Effects of Microencapsulated *Dunaliella Salina* Algae on Sensory Evaluations, Omega-3 Fatty Acid and Nutritional Compositions Value of Sago Bagea Cookies

Kevin, Athala; Raya, Indah*+; Natsir, Hasnah; Anshar, Andi Muhammad; Musa, Bulkis; Mayasari; Erna

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Hasanuddin University, INDONESIA

ABSTRACT: *This study aims to find the well-coated Dunaliella Salina Microcapsule (DSM) and use it as a supplement for sago bagea cookies. DSM formulas will be produced from the encapsulation of maltodextrin and Arabic gum by spray drying method, and their morphologies will be analyzed using a Scanning Electron Microscope (SEM). The best formula is chosen based on wrinkles that appeared and agglomeration on the particles of the produced microcapsules. Based on the SEM result, it is obtained that F3 is the well-coated microcapsule due to its less-wrinkled surface with no agglomeration. F3 will be used to fortify sago bagea cookies with ratios of 10%, 20%, and 30%. In this study, four sago bagea cookie formulas were made (Control, DSM 10%, DSM 20%, and DSM 30%). These formulas will be tested by sensory evaluations to determine the preferred cookies, which will be analyzed further for their nutritional values. Based on the sensory analysis, DSM 10% will be chosen due to panelist preference, with the highest overall acceptability score of 3.94 ± 0.71. DSM 10% will be analyzed on its omega-3 fatty acids (docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)) and nutritional compositions. In conclusion, the analysis showed DSM 10% has a higher content of DHA (4.705 mg/g to 7.305 mg/g), EPA (15.18 mg/g to 20.18 mg/g), ash (0.71% to 0.77%), fat (13.84% to 15.56%), protein (2.72% to 3.89%), crude fiber (1.33% to 1.34%) and total energy (456.28kcal/100g to 467.16kcal/100g) but lower in moisture (2.52% to 1.80%) and carbohydrate (80.21% to 77.98%) than control.*

KEYWORDS: *Sago bagea cookies; Dunaliella salina; encapsulation; Omega-3; DHA/EPA; spray drying; maltodextrin; Arabic gum; Sensory evaluations.*

INTRODUCTION

Omega-3 fatty acids such as docosahexaenoic acid (22:6n-3, DHA) and eicosapentaenoic acid (20:5n-3, EPA) are known for their significant biochemical and physiological effects on the human body, especially in giving a positive impact on human nutrition and health [1]. DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) exhibit anti-inflammatory and healthy properties that could reduce the risk of cardiac diseases such as arrhythmia and stroke, valid for the treatment of high blood pressure or rheumatoid arthritis and contribute

* *To whom correspondence should be addressed.*

+ *E-mail: indahraya@unhas.ac.id*

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on maintaining the normal brain function and adequate eye vision [2-4]. In addition, EPA has been seen to prevent coronary heart disease and lower blood cholesterol. Meanwhile, DHA is essential in developing babies' central nervous system [5]. These omega-3 fatty acids' functions have attracted the consumer's attention to applying DHA and EPA fatty acids in functional foods [1].

DHA and EPA global demands were predicted to increase to 241,000 tonnes by 2020 [6]. This demand outstrips the supply of DHA and EPA, posing a challenge to find new sustainable alternatives to relieve pressure on fisheries [7]. Omega-3 from fish was derived initially from microalgae consumed by fish. This realization has turned microalgae into a potential alternative source of Omega-3, especially DHA and EPA. Microalgae is not only used for food diversification but also to empower agricultural land, which is not feasible. Indonesia is a country that has suitable temperatures and contains a high level of salt, so it is feasible for the growth of microalgae [4].

Microalgae such as *Dunaliella*, *Chlorella*, and *Spirulina* are rich sources of lipids and PolyUnsaturated Fatty Acids (PUFAs) that are capable of rapid growth and can produce high biomass with simple nutritional requirements [8]. *Dunaliella salina* is one type of microalgae focused on production for the food industry because of its nutritional compounds. *Dunaliella salina* is a unicellular green alga (which can be green, orange, or red) with two flagella. This microalga provides various nutrients such as protein, carbohydrates, lipids, and pigments [9]. *Dunaliella salina* contains 6.63% moisture, 48.74% ash, 10.03% proteins, 3.49% lipids, and 3.46% carotenoids. *Dunaliella salina* could be used in capsule forms, solid forms, food bars or cookies, and liquid forms, such as antioxidant drinks or juices [10]. Microalgae also contain DHA and EPA, which are suitable for food fortification but have an undesired taste and odor. Also, these DHA and EPA are chemically unstable and susceptible to oxidation, which could affect the quality of the product, such as shelf-stability and the sensory properties of the powdered microalgae. Microencapsulation was conducted to prevent oxidation and protect microalgae's bioactive compounds by using encapsulation matrix/wall materials [11]. Microencapsulation has become an alternative to reduce the sensitivity to changes caused by external factors, such as light, oxygen, and temperature, and being prone to evaporate [12]. Microencapsulation is a method to extend drug release from dosage forms and to reduce its adverse effects [13]. Microencapsulation is used to provide protection against the degradative reaction by carrier matrices, prevent the loss of volatile compounds and enhance the stability of the core materials [14]. By microencapsulation technique, foods' shelf life could also be increased [15].

Cookies are one of the most popular bakery products consumed by almost all levels of society due to their taste and availability [16]. Because of its popularity, Indonesia's average consumption of cookies or biscuits increased by 33.3% from 2014-2018 [17]. The high consumption of these products caused an increase in wheat imports by up to 10.1% from 2014-2018 [18]. This import happens because the main ingredient of cookies is wheat flour obtained from wheat. To reduce wheat flour consumption and wheat imports, it is necessary to utilize local resources to replace wheat flour. Sago is one of Indonesia's local resources that could be used as a substitute material for cookies. In Southeast Sulawesi, a sago flour-based cookie is called a sago bagea cookie.

Sago bagea cookies are traditional cookies from several regions in eastern Indonesia, including Southeast Sulawesi [19]. Sago bagea cookies have a dry, crispy texture and are pale brown. The main ingredients for making sago bagea cookies are sago starch and other ingredients like sugar, vegetable/cooking oil, and eggs. Sago bagea cookies are generally topped with nuts [20]. Because of using sago flour/starch as their primary ingredient, the nutritional content in these cookies is primarily carbohydrates. To improve the quality of sago bagea cookies, it is necessary to substitute flour with other food ingredients [19]. Modern society today expects sago bagea cookies not only to have a delicious and filling taste but also to have functional properties for health [21]. Research about fortifying sago bagea cookies has been done by some researchers that fortify bagea sago cookies using sweet potato flour [19] and moringa leaf powder composite [20], but fortifying sago bagea cookies by using encapsulated microalgae has never been done before. Therefore, this research was conducted to find a well-coated formula for the microencapsulated *Dunaliella salina* based on the ratio of maltodextrin and Arabic gum was used to fortify sago bagea cookies' nutritional content as a supplement.

EXPERIMENTAL SECTIONS

Materials and apparatus

The materials used in this research were sago flour, eggs, butter, cooking oil, cashew nuts, sugar, and salt obtained from a local market in Makassar, Indonesia. The *Dunaliella salina* powder was purchased from Xi'an Herbsens China Co., Ltd. Maltodextrin (food grade) with DE = 10 was purchased

Table 1: Materials specification

Chemical materials	Specification	Degree of purity
Maltodextrin	Food grade with DE = 10	99%
Arabic gum	Food grade	99%
Docosahexaenoic acid (DHA) standard	Analytical grade (Merck)	98%
Eicosapentaenoic acid (EPA) standard	Analytical grade (Merck)	99%
n-hexane	Analytical grade (Merck)	99%
Hydrochloric acid (HCl)	Analytical grade (Merck)	37%
Sulfuric acid (H_2SO_4)	Analytical grade (Merck)	98%
Sodium hydroxide (NaOH)	Analytical grade (Merck)	98%
Pottasium hydroxide (KOH)	Analytical grade (Merck)	85%
Boric acid (H_3BO_3)	Analytical grade (Merck)	99,5%
Ethanol (C_2H_5OH)	Analytical grade (Merck)	95%
Selenium reagent mixture : - Copper (II) sulfate - Selenium	Analytical grade (Merck)	 1% 1%
Phenolphthalein indicator ($C_{20}H_{14}O_4$)	Analytical grade (Merck)	100%

DE = 10 was purchased from Globalchemical Factory Co., Ltd in China, and Arabic gum (food grade) was purchased from Newgreen Health Industry Co., Ltd. in China and aquadest. Other chemicals, namely DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) standard, n-hexane (C_6H_{14}), concentrated hydrochloric acid (HCl), concentrated sulfuric acid (H_2SO_4), sodium hydroxide (NaOH) or potassium hydroxide (KOH), boric acid 3% (H_3BO_3) (diluted from pure boric acid), ethanol (C_2H_5OH), selenium reagent mixture (mixture of copper (II) sulfate ($CuSO_4$) and selenium) and phenolphthalein indicator ($C_{20}H_{14}O_4$) were purchased from Sigma-Aldrich Chemicals. All of the reagents and solvents used were analytical grade. The specification of the used materials can be seen in Table 1.

Apparatus used in this research were beaker glass, Erlenmeyer flask, Kjeldahl flask, burette, and a stative, digital scale, Buchner funnel, laboratory vacuum pump, shaker (Biologix SK-L180-E), soxhlet extractor, desiccator, glass vials, porcelain dish, electric oven (Kirin KBO-600RA), electric furnace (Thermo Scientific Type f6010), mixer (EHSM 2000), baking tray, hotplate, spray dryer (LabPlant SD-05), ultrasonic cleaner (Elmasonic S 40H), spectrophotometer Uv-Vis (Shimadzu UV-2600), Scanning Electron Microscope/SEM (JEOL NeoScope JCM-6000Plus) and IBM SPSS Statistic 26 application.

Determination of DHA and EPA linear regression lines for Uv-Vis analysis

The determination of DHA and EPA linear regression lines was described in *Aulia* (2016) [22]. DHA and EPA concentrations could be analyzed by measuring the absorbance on the wavelength of 310 nm for EPA content and 272 nm for DHA content. DHA and EPA content was measured by inserting the obtained absorbance of DHA and EPA with the linear regression line equation from the standard curve. The standard curve was carried out using DHA and EPA standard solutions with a series concentration of 0 ppm, 1200 ppm, 1400 ppm, 1600 ppm, 1800 ppm, and 2000 ppm, respectively. The DHA and EPA content could be obtained by calculating these absorbances in the linear regression lines.

Microencapsulation of Dunaliella salina by spray drying method

Microencapsulation was measured by modifying the method described by *da Silva et al*. (2019) [23]. In this research, five *Dunaliella salina* microcapsule formulas were made with maltodextrin and Arabic gum as coating materials at different ratios. These formulations aimed to find a formula that could better wrap the *Dunaliella salina* powder. The compositions of microcapsule formulas can be seen in Table 2.

Table 2: Compositions of Dunaliella salina microcapsule formulas

Materials	Formulas (F)					
	F0	F1	F2	F3	F4	F5
Dunaliella salina powder (g)	5	5	5	5	5	5
Maltodextrin (g)	-	5	-	2.5	3.75	1.25
Arabic gum (g)	-	-	5	2.5	1.25	3.75
Aquadest (mL)	500	500	500	500	500	500

Table 3: Sago bagea cookies formulas with addition of dunaliella salina microcapsule (DSM)

Ingredients	Treatments			
	Control	DSM 10%	DSM 20%	DSM 30%
Sugar (g)	120	120	120	120
Egg (g)	50	50	50	50
Cooking oil (mL)	50	50	50	50
Cashew nut (g)	75	75	75	75
Salt (g)	1	1	1	1
Sago flour (g)	100	90	80	70
DSM (*Dunaliella salina* microcapsule) (g)	-	10	20	30

The compositions of the formulas were taken from previous research of *da Silva et al*. (2019) [23] with some modifications by using the *Dunaliella salina* powder to combined wall materials (maltodextrin and Arabic gum) with a ratio of 1:1 (w/w) and for the ratio of the combined wall materials were taken from the reference of *Antonio-Gómez et al*. (2021) [24] with slight modifications with the ratio of maltodextrin : Arabic gum (100%:0%, 0%:100%, 50%:50%, 75%:25%, and 25%:75% (w/w)).

First, all the materials (*Dunaliella salina* powder, maltodextrin, and Arabic gum) were put in each beaker glass labeled F0 to F5. Briefly, all of the formulas were dissolved in 500 mL of aquadest. Then the mixtures were homogenized using a shaker for 30 minutes at 250 rpm until a stable emulsion was formed. Then the formulas were inserted into the spray dryer immediately at flow rates of 0.5 L/h and atomization pressure at 2 bars with inlet and outlet temperatures of 160ºC and 60ºC, respectively. The formed microcapsules will be named DSM (*Dunaliella salina* microcapsule). Each formula was put in the glass vials and stored in a refrigerator with a temperature of 5ºC for further analysis.

Microcapsule morphology analysis

Microcapsules that have been formed through the spray drying process were then analyzed for their morphologies using a Scanning Electron Microscope (SEM) at 15 kV with a magnification of 10,000 times and 1,300 times to compare and determine the compositions of the well-coated microcapsules among the five formulas that have been formed. The well-coated formula will be used as an ingredient to fortify the sago bagea cookies.

Formulation of sago bagea cookies enriched by Dunaliella salina microcapsule

Sago bagea cookie formulations were measured by modifying the method described in *Hasriani et al.* (2018) [19]. The microcapsule used in this production was the formula with the best morphology that had been analyzed by SEM before. *Dunaliella salina* Microcapsule (DSM) addition uses the percentage of the microcapsule weight (w/w). The proportions of materials used to produce sago bagea cookies can be seen in Table 3.

The formulation compounds in Table 3 are selected based on the reference of *Hasriani et al.* (2018) [19] with a slight modification. The concentrations of 10%, 20%, and 30% were chosen in this research based on the previous research of *Christanti* (2013), which showed that substituting microalgae biomass up to 40% (w/w) and over in cookies fortification will decrease the cookies' physical

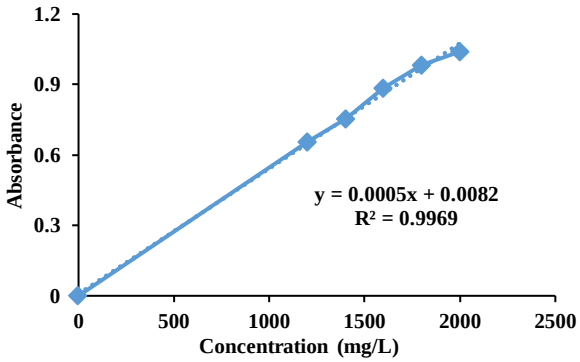

Fig. 1: EPA standard curve on wavelength of 310 nm

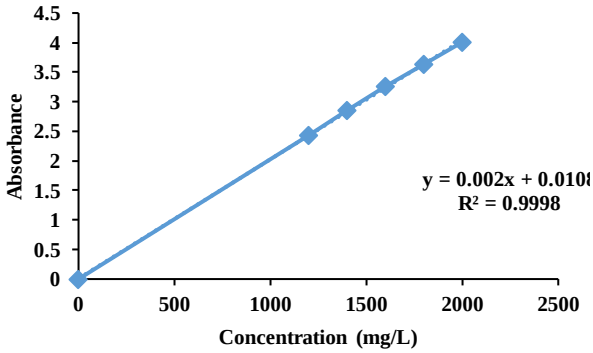

Fig. 2: DHA standard curve on wavelength of 272

appearance in color and its hardness. Moreover, the sensory analysis of the cookies will gradually decrease in taste due to the increasing amount of the microalgae substituted. On the other hand, the addition of microalgae with a concentration ranging from 10 grams – 30 grams is still accepted by panelists based on sensory evaluations [25].

Sago bagea cookies were prepared by roasting the cashew nuts and mashing them using a blender. Then the ingredients were mixed in order by mixing eggs and the sugar first, then adding mashed cashew nut, salt, and cooking oil until the batter was homogeneous. Next, the sago flour and the *Dunaliella salina* microcapsule were added to the batter gradually until the batter was getting soft. The batter was then set by giving space per cookie dough on a baking tray that had already been greased with butter. The final step was baking the cookie batter in the oven at a temperature of 150ºC for 30 minutes.

Sensory evaluation and statistical analysis

Sensory evaluation was used to determine the panelists' acceptance level of bagea cookies. The preference test of sago bagea cookies is carried out by using a scale of 1 (really dislike), 2 (dislike), 3 (neutral), 4 (like), and 5 (really like). In this research, the organoleptic test involved 25 untrained sensory panelists. The parameter assessed in this research includes a test of preference for color, taste, odor, and texture of sago bagea cookies.

The sensory evaluation result will be analyzed statistically with univariate statistics in the form of a descriptive test to determine the data's frequency and percentage. Meanwhile, the differences in data between groups were tested with the Kruskal-Wallis test.

Lipid extraction, DHA/EPA analysis, and proximate analysis

Lipid extraction and DHA/EPA analysis were measured by modifying the method described in Aulia (2016) [22]. The sample (sago bagea cookies) was weighed as much as 1 g. The sample was added to the Erlenmeyer flask, and then n-hexane was added with a volume ratio of 50 mL. The formed mixture was sonicated by ultrasonic cleaner for 1 hour at a temperature of 30ºC. The obtained lipid extract will be analyzed using a spectrophotometer Uv-Vis to investigate the DHA and EPA content (the result will be obtained in mg/L unit). The obtained DHA/EPA content will be converted into the unit of mg/g. The conversion of the unit was calculated by using the following formula:

$$\text{DHA/EPA content (mg/g)} = \frac{\text{DHA/EPA content (mg/L) x n-hexane volume used (L)}}{\text{weighed sago bagea cookies (g)}}$$

Official methods of analysis (AOAC, 2005) were used to determine moisture (AOAC 930.15), ash (AOAC 942.05), fat (AOAC 920.39), protein (AOAC 984.13), and crude fiber (AOAC 962.09) content of sago bagea cookies [26]. Carbohydrate content was calculated by difference using the following formula [27]:

$$\%Carbohydrate = (100\% - \%moisture + \%ash + \%fat + \%protein)$$

Furthermore, for total energy (per 100 grams) was calculated by using the following formula [27]:

$$Total\ energy\ per\ 100\ gram = ((9kcal\ x\ \%fat) + (4kcal\ x\ \%protein) + (4kcal\ x\ \%carbohydrate))$$

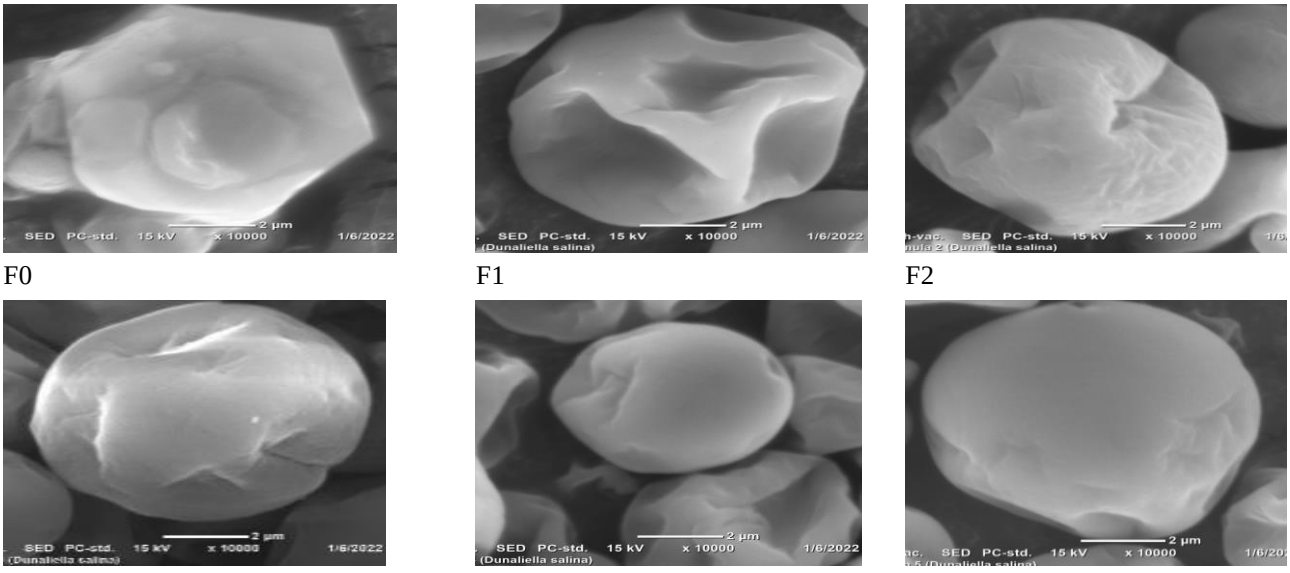

Fig. 3: Dunaliella salina microcapsules (DSM) morphologies with magnification of 10,000 times

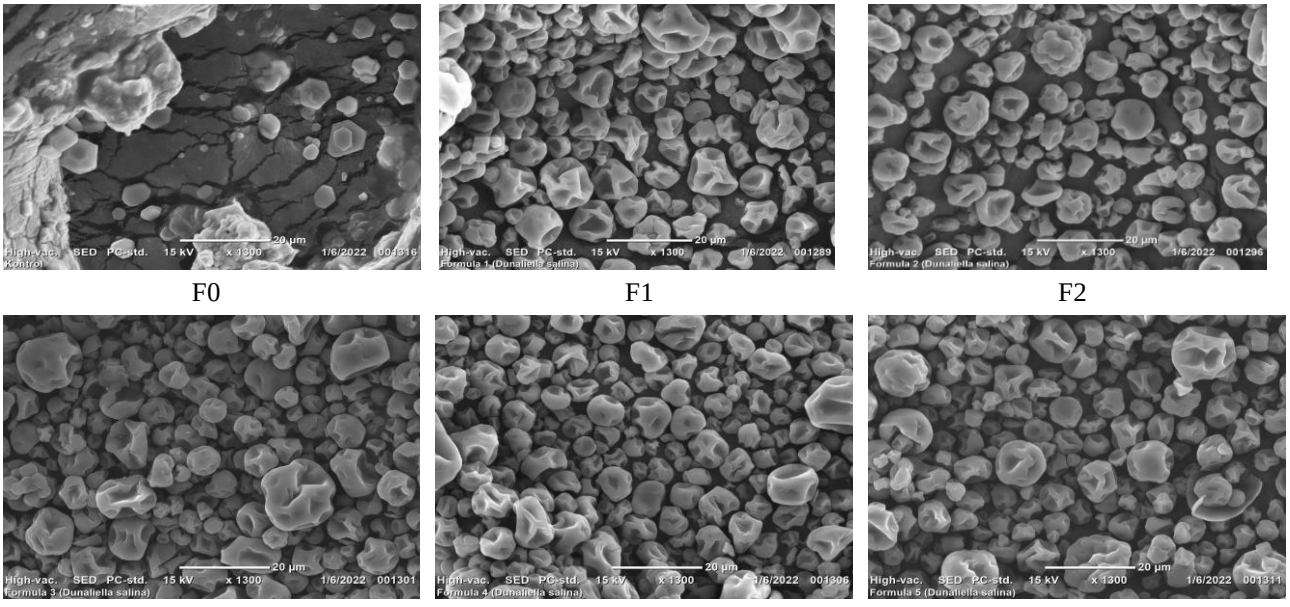

Fig. 4: Dunaliella salina microcapsules (DSM) morphologies with the magnification of 1,300 times

RESULTS AND DISCUSSION

DHA and EPA line regression

The results of EPA and DHA standard curve were shown in Fig. 1 and Fig. 2.

The linear regression equations derived from these two Fig.s were y = 0.0005x + 0.0082 for EPA and y = 0.002x + 0.0108 for DHA. These linear regression lines will be used for measuring the DHA and EPA contents of the sago bagea cookies by inserting the DHA and EPA absorbances (y) to get the concentration (x). The concentrations were calculated by using the following formula:

$$y = ax + b$$

$$x = \frac{y-b}{a}$$

Dunaliella salina microcapsules morphologies

The morphologies of *Dunaliella salina* microcapsules

can be seen in Fig. 3 for a magnification of 10,000 times and in Fig. 4 for a magnification of 1,300 times

SEM images were used to analyze and compare the sizes and morphologies of the produced Dunaliella Salina Microcapsule (DSM) formulas. The analysis was based on previous research by *Castejón et al*. (2021), who stated that by analyzing the morphology of microcapsules, the characteristics that affect the protection quality of the active ingredient of the produced microcapsules, such as concave shapes, wrinkled shapes, and aggregations could be observed and could be used as data to determine a microcapsule that provides better protection of the active ingredient based on the formed microcapsule shape [28]. Moreover, *Khazaei et al*. (2014) described a similar statement that SEM analysis could be used to observe the surface of the produced microcapsules and the desirable microcapsule could be determined by its surface because the smoother the surface, the better it could provide stability, protection, and control over the release of the encapsulated material [29].

The results of the SEM analysis on the formulas showed morphologies with shapes close to perfect globular shapes with different sizes (diameter of the microcapsule). According to *Botrel et al.* (2014) [30], the microencapsulation technique through spray drying will generally produce spherical products. The size of produced microcapsules obtained in consecutive are ranged from 14.57-21.16 μm (F0), 15.90-23.38 μm (F1), 22.45-32.33 μm (F2), 17.08-26.67 μm (F3), 16.75-24.50 μm (F4), and 18.77-28.68 μm (F5). The produced sizes of the microcapsules followed the result reported by *Nedovic et al*. (2011), who stated that the size of microcapsules obtained by the spray drying method ranged up to 50 μm [31].

F0 microcapsule in this study is the control of microcapsule formula (without the addition of wall material). Figs 3 and 4 show that the F0 had an irregular shape, and some particles were agglomerated. The result of F0 following research done by *Curi-Borda et al.* (2019) that stated an uncoated spray-dried microcapsule would be agglomerated into some aggregates [32].

On the other hand, the microcapsules produced using wall materials had a spherical shape that collapsed at F1 and F2, while F3, F4, and F5 had a wrinkled round shape. A ballooning event causes the deflated and wrinkled shape of the product. According to *Juniawati et al.* (2019) [33], ballooning is an event where the microcapsule particles bubble due to the formation of water vapor in the microcapsule structure during the spray drying process. When the capsule walls are not strong enough to withstand the pressure inside the microcapsule particles, the walls will break, and the particles will deflate. This deflating condition can cause a decrease in the efficiency of the microcapsules.

Ballooning in microcapsules can occur due to several factors, one of which is the high inlet temperature during spray drying. In formulas F3, F4, and F5, the morphology of the microcapsules tends to be round and looks better than F1 and F2. The F1 microcapsule appeared to be deflated because of F1 microcapsule is based on maltodextrin coating that can experience ballooning on the capsule walls. Ballooning in F1 microcapsules was caused by the maltodextrin coating material, which was unsuitable for the inlet temperature conditions of the spray dryer. Maltodextrin can form a microcapsule wall, but the resulting wall is less intense, making it easy to crack. This result followed the research of *Huda* (2020), which described crack or leak appearing on the surface of a spray-dried microcapsule that used a sole coating material such as maltodextrin due to its lack of ability to withstand the pressure of water that evaporated in the microcapsules during the drying process and resulted in a deflated microcapsule [34].

F2 microcapsules using Arabic gum as the sole coating material also experienced ballooning on some of the walls of the microcapsules. As can be seen in Fig. 4, there are deflated particles. These deflated particles indicate that Arabic gum coating material is unsuitable for the inlet temperature conditions of the spray dryer during the drying process. This result was following the research of *Khasanah et al.* (2015), who stated that Arabic gum does not have an excellent ability to emulsify because Arabic gum only has a protein content of up to 2%. Because of that, some particles in F2 are deflated [35].

The well-coated microcapsule parameters were analyzed based on the minor wrinkles in the particles of the produced microcapsule. A particle with slight wrinkles indicates that the active ingredients are still well-encapsulated [35-36]. Based on this statement, the active ingredients in F3, F4, and F5 are still well-encapsulated. If seen in Fig. 4, it can be seen visually that some of the

Table 4: Sensory evaluation of sago bagea cookies formulas

Formula	Color	Taste	Odor	Texture	Acceptability
Control	3.80 ± 0.86^{a}	4.36 ± 0.81^{a}	3.88 ± 0.72^{a}	4.04 ± 0.67^{a}	4.02 ± 0.53^{a}
DSM 10%	3.52 ± 0.87^{a}	4.28 ± 0.73^{a}	3.80 ± 0.95^{a}	4.16 ± 0.98^{a}	3.94 ± 0.71^{a}
DSM 20%	3.56 ± 0.82^{a}	4.08 ± 0.90^{a}	4.04 ± 1.02^{a}	3.76 ± 1.01^{ab}	3.86 ± 0.70^{a}
DSM 30%	3.64 ± 0.75^{a}	3.64 ± 0.75^{b}	3.52 ± 0.96^{a}	3.40 ± 0.95^{b}	3.55 ± 0.62^{a}

Datas reported are mean ± standard deviation of 25 panelist. Means with different superscript letters within the same column differ significantly ($p < 0.05$)

Control DSM 10% DSM 20% DSM 30%

Fig. 5: Appearance of sago bagea cookies

microcapsules in F4 had microcapsules accumulation in their morphology. According to *Jelita* (2019) [37], the accumulation of microcapsules can cause microcapsules to agglomerate.

Fig. 4 shows that the F5 microcapsule had a clump in some of the microcapsules with a large particle size. This phenomenon is due to the use of higher concentrations of Arabic gum. Arabic gum has a high viscosity, so it can increase the water content in the formed microcapsules, which has an opportunity to cause the microcapsules to clump. According to *Susianti et al*. (2020) [38], the higher the use of Arabic gum in an ingredient, the higher the water content in that material. Thus it affects the particle size to be larger. Microcapsules can be clumped if the water content is high [39-40]. This case is appropriate to the research of *Ho et al*. (2015), who experienced clumping or agglomeration due to the presence of big particles in the encapsulated Sim (*Rhodomyrtus tomentosa*) juice [39].

Kadarisman and Nurhasanah (2020) [41] stated that agglomeration in a material could affect its physical and chemical properties. Agglomeration can cause microcapsules to lose their stability; therefore, F4 and F5 microcapsules are unsuitable for making sago bagea cookies. Based on this statement, the F3 microcapsule with 50% maltodextrin and 50% Arabic gum ratio is the well-coated formula because it has the slightest wrinkle on its particle, and no agglomeration occurred. This finding was in accordance with research done by *Baygan et al.* (2022) about encapsulated *Ziziphora clinopodioides* essential oil, who stated that the suitable ratio for encapsulation was a combination of wall materials, including maltodextrin and gum Arabic at the ratio of 1:1 [42]. Then, F3 will be used as fortification materials to manufacture sago bagea cookies.

Appearances and sensory characteristics of sago bagea cookies

The appearance of the produced sago bagea cookies formulas are shown in Fig. 5.

As shown in Fig. 5, the addition of *Dunaliella salina* microcapsule affected the appearance of the color of the produced sago bagea cookies. The addition of the microcapsule changed the color of the cookies to greenish. The increasing microcapsule substitution on the formula will make the sago bagea cookies darker. The chlorophyll content in the *Dunaliella salina* microcapsule caused the discoloration of these formulas.

The manufactured sago bagea cookies formulas will be given to panelists for sensory evaluation to determine their acceptance levels of bagea cookies**,** and the most accepted cookies formula will be analyzed for its nutritional compositions. The result of the sensory evaluation of sago bagea cookies are presented in Table 4.

Based on the analysis result, the *Dunaliella salina* microcapsule addition did not affect the panelist acceptability of sago bagea cookies in terms of color and odor attributes. However, it significantly affected taste and texture ($p<0.05$).

Table 5: DHA/EPA content and nutritionals value of sago bagea cookies

Composition	Control	DSM 10%
DHA (mg/g)	4.705	7.305
EPA (mg/g)	15.18	20.18
Moisture (%)	2.52	1.80
Ash (%)	0.71	0.77
Fat (%)	13.84	15.56
Protein (%)	2.72	3.89
Crude fiber (%)	1.33	1.34
Carbohydrate (%)	80.21	77.98
Total energy (kcal/100g)	456.28	467.16

As shown in Table 4, the increasing amount of microcapsules used in the cookie dough will lower the panelist acceptance level in taste and texture parameters. The higher the substitution of *Dunaliella salina* microcapsule in the sago bagea dough, the lower the acceptance level of the panelist in the parameters of taste and texture. The preference level of the panelists was decreasing due to the hard texture and the bitter taste of the sago bagea cookies formula. The addition of the microcapsule in the cookie dough will make the produced cookies' texture harder. The bitter taste was caused by some active compounds in the *Dunaliella salina* microcapsule, such as alkaloids and tannins [43]. This result was in accordance with previous research by *Christanti* (2013), who experienced decreasing acceptance of cookies in taste and texture along with the increasing ratio of microalgae used [25].

Based on the sensory evaluation of the assessed parameter that had been analyzed on the Kruskal Wallis test in Table 4, it could be concluded that DSM 10% is the best-preferred formula with the highest mean value of 3.94 near the mean value of the control on 4.02. At the same time, DSM 20% and DSM 30% mean values were 3.86 and 3.55, respectively. The results of the sensory analysis were in accordance with the research of *Christanti* (2013), which also showed that cookies with the addition of 10% microalgae (w/w) were the most accepted cookies by the panelist in terms of overall acceptability [25]. Then, DSM 10% will be further analyzed for its nutritional values and DHA/EPA content.

DHA/EPA content and nutritional value of the produced sago bagea cookies

DHA/EPA content and nutritional value of the produced sago bagea cookies control and DSM 10% are shown in Table 5.

Based on Table 5, it could be known that the addition of *Dunaliella salina* microcapsule affected the nutritional value of the produced sago bagea cookies. The addition of the microcapsule increases the DHA from 4.705 mg/g to 7.305 mg/g. Meanwhile, the EPA increased from 15.18 mg/g to 20.18 mg/g. DSM 10% omega-3 content (DHA+EPA) is 27.485 mg/g or 274.85 mg/100g. This founding result was lower than the DHA+EPA content reported by *Babuskin et al.* (2014), who used freeze-dried *Nannochloropsis oculata* as a source of omega-3 on cookies with DHA+EPA content of 298 mg/100g [44]. This phenomenon might occur due to different drying methods and microalgae types.

The ash content of DSM 10% (0.77%) was slightly higher than the control (0.71%). The ash content in this study is higher than the reported research of Chirstanti (2013), who fortified cookies using *Spirulina platensis* with ash content ranging between 0.03 – 0.07% [25].

The obtained fat content of DSM 10% is also higher than the control, with a DSM 10% fat percentage of 15.56%. These findings had higher fat content than the reported research of *Batista et al.* (2017), with a fat content ratio of 11.5 – 13.1% [45].

In terms of protein, DSM 10% also showed an increase in its content. These findings were following the research of *Batista et al.* (2017), who reported that the cookies substituted by microalgae would increase the protein content of the produced cookies [45].

The total energy of DSM 10% is higher than control (456.28 kcal/100g for control and 467.16 kcal/100g for DSM 10%). The result was higher than the research done by *Batista et al.* (2017), who reported that the

fortified cookies by substituting some microalgae, gave a different result of total energy ranging between 451 – 460 kcal/100g [45].

Regarding the crude fiber obtained, the DSM 10% did not show a significant increase, with a percentage of 1.34%. Compared to a previous study by *Batista et al.* (2019), the DSM 10% crude fiber was lower than *Batista et al*. (2019) reported (ranging from 4.4 – 6.7%) [46].

Regarding the moisture content of the sago bagea cookies, the DSM 10% showed a lower moisture content than the control. The result was in accordance with the research of *Junianto* (2022), which showed a similar decrease in water content along with the addition of microalgae to donut products [47].

Regarding carbohydrate content, DSM 10% had lower content than the control. The decreasing carbohydrate content was also reported by *Batista et al*. (2017) and *Lucas et al*. (2018) when the microalgae biomass was added to cookies and snacks [45, 48]. The decrease in the observed carbohydrate content is related to the removal of sago flour, which is rich in carbohydrates [19].

CONCLUSIONS

This research was conducted to formulate and find the well-coated microcapsule based on its morphology. This research found that microcapsule F3 with the combined wall material of maltodextrin and Arabic gum at the ratio of 1:1 was the well-coated microcapsule due to F3 being the microcapsule having the slightest wrinkle and no agglomeration occurred in its particle than the other formulas. Thus, the F3 was chosen as a material to enrich the sago bagea cookies formula. The formulated cookies in this research consist of four formulas, namely control, DSM 10%, DSM 20%, and DSM 30%, that will be analyzed by sensory analysis to determine the preferred cookies formula. The research results in sensory analysis of the formulated bagea sago cookies showed that the addition of *Dunaliella salina* microcapsule did not affect the panelist acceptability of sago bagea cookies in color and odor attributes but showed a significant effect in taste and texture ($p<0.05$) due to the increasing amount of microcapsule used in the cookies dough. Regarding overall acceptability, DSM10% got the highest mean score of 3.94 ± 0.71, making DSM 10% the most preferred formula. Then, the DSM 10% was further analyzed on its omega-3 (DHA+EPA) and nutritional value. The analysis result showed that DSM 10% has a higher content of DHA (4.705 mg/g to 7.305 mg/g), EPA (15.18 mg/g to 20.18 mg/g), ash (0.71% to 0.77%), fat (13.84% to 15.56%), protein (2.72% to 3.89%), crude fiber (1.33% to 1.34%), and total energy (456.28 kcal/100g to 467.16 kcal/100g) but lower in moisture (2.52% to 1.80%) and carbohydrate (80.21% to 77.98%) than the control.

Abbreviations

w/w	Weight per weight
AOAC	Association of official analytical chemists
F	Formula
DE	Dextrose equivalent
DHA	Docosahexaenoic acid
DSM	*Dunaliella salina* microcapsule
EPA	Eicosapentanoic acid
PUFAs	Polyunsaturated fatty acids
SEM	Scanning electron microscope

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