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Research Article

Effect of Homogenization Process on the Production of *Arthrospira platensis* Carotenoid Nanocapsules Encapsulated with Arabic Gum and Whey Protein Concentrate

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ARTICLE INFO

Received: December 07, 2021
Accepted: February 24, 2022
Published: February 25, 2022

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Keywords:

Arthrospira platensis
Homogenization Process
High Shear Homogenizers
Ultrasound
Nanocapsules

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Abstract

Arthrospira platensis contains a high concentration of carotenoids mainly 80 % β -carotene. The use of β -carotene in functional food and nutritional supplements was constrained by its lack of chemical and physical stability. Therefore, efforts were needed to protect carotenoids from damage by using an encapsulation system. The emulsion preparation before the encapsulation process had an influence on the stability of the oil-in-water emulsion. Various methods were used to prepare emulsions, such as high shear homogenizers (HSH) and ultrasound (US) treatment. The homogenization step was critical because it influenced the chemical stability of any encapsulated compounds. The purpose of this work was to generate nanocapsules of β -carotene with minimum carotenoid degradation, high efficiency of encapsulation, and small particle size, with variation in the homogenization process. This study consisted of 5 treatments, including the homogenization processes used HSH 24.000 rpm for 90 s ; HSH 24.000 rpm for 60 s, amplitude 55 % for 120 s ; amplitude 94 % for 138 s ; amplitude 55 % for 138 s ; and amplitude 55 % for 120 s . Each parameter was analysed by ANOVA followed by Tukey pairwise comparisons with 95% confidence level and $p < 0.05$. The particle size was influenced by the emulsification process, such as homogenization treatment, homogenization time, amplitude, and time of sonication. The sample that was homogenized using both HSH and US had a smaller particle size and the highest efficiency in encapsulation than others. The combination of homogenization process could decrease particle size.

Cite this as: Alfionita, K., Budhiyanti. S. A., & Ekantari, N. (2022). Effect of Homogenization Process on the Production of *Arthrospira platensis* Carotenoid Nanocapsules Encapsulated with Arabic Gum and Whey Protein Concentrate. *Jurnal Ilmiah Perikanan dan Kelautan*, 14(1):12–24. <http://doi.org/10.20473/jipk.v14i1.30074>

1. Introduction

Arthrospira is a photosynthetic, filamentous, spiral-shaped, multicellular blue-green microalgae and an essential microalga with a high content of valuable pigment. *Arthrospira platensis* has a high protein content, carotenoids (mainly β -carotene), essential fatty acids, vitamins, and minerals (Belay, 2007; Mani et al., 2008). Carotenoids are responsible for the red and yellow hues in nature, with an average of 3.4 to 4.0 g/kg in *A. platensis*. β -carotene content is 80% of the carotenoids content in *Arthrospira*, which is converted into vitamin A (Sotiroudis and Sotiroudis, 2013). Rather than its provitamin A function, β -carotene can perform as an antioxidant (Kasperczyk et al., 2014), decreasing the risk of type 2 diabetes, promoting immune system function, and lowering the risk of cardiovascular disease (Tanaka et al., 2012). Nevertheless, β -carotene's use in functional foods and nutritional supplements is constrained by its lack of physical and chemical stability (Chen and Zhong, 2015).

Carotenoids are easily oxidized and damaged due to heat, which affects their stability in the product. Due to the presence of highly conjugated double bonds in its structure, a bioactive chemical such as carotenoid is easily oxidized and susceptible to light, heat, and singlet oxygen during food processing and storage, resulting in the loss of both its color and bioactivity. Therefore, efforts are needed to protect carotenoids from damage due to heat, oxidation, light, or isomerization by using an encapsulation system (Ferreira and Nunes, 2019). Encapsulation is the process of encasing bioactive compounds in a heterogeneous or homogeneous matrix to form small capsules with microscales (> 1000 nm) or nanoscales (<1000 nm) (Rodríguez et al., 2016; Ricaurte et al., 2016; Sanguansri and Augustin, 2006). Encapsulation is a potential approach in which a bioactive compound is protected by a wall material that preserves the natural bioactive properties over time. The form of bioactive encapsulation will maintain, release, stabilize, and control the release of the bioactive compound. Incorporating β -carotene into the emulsion before the spray drying process can improve its functional properties. β -carotene bioaccessibility may be enhanced by the incorporation of oil-in-water emulsions due to the higher solubility of β -carotene in food-grade oils than in water (Qian et al., 2012; Salvia et al., 2013a; Salvia et al., 2013b). It can be dissolved in the oils and subsequently diffused in an aqueous phase in the form of smaller lipid droplets utilizing homogenization techniques (McClements and Rao, 2011).

Emulsions are thermodynamically unstable systems in which oil and water rapidly separate from

one another. The emulsifier stabilized the system by producing a thin layer surrounding the dispersed phase globules (Ghosh and Rousseau, 2011). Different densities between oil and the aqueous phase, combined with the unfavorable interaction of oil and water molecules, result in the formation of distinct layers (Kerkhofs et al., 2011; Maphosa et al., 2017). Thus, the production of emulsion-based materials requires an understanding of the rheology, stability, and bioavailability of active chemicals in order to produce products with the desired physical properties. The method had an effect on the oil-in-water emulsion's stability. Alcântara et al. (2019) reported that the efficiency of chia oil encapsulation was higher with the combination of the homogenization process (ultraturrax and ultrasound). A combination of homogenization and sonication time for solid lipid nanoparticle was also reported by Behbahani et al. (2017), that emulsion's particle size was affected by homogenization time. Other research about amplitude and sonication time was reported by Gaiwad and Pandit (2008), Higuera-Barraza et al. (2017), and Hussathirak et al. (2019). At the moment, emulsions are prepared using a variety of processes, including high shear homogenizers and ultrasonic (US) treatment. The shear force was generated by the cavitation of US power, which dispersed oil droplets into microscopic particles with a limited size distribution. Additionally, numerous investigations indicated that US power could produce stable oil-in-water emulsions. Kumalasari and Budhiyanti (2015) and Alfionita et al. (2017) suggested that a high shear homogenizer (HSH) might be used to minimize the size and homogeneity of droplets in order to maintain emulsion stability.

The important aspect of emulsion for commercial or industrial applications was to transform a nutraceutical delivery system's liquid into a powder to permit easy handling and utilization while also extending shelf life (Maher et al., 2014). Spray drying is a low-cost encapsulation technology widely used in the food and pharmaceutical industries because it is suitable for continuous large-scale powder production, relatively simple, inexpensive, fast, and reproducible. This short operational duration may lessen the risk of heat-sensitive components being damaged (Dias et al., 2015). Previous studies suggested that optimizing emulsification process conditions and product formulation was required for successful formulation of a powdered delivery system (core material and wall material) (Mao et al., 2010; McClements and Rao, 2011; Behbahani et al., 2017). The incorporation of wall material into an emulsion prior to dehydration was significant because it raised the glass transition temperature of the solid matrix around the oil droplets, boosting the chemical stability of any

encapsulated compounds (Mosquera *et al.*, 2012).

Arabic gum and whey protein concentrate were used for wall material due to their high encapsulation efficiency and good volatile retention (Carneiro *et al.*, 2013; Kumalasari and Budhiyanti, 2015; Hidayat *et al.*, 2016; Vahidmoghdam *et al.*, 2019). Arabic gum has been utilized as a wall material in various encapsulation processes, for example, in the microencapsulation and nanoencapsulation of oils, volatile bioactive chemicals, and flavorings, among other applications (Krishnan *et al.*, 2005; Soottitantawat *et al.*, 2005; Fang *et al.*, 2005; Vaidya *et al.*, 2006) and Prata *et al.* (2013) also found that whey protein concentrates had the greatest emulsifying potential. β -carotene nanoencapsulation with arabic gum and whey protein concentrate is expected to maintain the quality of carotenoids, allowing for greater use of β -carotene nutrients. The objective of the current study was to develop nanocapsules of β -carotene with minimum carotenoid degradation, high efficiency of encapsulation, and small particle size, with variation in the homogenization process.

2. Materials and Methods

2.1 Material

The materials in this research are *A. platensis* (purchased from PT. Trans Pangan Spirulindo, Indonesia), n-hexane (Merck, Germany), ethanol (Merck, Germany), virgin coconut oil or VCO (CV. Pusat Pengolahan Kelapa Terpadu, Indonesia), Arabic gum (Medika, Indonesia), whey protein concentrate or WPC (Carnivor, Indonesia), and distilled water.

2.2 Sample Preparation

Arthrospira platensis was extracted according to Wahyu and Yanuar (2010) and Pahlevi *et al.* (2008). The extraction process was started by weighing 9 grams of *A. platensis*, then placed in the three-neck flask layered aluminium foil, attached to the spiral condenser and hot plate stirrer, then added with 27 mL ethanol and 270 mL hexane solvent. The extraction process took 3 hours and 24 minutes, with a hot plate stirrer set at 1500 rpm and 51.9°C. After that, the carotenoid extract was separated from the pellet and evaporated using a rotary vacuum evaporator for 20 minutes at 45°C. The extract was sprayed with nitrogen to evaporate all solvent and to produce the condensed carotenoid extract. The carotenoid extract was kept at 0±2°C in a glass-wrapped by aluminium foil until it used.

2.3 Emulsion Production

First, 0.36 g carotenoid extract was diluted

in 10 mL VCO in a volumetric flask and sealed with aluminum foil to protect it from light. After that, 18 g of Arabic gum and 9 g of WPC was diluted into 90 mL distillate water as a mix of encapsulating material. The mix was stirred at 60°C and 600 rpm for 30 minutes, then decreased in temperature to 45°C (Rosanita, 2014; Kumalasari and Budhiyanti, 2015). Finally, the carotenoid dilution and encapsulating material were mixed with various methods using a high-speed homogenizer and or ultrasound (Table 1).

2.4 Spray Drying

The spray drying process was used to convert the carotenoid emulsion into encapsulated powder. The carotenoid emulsion was pumped into a spray dryer chamber. The drying process was controlled to keep 150°C for inlet temperature and 70°C for outlet temperature using Büchi Mini Spray Dryer B-290 (made from Switzerland). The encapsulated powder was collected and placed in a dark glass bottle, and kept frozen before use for further analyzed.

2.5 Nanocapsules Characteristics Analysis

2.5.1 Emulsion stability analysis

Emulsion stability was analyzed using creaming index after the homogenization process (Cho *et al.*, 2008). The emulsion was placed at a conical bottle then were centrifuged at 2300 rpm for 15 minutes. The emulsion stability was expressed as the percentage of the emulsified layer volume remaining in the original emulsion volume.

2.5.2 Carotenoid content analysis

2.5.2.1 Total carotenoid

Carotenoid content analysis used the Desorby *et al.* (1997) method with modification. Total carotenoid, surface carotenoid, and retention of carotenoid were evaluated respectively after the spray drying process. Total carotenoid was measured by placing 50 mg of capsule powder into an Erlenmeyer flask, then adding 0.25 mL of dilute water and 2.5 mL of n-hexane for analysis. It was stirred at 500 rpm for 30 minutes. The supernatant was read by a spectrophotometer (Genesys 10 UV-VIS) at a wavelength of 450 nm. The absorbance was converted to the standard curve of the carotenoid. Total carotenoid was calculated by using the following equation:

$$\text{Total carotenoid } (\mu\text{g}/\text{mg db}) = \frac{\text{Total carotenoid}}{100 - K_a} \times 100$$

Table 1. Various homogenization processes of *Arthrospira platensis* carotenoid emulsion

Sample code*	Homogenization process			References
	High speed homogenizer (HSH) 24,000 rpm Time (s)	Ultrasound		
		Amplitude (%)	Time (s)	
A	90	---	---	Kumalasari and Budhiyanti (2015)
B	60	55	120	Hidayat <i>et al.</i> (2016)
C	---	94	138	Hashtjin and Abbasi (2015)
D	---	55	138	Optimization
E	---	55	120	Optimization

*Each sample code was replicated two times.

Table 2. Carotenoid contents of *A. platenis* carotenoid encapsulated with Arabic Gum and Whey Protein Concentrate

Treatment	Surface carotenoid	Total carotenoid	Encapsulation retention	Efficiency encapsulation
A	28.20 ± 1.17 ^d	73.04 ± 0.35 ^b	72.35 ± 0.35 ^b	61.39 ± 1.41 ^b
B	17.56 ± 0.13 ^c	66.53 ± 1.93 ^c	65.89 ± 1.91 ^c	73.58 ± 0.96 ^a
C	72.81 ± 0.49 ^a	122.3 ± 1.76 ^a	121.13 ± 1.74 ^a	40.46 ± 0.46 ^d
D	59.30 ± 0.7 ^b	66.83 ± 0.36 ^c	66.19 ± 0.35 ^c	11.26 ± 0.57 ^c
E	38.49 ± 0.69 ^c	75.32 ± 25.45 ^b	74.60 ± 0.48 ^b	48.9 ± 0.59 ^c

Description: Different superscripts in the same column shows that there are significant differences ($p < 0.05$): A : High Speed Homogenizer (HSH) 24,000 rpm, 90 s; B : HSH 24,000 rpm, 60 s, Ultrasound (US) Amplitude 55 %, 120 s; C : US Amplitude 94 %, 138 s; D : US Amplitude 55 %, 138 s; E US Amplitude 55 %, 120 s

2.5.2.2 Surface Carotenoid

The carotenoid surface was calculated by placing 50 mg of powder into an Erlenmeyer flask, adding 0.25 mL of dilute water and 2.5 mL of n-hexane for analysis, then stirring at 100 rpm for 15 seconds. The sample was centrifuged at 1000 rpm for 1 minute. The supernatant was analyzed by a spectrophotometer at a 450 nm wavelength. Carotenoid surface was calculated by the following equation:

$$\text{Surface carotenoid } (\mu\text{g/mg db}) = \frac{\text{Surface carotenoid}}{100 - K_a} \times 100$$

2.5.2.3 Carotenoid retention

The carotenoid retention ratio was a ratio between the carotenoid levels in the nanocapsules after spray drying with the initial carotenoid extract levels before the nanoencapsulation process (Desorby *et al.*, 1997). Carotenoid retention was calculated by using the following equation:

$$\% \text{ carotenoid retention} = \frac{\text{Total carotenoid}}{\text{Initial carotenoid extract}} \times 100$$

2.5.2.4 Encapsulation efficiency (EE)

The encapsulation efficiency was carried out to measure the effectiveness of the nanoencapsulation process in trapping the core material (Alcântara *et al.*, 2019). Encapsulation efficiency was expressed by using the following equation:

$$\% \text{ EE} = \frac{\text{Total carotenoid} - \text{Surface carotenoid}}{\text{Total carotenoid}} \times 100$$

2.5.3 Particle size distribution and droplet size

Particle size distribution and droplet size was analyzed from encapsulated powder. Particle size analysis was carried out using a particle size analyzer or PSA (Horiba scientific SZ-100 type made from Japan) according to Liang *et al.* (2013). Five milliliters sample was placed into a cuvette by pipette then the cuvette was put into PSA tool. The result of particle size and polydispersity index was displayed on the computer.

2.5.4 Antioxidant activity

Antioxidant activity was analysed using the scavenging effect of antioxidants on 2,2-diphenyl-1-picrylhydrazyl (DPPH). The encapsulated powders were diluted in methanol at a 1 mg/ml concentration. 1 mL of DPPH dilution was mixed with 1 mL of encapsulated powder (O'Sullivan, 2013). After incubating the sample in a dark room for 15 minutes, the absorbance was measured using a spectrophotometer with a 515 nm wavelength. DPPH solution and methanol were used as a control with the same amount of sample. The absorbance value was used to calculate radical scavenging activity (% of inhibition) with the formula:

$$\% \text{ inhibition} = \frac{(\text{Abs control} - \text{Abs encapsulated powders})}{\text{Abs control}} \times 100\%$$

2.5.5 Statistical analysis

Each parameter was analysed by one-way ANOVA followed by Tukey pairwise comparisons post hoc using Tukey's method with 95% confidence level and $p < 0.05$. Statistical analysis was performed using Minitab 19 software.

3. Result and Discussion

3.1 Emulsion Stability

The emulsion-making process used by HSH and US in various conditions was shown in Table 1, and the emulsion stability of the carotenoid emulsion was shown in Figure 1. The sample code E (55% amplitude level and 120 seconds) had the highest stability (72%) compared to the others. However, it was not significantly different from sample C (94% amplitude level for 138 seconds) (65%). On the other hand, sample C was not significantly different from sample A (high speed homogenizer for 90 seconds) (57%), B (high-speed homogenizer for 60 seconds and ultrasound at 55% amplitude level for 120 seconds) (58%), and D (55% amplitude level for 138 seconds) (59%). The result showed that HSH had no effect on the formation of a stable emulsion. The cavitation of US power generated the shear force, which could disperse oil droplets into small particles with a narrow size distribution. Also, many studies mentioned that US power could produce oil-in-water emulsions with good stability (Chemat *et al.*, 2011; Bajac *et al.*, 2019). The instability of the emulsion system was shown by the creaming in the system. Based on the results, sample E (55% amplitude level and 120 seconds) had the highest stability emulsion, which was processed by US. It may be because sonication could be able to form a stable emulsion through cavitation,

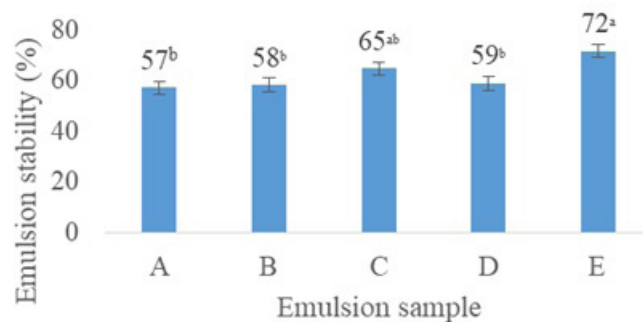


Figure 1. The emulsion stability of carotenoid *A. platensis* emulsion. Description: Different superscripts shows that there are significant differences ($p < 0.05$): A : High Speed Homogenizer (HSH) 24,000 rpm, 90 s; B : HSH 24,000 rpm, 60 s, Ultrasound (US) Amplitude 55 %, 120 s; C : US Amplitude 94 %, 138 s; D : US Amplitude 55 %, 138 s; E US Amplitude 55 %, 120 s

but based on the particle size powder obtained from the spray drying process, sample E had the highest surface oil. Emulsion stability was one of the parameters for knowing the emulsion resistance during the waiting period before spray drying.

The stability of carotenoid emulsions could be determined by monitoring the layer's height over time. Typically, the droplets in an emulsion had a different density than the liquid that surrounds them (McClements, 2005). Because the emulsifier had a higher density than the oil in this study, the oil droplets tended to rise upward, a phenomenon referred known as "creaming" (Tadros, 2013). Due to the fact that the densities of the majority of edible oils were lower than those of water, oil tended to accumulate at the top of an emulsion and water at the bottom. By centrifuging an emulsion at a constant speed for a specified period of time, the creaming instability of the emulsion could be enhanced (Sherman, 1995). The emulsion preparation method had a great influence on the stability of oil in water emulsion. Different emulsion preparation procedures tend to produce emulsions with varying degrees of stability. Numerous breakdowns of emulsions were possible, including creaming or sedimentation, which occurred when two or more droplets mix to produce a droplet with a larger volume but a smaller area, and flocculation caused by Van der Waals attraction, which occurred when the droplets lack adequate repulsion (McClements, 2005). Additionally, the breakdown could be triggered by Ostwald ripening, which was caused by differences in the chemical potentials of the materials within the small and large droplets; coalescence, which was triggered by the thinning and disruption of the liquid film between

Table 3. Particle size of *A. platenis* carotenoid encapsulated with with Arabic Gum and Whey Protein Concentrate

Treatment	Capsule particle size (nm)	Polydispersity Index
		(PDI)
A	839.9667 ± 11.38 ^a	0.519 ^a
B	743.1667 ± 6.08 ^a	0.503 ^a
C	1420.467 ± 0.92 ^c	0.455 ^a
D	1321.533 ± 40.8 ^{bc}	0.538 ^a
E	1260.767 ± 14.14 ^b	0.508 ^a

Description: Different superscripts in the same column shows that there are significant differences ($p < 0.05$). A : High Speed Homogenizer (HSH) 24,000 rpm, 90 s; B : HSH 24,000 rpm, 60 s, Ultrasound (US) Amplitude 55 %, 120 s; C : US Amplitude 94 %, 138 s; D : US Amplitude 55 %, 138 s; E US Amplitude 55 %, 120 s

the droplets; or phase inversion, which occurs when the dispersion phase and medium exchange positions (Tadros, 2004).

Meybodi *et al.* (2014) mentioned that the presence of oil oxidation could increase instability of the emulsion due to droplet aggregation. In the emulsion phase, physical characteristics such as stability were key issues that should be considered. Emulsion stability referred to the ability of the emulsion to withstand changes in its characteristics over a certain period of time. The level of emulsion size was determined by several factors, including particles, particle size distribution, and density between the dispersed phase and the continuous phase (Given, 2009). In addition, emulsion stability was needed to predict the presence of droplet aggregation over a certain period of time.

3.2 Carotenoid Content

Carotenoid contents were the chemical parameter of *A. platenis* carotenoid nanocapsules, including surface carotenoid, total carotenoid, carotenoid retention, and encapsulation efficiency. The difference in carotenoid contents is due to different homogenization processes (Table 2). The C sample (94% amplitude level for 138 seconds) had the highest total carotenoid and carotenoid retention but had low encapsulation efficiency. The encapsulation efficiency in sample B (high-speed homogenizer for 60 seconds and ultrasound at 55% amplitude for 120 seconds) was higher than others, indicating that the emulsion process fit the carotenoid emulsion. The other fact was that the surface carotenoid in the B (high speed homogenizer for 60 seconds and ultrasound at 55% amplitude for 120 seconds) sample was lower than in the others. It indicated that the homogenization process produced

good emulsion stability, the wall material could entrap the core material, and prevent damage during the spray drying process. Reineccius (2004), Li *et al.* (2016), and Vahidmoghadam *et al.* (2019) stated that one of the important parameters to determine good encapsulation process was surface oil content and encapsulation efficiency because it was related to the quality of the resulting capsule structure.

The homogenization processes of B and C were different. B used a 24.000 rpm high-speed homogenizer (HSH) for 60 seconds and ultrasound with a 55% amplitude for 120 seconds, but C used ultrasound with a 94% amplitude for 138 seconds without a high-speed homogenizer. Hashtjin and Abbasi (2015) explained that nanoemulsion of peeled orange essential oil was used with ultrasound at 94% amplitude and for 138 seconds as the optimum homogenization condition to produce an emulsion with Tween 80 emulsifier, but in this research, the emulsion method was not appropriate for applying carotenoid extract emulsion with arabic gum and whey protein concentrate based on carotenoid contents and particle size.

The D sample that was homogenized by ultrasound with 55% amplitude for 138 seconds showed the high content of surface carotenoid (59.30 µg/mg db) and the lowest efficiency of encapsulation (11.26%). Compared to the A sample, there was a significant difference between surface carotenoid and encapsulation efficiency, which had 28.20 g/mg db and 61.39%, respectively. This data revealed that the high-speed homogenizer played an essential role in the homogenization process of the carotenoid emulsion. McClements (2004) stated that stirring at a certain speed and time in the emulsion system could increase the absorption of the emulsifier so that the emulsifier could cover the oil components and reduce the droplet

interfacial tension. The oil component, which was well adsorbed by the emulsifier, caused the oil content on the capsule surface to be lower and the encapsulation efficiency to be higher. In addition, energy input during the emulsification process also played an important role in the stability of the emulsion formed. Stable emulsion could produce stable capsule powder after passing through the spray drying process (Mohammed *et al.*, 2020). The B sample (high-speed homogenizer for 60 seconds and ultrasound at 55% amplitude for 120 seconds) got more energy input because there were two processing times during emulsion formation. Based on this, it could be concluded that B sample emulsion and powder were more stable than the other four samples. This was evidenced by the low carotenoid content and high encapsulation efficiency in sample B. Similar results were shown by the study of Alcântara *et al.* (2019) using a high speed homogenizer with ultrasonication as a homogenizer for microencapsulation of chia seed oil (*Salvia hispanica* L.). Alcântara *et al.* (2019) showed the results of the lowest surface oil content and the highest encapsulation efficiency when using a high speed homogenizer and ultrasonication during emulsion formation.

3.3 Particle Size

The A sample (high-speed homogenizer for 90 seconds) and B sample (high-speed homogenizer for 60 seconds and ultrasound at 55% amplitude for 120 seconds) showed smaller particle size than C (94% amplitude for 138 seconds), D (55% amplitude level for 138 seconds), and E (55% amplitude level for 120 seconds) samples. Based on statistical analysis (Table 3), the particle size of B had no significant difference with that of A, where the homogenization process was done without ultrasound. The A used HSH only for 90 seconds and the B used HSH at 24,000 rpm for 60 seconds and ultrasound 55% amplitude for 120 seconds. On the other hand, the C sample was homogenized only by ultrasound at 94% amplitude for 138 seconds. The particle size of the C was twice that of the B. This revealed that the HSH was an important step in producing small particles of carotenoid encapsulated by arabic gum and whey protein concentrate. Higher sonicator intensities, on the other hand, may result in faster droplet coalescence (Jafari *et al.*, 2006; Li and Chiang, 2012). Longer sonication (more than 120 seconds) and higher amplitude (more than 55 percent) may result in larger droplets forming due to coalescence.

Nanoparticles were produced from bioactive compounds or native oil and recovered or enclosed by a homogeneous or heterogeneous matrix as a wall

material to create small particles with a size of 10-1000 nm (Mohanraj and Chen, 2006). The particle size and size distribution of the particles were the most critical aspects to consider in order to ensure stability, targeting ability, biological destiny, loading, and release of the bioactive during application. There is a possibility that it will determine the stability and bioavailability of nanoparticles. The Particle Size Analyzer (PSA) was utilized for particle size analysis, and the polydispersity index was used to describe the size distribution of the particles (PDI). It was discovered by Gaumet *et al.* (2008) that size (mean diameter or z-average) and size distribution were important parameters for nanoencapsulation evaluation because of their relationship to distribution, physicochemical changes of the encapsulated compounds, viscosity, and surface area of the nanoparticles, among other things.

The preparation method to obtain a good emulsion always involves homogenization. The control of homogenization conditions (speed, pressure, temperature, and cycles) is required to obtain emulsions with the desired properties (droplet size, stability, encapsulation, and biocompound delivery (Yuan *et al.*, 2008). At greater homogenization speeds, a higher energy density was applied to the solution, which resulted in a reduction in the size of the emulsion droplet. Shear stress was also shown to be inversely proportional to the size of the emulsion droplets. Increasing shear stress results in a reduction in droplet size and the formation of nanodroplets. After migrating through the rotor-stator area, the mean droplet size decreased relatively little, although it varied according to speed, time, and emulsion components (Hakansson and Rayner 2018). Ultrasound was a technique that can be employed in liquid systems. It was characterized as high-frequency waves followed by the breakup of droplets due to cavitation effects (Létang *et al.*, 2001; Peshkovsky and Bystryak, 2014). Sound waves may cause particle size reduction, resulting in inhomogeneous particles ranging non size from micro to nano. To achieve tiny particles, both US and HSH were utilized in the homogenization process during emulsification. Tadros (2004) said that the stability, appearance, color, and texture of emulsion systems are dependent on the emulsion droplets and particle size distribution. According to Stokes' Law, droplet diameter was critical in determining oil-in-water emulsion stability because creaming and sedimentation occur due to the gravitational effect. There was a direct correlation between oil droplet diameter and emulsion stability. As mentioned before, McClements and Decker (2000) also stated that reducing droplet size will enhance the emulsion stability and is thermodynamically stable.

3.4 Yield

The yield of capsule powders produced by spray drying (Figure 2). Differences in the homogenization process were not significantly different among the five treatments on A, B, C, and D samples. The data showed that the homogenization process did not significantly affect the yield, as Tontul and Topuz (2015) and Tontul and Topuz (2013) mentioned that yield was not affected by the homogenization during the emulsification process but the wall materials.

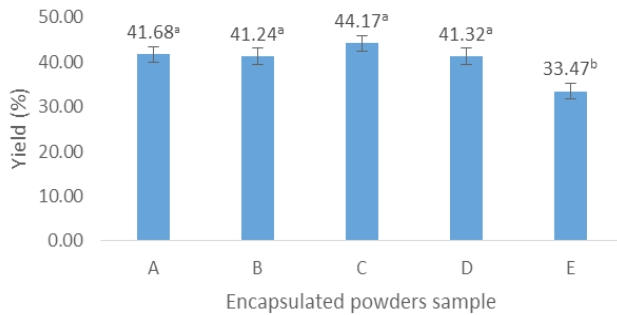


Figure 2. The yield of *A. platenis* carotenoid encapsulated with Arabic Gum and Whey Protein Concentrate.

Description: Different superscripts in the same column shows that there are significant differences ($p < 0.05$) A : High Speed Homogenizer (HSH) 24,000 rpm, 90 s; B : HSH 24,000 rpm, 60 s, Ultrasound (US) Amplitude 55 %, 120 s; C : US Amplitude 94 %, 138 s; D : US Amplitude 55 %, 138 s; E US Amplitude 55 %, 120 s

3.5 Moisture Content

Moisture content is a critical quality control factor, affecting the appropriateness, storage behaviour, and stability of the powder. The moisture content of the powder ranged from 2.68 to 5.30 percent (Figure 3). Sample E had the lowest moisture content of the others. Samples A and B presented no significant difference by ANOVA analysis ($p < 0.05$). Higher moisture content was affected by the manufacturing of the capsules. Due to insufficient powder flow during the spray drying process, capsule performance would be inconsistent. Additionally, it may result in capsule surface adhesion problems (Tomar et al., 2017). Crouter and Briens (2014) indicated that powder characteristics and behaviours were crucial for efficient and successful powder manufacture. The hygroscopicity of a powder might indicate its capacity to absorb water vapor from the surrounding atmosphere. According to the data in Figure 4, different homogenization methods can have an effect on the moisture content.

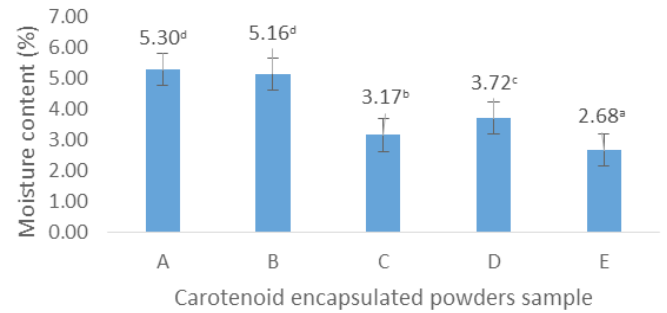


Figure 3. The moisture content of *A. platenis* carotenoid encapsulated with Arabic Gum and Whey Protein Concentrate. Description: Different superscripts shows that there are significant differences ($p < 0.05$): A : High Speed Homogenizer (HSH) 24,000 rpm, 90 s; B : HSH 24,000 rpm, 60 s, Ultrasound (US) Amplitude 55 %, 120 s; C : US Amplitude 94 %, 138 s; D : US Amplitude 55 %, 138 s; E US Amplitude 55 %, 120 s

3.6 Antioxidant Activity

The radical scavenging activity of the *A. platenis* carotenoid capsule from five different emulsification processes was in the range of 37.14 – 66.26 % (Figure 4). Based on statistical analysis by ANOVA, the data showed that sample D (55% amplitude level for 138 seconds) had the highest antioxidant activity and sample E (55% amplitude level for 120 seconds) was in second place, but there was no significant difference with sample B ($p > 0.05$).

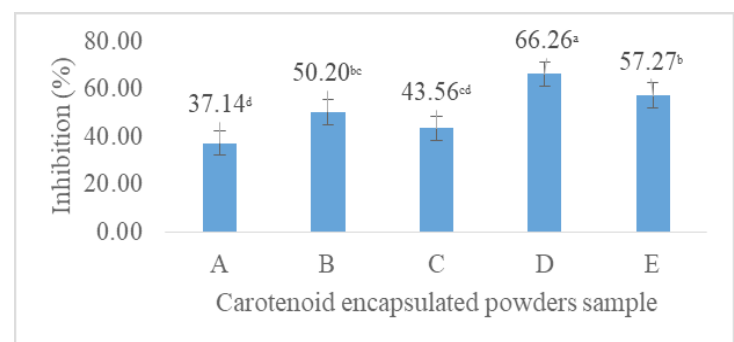


Figure 4. Antioxidant activity of *A. platenis* carotenoid nanocapsules encapsulated with Arabic Gum and Whey Protein Concentrate. Description: Different superscripts shows that there are significant differences ($p < 0.05$): A : High Speed Homogenizer (HSH) 24,000 rpm, 90 s; B : HSH 24,000 rpm, 60 s, Ultrasound (US) Amplitude 55 %, 120 s; C : US Amplitude 94 %, 138 s; D : US

Samples D and E undergo homogenization by the US with an amplitude of 55% for 138 seconds and 55%

for 120 seconds, respectively. Compared with sample B, which undergo homogenization by HSH 24,000 rpm for 60 seconds and the US with an amplitude of 94% for 138 seconds, there was no significant different with sample E ($p > 0.05$). The scavenging activity did not rise linearly with increasing amplitude of US applied to the emulsion phase, indicating that the energy input mechanism was not the only factor affecting the carotenoid capsules' total antioxidant activity. [Tan and Nakajima \(2005\)](#) and [Yi et al. \(2014\)](#) hypothesize that emulsions with smaller particle sizes may promote the oxidation of the oil phase and decrease the bioactivity of the oil droplets. [McClements and Decker \(2000\)](#) mentioned that lipid oxidation can be accelerated by reactions that take place at the surface of the emulsion droplets. The carotenoids lost in the microcapsules can be directly attributed to oxygen exposure during encapsulation formation by the homogenization speed, which may have caused the oxidation. The crucial factor of the microencapsulation production is the controlled release, which ensures the release of carotenoid compounds in the human body after consumption ([Lima et al., 2019](#)).

4. Conclusion

The current study confirmed that the homogenization process depends on material from both the core and wall material to produce an emulsion and capsules with good stability and small particle size. The particle size was influenced by the emulsification process, such as homogenization treatment, homogenization time, amplitude, and time of sonication. The sample that was homogenized using both high-speed homogenizer and ultrasound had a smallest particle size and best encapsulation effectiveness. In general, combining the process homogenization can improve particle size reduction efficacy.

Acknowledgment

Thank you to the Department of Fisheries Faculty of Agriculture Universitas Gadjah Mada for supporting and facilitating this research.

Authors' Contribution

All authors have contributed to the final manuscript. The contributions of each author are as follows, Khusnul Alfionita; carried out the experiment, collected data, and wrote the manuscript. Siti Ari Budhiyanti; designed the research and wrote the manuscript. Nurfitri Ekantari; designed the research,

analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors stated and declared that there is no conflict of interest in this research.

Funding Information

This research was funded by Featured Research Grants Faculty of Agriculture Universitas Gadjah Mada with Grant Number 1688/PN/PT/2019.

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