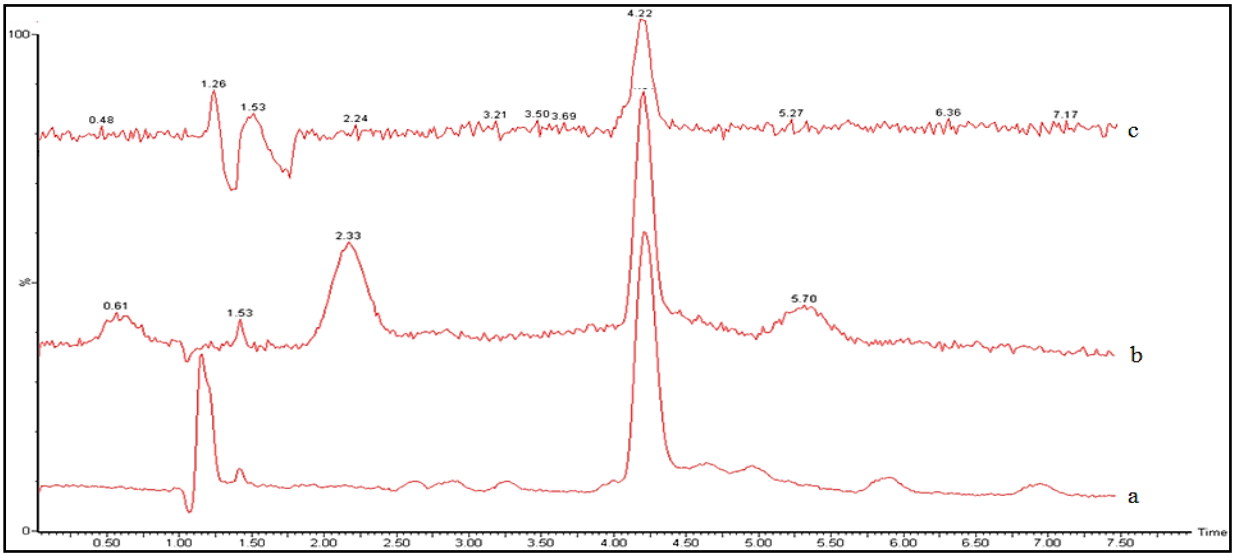

Figure 4. The chromatogram of caffeine standard (a), EtOH extract (b), and microcapsule (c). Positive ion mode of Time-of-flight mass spectrometry electrospray ionization (TOF MS ESI+). Retention time 4.22 min

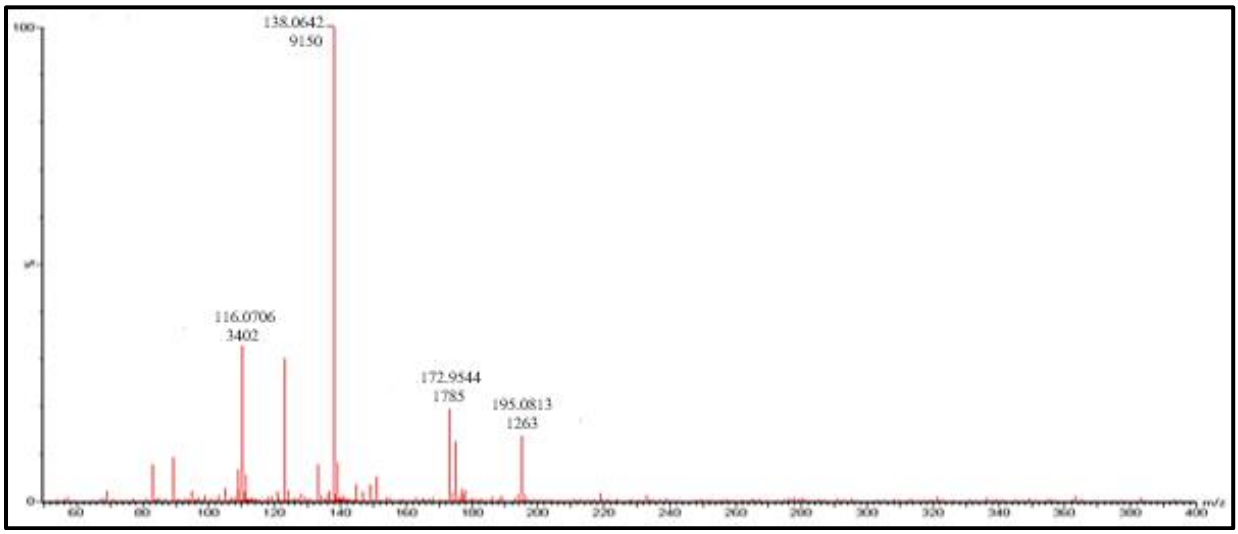

Figure 5. The spectrum of caffeine standard. Positive ion mode of Time-of-flight mass spectrometry electrospray ionization (TOF MS ESI+). Ion fragmentation m/z 195.081

DOI: https://doi.org/10.31351/vol31iss1pp20-31

Chlorogenic acids content

Based on the chromatogram in the Figure 6a, the retention time of CGA was at 4.79 min with the molecular ion m/z 353.14 in the negative ion mode (Figure 7). This finding is in line with previous publications showing the retention time of CGA was 4-5 min with m/z of the molecular ion were 352.7[39] and 353[27].

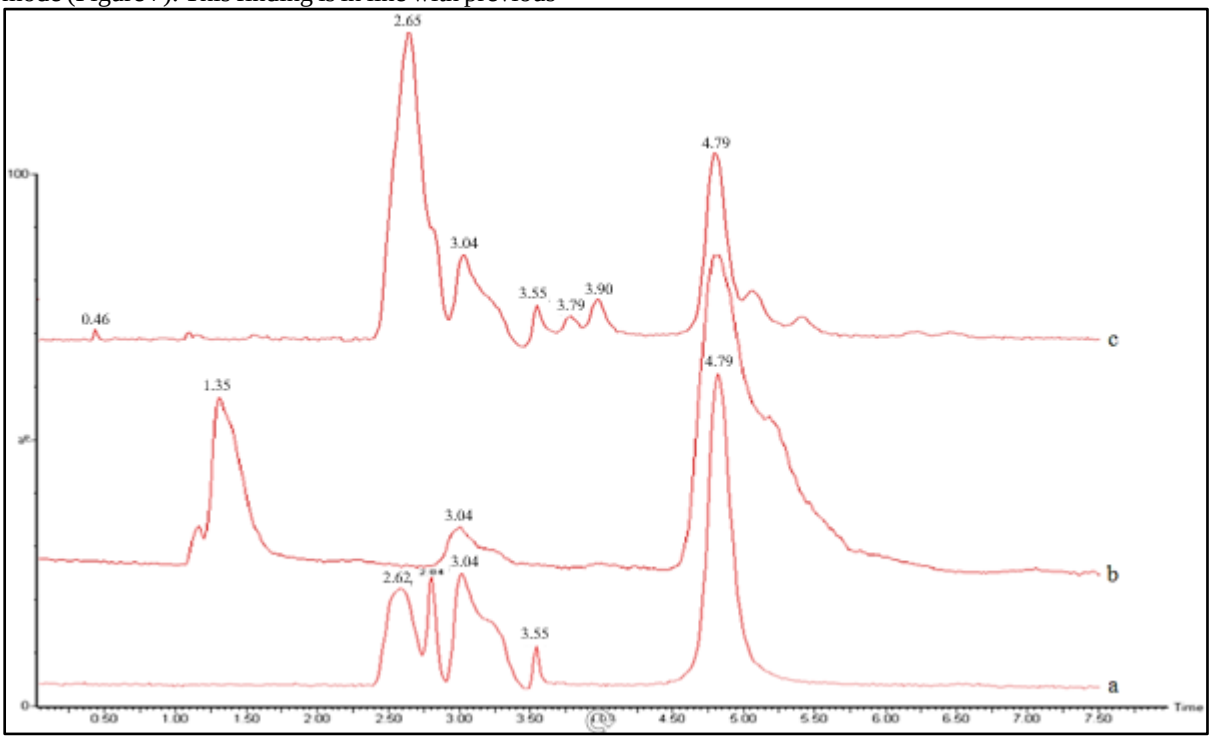

Figure 6. The chromatogram of the CGA standard (a), EtOAc extract (b), and microcapsule (c). Negative ion mode of Time-of-flight mass spectrometry electrospray ionization (TOF MS ESI-). Retention time 4.79 min

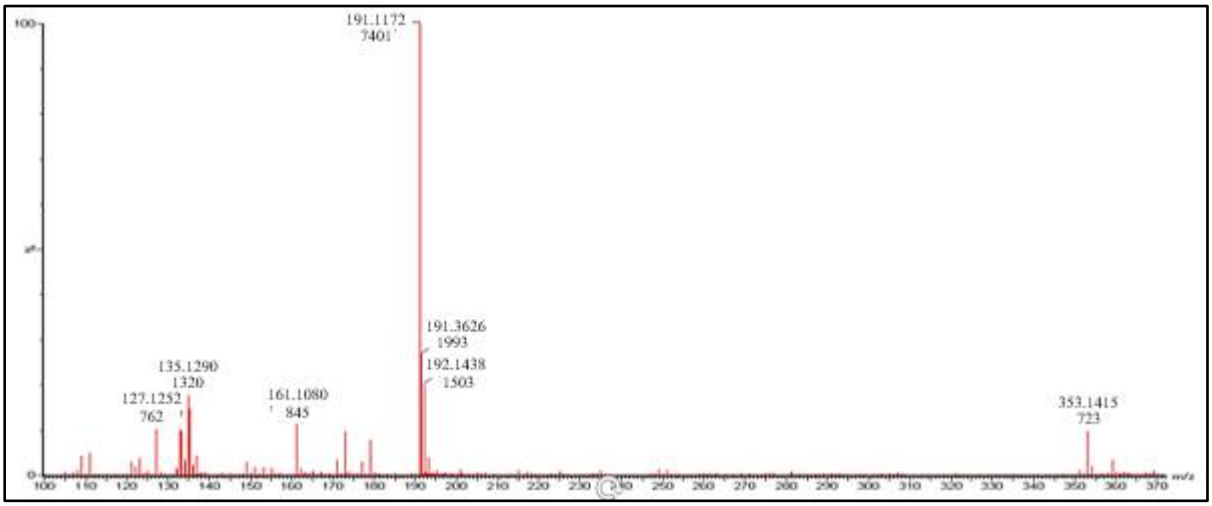

Figure 7.The spectrum of CGA standard. Negative ion mode of Time-of-flight mass spectrometry electrospray ionization (TOF MS ESI-). Ion fragmentation m/z 353.14

The regression curve of CGA standard (10-50 µg/mL) was obtained Y = 1426.8x + 7848.8 (R^2 = 0.999). This linear regression curve was used for the determination of CGA content in the microcapsule and extract. We found that the CGA content of the extract and microcapsule was 10.88 and 8.52 %, respectively (Table 2). CGA content of the microcapsule was lower than the extract (0.28 times). Our data were in agreement with the other studies reporting the decrease of CGA content after microencapsulation. Similar with the decrease in caffeine content, the CGA content was also affected by the difference in wall material and type of coffee extract. A previous study demonstrated that the

DOI: https://doi.org/10.31351/vol31iss1pp20-31

CGA content for the green coffee extract and microcapsule (using maltodextrin) were 10.23 and 9.9 mg/g, respectively[28]. Abrahão et al[21] showed the CGA content for the spent espresso coffee ground extract and microcapsule (using WPC) were 3.71 and 1.57 mg/g, respectively. CGA is a thermo-labile phenolic compound that is susceptible to degradation. The loss of CGA content due to the encapsulation process in this study is 21.69% or 0.28 times lower than the extract. The encapsulation process might trigger isomerization, epimerization, lactonization, and other degradation that alter the chemical structure CGA[21,40].

Total phenolic contents

The total phenolic content of the extract and the microcapsule were 219 and 1794.7 mg GAE/100 g, respectively (Table 2). The total phenolic content of the microcapsule was higher than the extract (7.19 times). The result was in line with other studies that reported the increase of TPC is associated with the use of WPC as a wall material. Díaz-Bandera et al[41] reported the utilization of WPC in the microencapsulation of Roselle extract that increased the TPC from 72.06 to 89.09 mg GAE/100 g (0.24 times). Abrahão et al[21] also reported the same showing that the microencapsulation of spent espresso coffee grounds using WPC increased the TPC from 757 to 1284 mg GAE/100 g (0.7 times). Interestingly, the enhancement of TPC in the microencapsulated extract in this study was an abundant relatively. On contrary, other studies have reported a decrease in TPC after microencapsulation of GCB using maltodextrin. Calva-Estrada et al[16] reported the utilization of WPC for the encapsulation of vanilla extract significantly reduced the TPC from 0.89 and 0.19 g GAE/100 g (3.68 times); whereas Desai et al[28] reported the decrease in TPC from 350.28 to 174.07 mg GAE/100 mL (1.01 times) in green coffee extract after microencapsulation. The changes in the TPC of the microcapsule extract could be due to the formation of polymerization of phenolic compounds as a result of the higher inlet temperature (180 °C) during the encapsulation process. Furthermore, the changes might be due to the physicochemical transformation of the phenolic compounds upon interaction with whey proteins as a wall material that lead to the formation distict chemical structures[21,41,42].

Table 2. Parameter values of the extract and the microcapsule

Parameters	GCB extract	Microcapsule
Radical scavenger (IC_{50} (µg/mL)	109.84 ± 0.17	179.23 ± 4.83
Total phenolic content (mg GAE/100 g)	219 ± 0.02	1794.7 ± 77
Chlorogenic acid content (%)	10.88 ± 0.11	8.52 ± 0.05
Caffeine content (%)	18.23 ± 0.08	15.25 ± 0.06

Data are expresed as mean ± SD (n = 3). GCB: green coffee bean; IC_{50}: half maximal inhibition concentration; GAE: gallic acid equivalent

Radical scavenging activities

To determine the antioxidant activity of the extract, we performed a radical scavenging activity using DPPH as a reagent. We found that the IC_{50} of the extract and microcapsule were 109.84 and 179.23 µg/mL, respectively. The IC_{50} of the microcapsule was higher than the extract (Table 2), indicated that the microcapsule has a lower antioxidant activity compared to the extract (0.62 times). The decrease in the radical scavenging activity was might be due to the effect of the encapsulation process and the use of WPC. The decrease in antioxidant activity after microencapsulation using WPC was also observed in the vanilla extract[16], but not in the microencapsulation using maltodextrin[31] and glucomannan[35]. This result suggested that utilization of WPC as a wall material in GCB extract microencapsulation was not as effective as maltodextrin or glucomannan in terms of its ability as a radical scavenger. The use of maltodextrin as a wall material in GCB extract microencapsulation increased the antioxidant capacity from 414.8 to 425.9 µmol Trolox/g[31]; whereas the use of glucomannan as a wall material in the microencapsulation of roasted coffee extract increased DPPH scavenging activity from 619.21 to 813.3 µM Trolox/g[35]. The radical scavenging activity of the microcapsule is determined by many factors, including the temperature during the process. As the process of microencapsulation in this study used the inlet and outlet temperature of 180 and 70 °C, respectively, the thermolabile compounds, including those that have radical scavenging activity might be degraded or altered. The high temperature during the encapsulation process could lead to pre-oxidative reactions and other reactions which could change the native compounds or generate artefacts[16,35]. Moreover, different types of wall material for the encapsulation could also affect the antioxidant potential due to its interaction with the phytochemical constituents[40,41]. Although the total phenolic content of the microcapsule was higher than the extract, the radical scavenging activity is lower than the extract. This indicated that the compounds generated by the microencapsulation process and interaction with WPC were not able to scavenge the DPPH radicals. Other antioxidant assays with a

DOI: https://doi.org/10.31351/vol31iss1pp20-31

distinct mechanism of action were required to assess the antioxidant potential of the microcapsule.

Conclusion

In summary, we found that WPC was effective as a wall material in GCB extract microencapsulation using spray drying. The microcapsule showed smaller and narrower span particles with microspheres geometry, compact morphology, and wringkled surface of particle structure. The microcapsule was able to carry and protect considerable amounts of bioactive compounds. It preserved GCB bioactive compounds including caffeine and chlorogenic acid. It also engulfed and packaged the unpleasant flavor and aroma in the GCB extract. This method can be applied to formulate innovative products based on coffee beans for healthy pharmaceutical ingredients, food, and beverages.

Conflicts of Interest

The authors declared no conflict of interest.

Acknowledgments

The authors thank Direktorat Jenderal Pendidikan Tinggi (Ditjen DIKTI) of Indonesia for financial support wthin Beasiswa Program Pascasarjana-Dalam Negeri (BPP-DN) No: 1405.29/E4.4/2015.

References

1. Bae J, Hong KH. Optimized dyeing process for enhancing the functionalities of spent coffee dyed wool fabrics using a facile extraction process. Polymers. 2019 Mar 28;11(4):574.
2. Ijanu EM, Kamaruddin MA, Norashiddin FA. Coffee processing wastewater treatment: a critical review on current treatment technologies with a proposed alternative. Appl Water Sci. 2019 Nov 18;10(1):11.
3. Gaascht F, Dicato M, Diederich M. Coffee provides a natural multitarget pharmacopeia against the hallmarks of cancer. Genes Nutr. 2015 Nov 17;10(6):51.
4. Satho T, Dieng H, Ahmad MHI, Ellias SB, Hassan AA, Abang F, et al. Coffee and its waste repel gravid Aedes albopictus females and inhibit the development of their embryos. Parasit Vectors. 2015 May 14;8:272.
5. Garg SK. Green Coffee Bean. In: Nutraceuticals. Elsevier. 2016;653–67.
6. Nieber K. The Impact of Coffee on Health. Planta Med. 2017 Nov;83(16):1256–63.
7. Evans J, Richards JR, Battisti AS. Caffeine. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2020.
8. Ágoston C, Urbán R, Király O, Griffiths MD, Rogers PJ, Demetrovics Z. Why Do You Drink Caffeine? The Development of the Motives for Caffeine Consumption Questionnaire (MCCQ) and Its Relationship with Gender, Age and the Types of Caffeinated Beverages. Int J Ment Health Addict. 2018;16(4):981–99.
9. de Melo Pereira GV, de Carvalho Neto DP, Magalhães Júnior AI, do Prado FG, Pagnoncelli MGB, Karp SG, et al. Chemical composition and health properties of coffee and coffee by-products. In: Advances in Food and Nutrition Research. Elsevier. 2020; 65–96.
10. Cid MC, de Peña M-P. Coffee: Analysis and Composition. In: Caballero B, Finglas PM, Toldrá F, editors. Encyclopedia of Food and Health. Oxford: Academic Press. 2016;225–31.
11. Vulić J, Šeregelj V, Kalušević A, Lević S, Nedović V, Tumbas Šaponjac V, et al. Bioavailability and Bioactivity of Encapsulated Phenolics and Carotenoids Isolated from Red Pepper Waste. Molecules. 2019 Aug 5;24(15):2837.
12. Ramos OL, Pereira RN, Rodrigues RM, Teixeira JA, Vicente AA, Malcata FX. Whey and Whey Powders: Production and Uses. In: Caballero B, Finglas PM, Toldrá F, editors. Encyclopedia of Food and Health. Oxford: Academic Press. 2016;498–505.
13. Tavares T, Malcata FX. Whey and Whey Powders: Protein Concentrates and Fractions. In: Caballero B, Finglas PM, Toldrá F, editors. Encyclopedia of Food and Health. Oxford: Academic Press. 2016;506–13.
14. Lee Y-K, Ganesan P, Baharin BS, Kwak H-S. Characteristics, stability, and release of peanut sprout extracts in powdered microcapsules by spray drying. Dry Technol. 2015;33(15–16):1991–2001.
15. Oliveira BE, Junior PCG, Cilli LP, Contini LRF, Venturini AC, Yoshida CMP, et al. Spray-drying of grape skin-whey protein concentrate mixture. J Food Sci Technol. 2018 Sep;55(9):3693–702.
16. Calva-Estrada SJ, Mendoza MR, García O, Jiménez-Fernández VM, Jiménez M. Microencapsulation of vanilla (Vanilla planifolia Andrews) and powder characterization. Powder Technol. 2018 Jan 1;323:416–23.
17. Premi M, Sharma HK. Effect of different combinations of maltodextrin, gum arabic and whey protein concentrate on the encapsulation behavior and oxidative stability of spray dried drumstick (Moringa oleifera) oil. Int J Biol Macromol. 2017 Jul 27;105(Pt 1):1232-1240
18. Sarabandi K, Peighambardoust SH, Sadeghi Mahoonak AR, Samaei SP. Effect of different carriers on microstructure and physical characteristics of spray dried apple juice concentrate. J Food Sci Technol. 2018 Aug 1;55(8):3098–109.
19. Rocha J de CG, de Barros FAR, Perrone ÍT, Viana KWC, Tavares GM, Stephani R, et al.

DOI: https://doi.org/10.31351/vol31iss1pp20-31

Microencapsulation by atomization of the mixture of phenolic extracts. Powder Technol. 2019 Feb 1;343:317–25.

20. Khoddami A, Wilkes MA, Roberts TH. Techniques for analysis of plant phenolic compounds. Molecules. 2013 Feb 19;18(2):2328–75.

21. Abrahão FR, Rocha LCR, Santos TA, Carmo EL do, Pereira LAS, Borges SV, et al. Microencapsulation of bioactive compounds from espresso spent coffee by spray drying. LWT. 2019 Apr;103:116–24.

22. Fikry M, Yusof YA, M. Al-Awaadh A, Abdul Rahman R, Chin NL, Ghazali HM. Antioxidative and quality properties of full-fat date seeds brew as influenced by the roasting conditions. Antioxidants. 2019 Jul 18;8(7):226.

23. Gonçalves B, Moeenfard M, Rocha F, Alves A, Estevinho BN, Santos L. Microencapsulation of a Natural Antioxidant from Coffee-Chlorogenic Acid (3-Caffeoylquinic Acid). Food Bioprocess Technol. 2017 Aug;10(8):1521–30.

24. Kusmayadi A, Adriani L, Abun A, Muchtaridi M, Tanuwiria UH. The microencapsulation of mangosteen peel extract with maltodextrin from arenga starch: formulation and characterization. J Appl Pharm Sci. 2019 Mar;9(3):33–40.

25. Kuck LS, Noreña CPZ. Microencapsulation of grape (Vitis labrusca var. Bordo) skin phenolic extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating agents. Food Chem. 2016 Mar 1;194:569–76.

26. Tine Y, Renucci F, Costa J, Wélé A, Paolini J. A method for LC-MS/MS profiling of coumarins in zanthoxylum zanthoxyloides (Lam.) B. Zepernich and Timler extracts and essential oils. Molecules. 2017 Jan 22;22(1):174.

27. Vinson JA, Chen X, Garver DD. Determination of total chlorogenic acids in commercial green coffee extracts. J Med Food. 2019 Mar;22(3):314–20.

28. Desai NM, Haware DJ, Basavaraj K, Murthy PS. Microencapsulation of antioxidant phenolic compounds from green coffee. Prep Biochem Biotechnol. 2019 Apr 21;49(4):400–6.

29. Lee I-C, Lee J-S, Lee J-H, Kim Y, So W-Y. Anti-Oxidative and Anti-Inflammatory Activity of Kenya Grade AA Green Coffee Bean Extracts. Iran J Public Health. 2019 Nov;48(11):2025–34.

30. Song SJ, Choi S, Park T. Decaffeinated Green Coffee Bean Extract Attenuates Diet-Induced Obesity and Insulin Resistance in Mice. Evid-Based Complement Altern Med ECAM. 2014;2014:718379.

31. Desai NM, Gilbert Stanley J, Murthy PS. Green coffee nanoparticles: optimisation, in vitro bioactivity and bio-release property. J Microencapsul. 2020 Jan 2;37(1):52–64.

32. Nosari ABFL, Lima JF, Serra OA, Freitas LAP. Improved green coffee oil antioxidant activity for cosmetical purpose by spray drying microencapsulation. Rev Bras Farmacogn. 2015 May;25(3):307–11.

33. Arpagaus C, John P, Collenberg A, Rütti D. Nanocapsules formation by nano spray drying. In: Nanoencapsulation Technologies for the Food and Nutraceutical Industries. Elsevier. 2017;346–401.

34. Arslan S, Erbas M, Tontul I, Topuz A. Microencapsulation of probiotic Saccharomyces cerevisiae var: Boulardii with different wall materials by spray drying. LWT - Food Sci Technol. 2015;63(1):685–90.

35. Sakawulan D, Archer R, Borompichaichartkul C. Enhancing antioxidant property of instant coffee by microencapsulation via spray drying. In: Proceedings of 21th International Drying Symposium. Universitat Politècnica València. 2018 Sept 11-14;8

36. Jacobs IC. Chapter 30 - Process Scale-up Considerations for Microencapsulation Processes. In: Gaonkar AG, Vasisht N, Khare AR, Sobel R, editors. Microencapsulation in the Food Industry. San Diego: Academic Press. 2014;391–8.

37. Perrone D, Donangelo C M, Farah, A. Fast simultaneous analysis of caffeine, trigonelline, nicotinic acid and sucrose in coffee by liquid chromatography–mass spectrometry. Food Chemistry. 2008;110(4):1030–1035

38. Davies NM, Wasan KM. Pharmacokinetics and drug metabolism in Canada: The current landscape. 2018.

39. Sapozhnikova Y. Development of liquid chromatography–tandem mass spectrometry method for analysis of polyphenolic compounds in liquid samples of grape juice, green tea and coffee. Food Chemistry. 2014;150:87–93.

40. Díaz DI, Beristain CI, Azuara E, Luna G, Jimenez M. Effect of wall material on the antioxidant activity and physicochemical properties of Rubus fruticosus juice microcapsules. J Microencapsul. 2015 Apr 3;32(3):247–54.

41. Díaz-Bandera D, Villanueva-Carvajal A, Dublán-García O, Quintero-Salazar B, Dominguez-Lopez A. Assessing release kinetics and dissolution of spray-dried Roselle (Hibiscus sabdariffa L.) extract encapsulated with different carrier agents. LWT - Food Sci Technol. 2015 Dec 1;64(2):693–8.

DOI: https://doi.org/10.31351/vol31iss1pp20-31

42. Chong S, Wong CW. Effect of spray dryer inlet temperature and maltodextrin concentration on colour profile and total phenolic content of Sapodilla (Manilkara zapota) powder. Int Food Res J. 2017 Dec 27;24:2543–8.

43. Ballesteros LF, Ramirez MJ, Orrego CE, Teixeira JA, Mussatto SI. Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. Food Chem. 2017 Dec 15;237:623–31.

44. Papoutsis K, Golding JB, Vuong Q, Pristijono P, Stathopoulos CE, Scarlett CJ, et al. Encapsulation of citrus by-product extracts by spray-drying and freeze-drying using combinations of maltodextrin with soybean protein and ι-carrageenan. Foods. 2018 Jul 19;7(7):115.