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Chorella vulgaris and Spirulina Platensis : Concentration of Protein, Docosahexaenoic Acid Chorella (DHA), Eicosapentaenoic Acid (EPA) and Variation Concentration of Maltodextrin via Microencapsulation Method

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Abstract

The research aimed to find concentration of Protein Docosahexaenoic Acid (DHA), Eicosapentaenoic Acid (EPA) and variation concentration of maltodextrin via microencapsulation method from *Chlorella vulgaris* and *Spirulina platensis*. *C.vulgaris* and *S.platensis* were phytoplankton that had high protein content primarily and fatty acid were Docosahexaenoic Acid (DHA), Eicosapentaenoic Acid (EPA). Because of that, they could be addition compound to many commercial products therefore it can be a good nutrition for human body. Analysis method was carried out by the sonication extraction for short and cheap lyses of phytoplankton biomasses; UV/VIS spectrophotometer to determine the concentration of DHA and EPA in from phytoplankton crude extract. The result indicated that concentration of crude protein from *Spirulina platensis* was higher than *Chlorella vulgaris* were 55,98 % and 13,64% respectly; DHA and EPA in *Chlorella vulgaris* were 36.53 and 123.46 mg/g DW. The concentration of DHA and EPA in *Spirulina platensis* was higher than *Chlorella vulgaris* were 72,345 mg/g and 331,07 mg/g DW. While SEM data showed that the quality of the resulting microcapsules for *Chlorella vulgaris* by using maltodextrin as a coating that

was with the addition of 30% of maltodextrin and 15% maltodextrin for *Spirulina platensis.*

Keywords:*Chlorella* vulgaris, *Spirulina platensis*, Protein, Eicosapentaenoic Acid (EPA), Docosahexaenoic Acid (DHA), Microencapsulation

INTRODUCTION

Nutrition was the basic capital development of the child's genetic potential optimally **[1]**, starting from pregnant women, infants, toddlers, adults to the elderly [2], 19.6% of children under five in Indonesia were malnourished, according to Riskesdas data (2010), the number of children under five in Indonesia malnourished reached 13% and 4.9% suffer from malnutrition [3,4].

Foodstuffs were more important in the baby biscuit complementary because this is very important as nutrition in addition to breastmilk. One of these requires complementary foods have high nutritional value. This is the underlying biscuit selected as an alternative product fortified with a rich phytoplankton microencapsulation DHA and EPA because biscuits were one of processed food products was much preferred by children, and not only that, to the elderly was loved. Long shelf life [1,5,6] was also a major influence to fortify by utilizing the nutrient composition of phytoplankton once expected to be the natural dyes (*Spirulina platensis*, *Phycocyanin* and *C.vulgaris* blue pigment, a green pigment) [7].

Microalgae had important rule as eukaryotic microorganism in natural product synthesis. Microalgae species had been used widely in developed countries because the growth was more modest than the higher plants [8]. The number of species of *Chlorella*, *Spirulina* and *Dunaliella* were three types of phytoplankton that have commercial potential that had been used to produce fats, proteins, and pigments [9,10]. Because of protein, DHA and EPA from phytoplankton, they could be a foodstuff to fortification in baby biscuit.

Research [11] that kind of Spirulina had long been used as food for protein and nutrients such as γ-linolenic acid, DHA and EPA [12]. This species is primarily used in the treatment of hyperlipidemia, hypertension, renal failure prevention, fueled the growth of Lactobacillus gut (intestine helps in the absorption of mutagenic) and suppression of blood sugar levels. Phytoplankton *C.vulgaris*, selected as a source of DHA and EPA [12,13]. Meanwhile, according to [14] stated that *C.vulgaris* in protein and fatty acids are high enough even so it has huge potential as a source of DHA and EPA [15].

Therefore, the protein content in the diet and omega-3 fatty acids, especially DHA and EPA were easily oxidized and hydrolyzed, it was necessary to

microencapsulation of the phytoplankton *S.platensis* and *C.vulgaris* to keep the quality of protein, DHA and EPA in this study.

MATERIAL AND METHOD

Pure culture of *Chlorella vulgaris* and *Spirulina platensis* (the cultivation of phytoplankton Jepara Jogyakarta), acetone pa, distilled water, Medium Conwy (FeCl3.6H2O, MnCl2.4H2O, H3BO3; NaEDTA; NaH2PO4. 2H2O; NaNO3; ZnCl2; CoCl2.6H2O; CuSO4.5H2O, (NH4) 6Mo7O24.4H2O); Vitamins (vitaminB1 and B12); Sodium borax, KIO_3, H_2SO_4, Potassium iodide, methanol, potassium hydroxide, HCl, $Na_2S_2O_3.5H_2O$, Na_2SO_4 anhydrous, oxalic acid. Phenolphthalein indicator, the indicator methyl red, 96% ethanol, I_2, starch, potassium ferrosianida, filter paper, aluminum foil, tissue, maltodextrin, n-hexane, 2-propanol, kloroforom, DHA and EPA standards, aluminum foil, paper Whatman number 40 and number 42 Whatman filter paper.

Commonly used glassware, microscopes, haemocytometer (Neubauer-improved), Hand counter, Salinometer, SPNISOSFD oven, digital balance Ohauss NO AP 110, FT-IR Shimadzu 820 1PC, centrifugation, Ultrasonic and UV-VIS spectrophotometer.

METHODS

Culture and preparation S.platensis and C.vulgaris

Materials used in this study consisted of phytoplankton *C.*vulgaris, S.*platensis* and maltodextrin-encapsulated of biomass. The first stage, *C.vulgaris* and *S.platensis* cultured in a room temperature and lighted as well as aeration controlled to achieve optimal growth. Stock *C.vulgaris* and *S.platensis*, cultured in two large container (approximately 10-14 days) with the addition of medium Conwy, vitamins. Harvesting were done by taking the residue phytoplankton.

Protein Analysis* of *C.vulgaris* and *S.platensis

Concentration of protein were measured with Kjeldhal method from phytoplankton *C.vulgaris and S.platensis* crude extract**.**

Lipid Extraction and Analysis of DHA and EPA C.vulgaris* and *S.platensis

As much as 0.1 g of dry biomass put in Erlenmeyer then added n-hexane, 50 mL, then extracted by means of ultrasonic (sonicator) with 40 kHz, then centrifuged to separate the extra biomass with n-hexane. The extract was then analyzed the content of DHA and EPA it by using UV-VIS spectrophotometer.

Biomass Microencapsulated C.vulgaris and S.platensis with Method Freeze Dryer

In this study, 5 microencapsulate formula and one control each to get an efficient formula as a mixture that showed in Table 3 below.

Table 1 : Composition of Microencapsulation Formula

Material	Control	Formula 10% (F1)	Formula 15% (F2)	Formula 20% (F3)	Formula 25% (F4)	Formula 30% (F5)
Wet Biomass of phytoplankton	1 g	1 g	1 g	1 g	1 g	1 g
Maltodextrin	-	0,1 g	0,15 g	0,2 g	0,25 g	0,3 g
Distilled water	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml

Homogeneous material were poured into petri dish and covered with aluminum foil, put in the freezer for 24 hours.. The encapsulation material was analyzed with SEM to view the best morphology of microencapsulation generated.

RESULT AND DISCUSSION

Research had been done on the growth of *Chlorella vulgaris* and *Spirulina platensis* and get the biomass in medium Conway. The culture were in sterile sea water at room temperature 25-29 °C and 28-30 ppt salinity. After 3 days in a small container *C.vulgaris* and *S.platensis* then transferred to the larger medium that has been given medium Conway, where the first day is the phase displacement preconditions.
Analysis of protein from crude extract of phytoplankton *C.vulgaris* and *S.platensis* were done by Kjeldhal method. The data for *C.vulgaris* was 13,64% and *S.platensis* was 55,98 %. The result showed that *S.platensis* was a spesies of phytoplankton had higher protein than *C.vulgari*s.

Harvesting biomass of *S.platensis* and *C.vulgaris* fitoplakton

Biomass of phytoplankton harvesting could be done in stationary phase condition. The stationary phase was the phase where the maximum biomass concentration, microalgae start light and nutrient deficiencies. Harvesting was done by means of centrifugation, the separation techniques using a mix of gravity with a speed of 1,200 rpm, 15 minutes. After separate the solid residue obtained washed using distilled water in the last rinse. This is done to reduce the levels of salt in the phytoplankton biomass and dried by using Freeze dryer to dry. Then the biomass obtained separated for analysis purposes and microencapsulated ingredients.

Figure 1. Biomass of phytoplankton

Protein Analysis* of *C.vulgaris* and *S.platensis

Analysis of protein from crude extract of phytoplankton *C.vulgaris* and *S.platensis* were done by Kjeldhal method. The data for *C.vulgaris* was 13,64% and *S.platensis* was 55,98 %. The result showed that *S.platensis* was a spesies of phytoplankton had higher protein than *C.vulgari*s.

Extraction of lipids and analysis DHA and EPA on C.vulgaris and S. platensis

Sonication extraction method utilizing ultrasonic waves, 42 kHz to accelerate the contact time between the sample and the solvent although at room temperature. The results of lipid extraction could be seen in Figure 2.

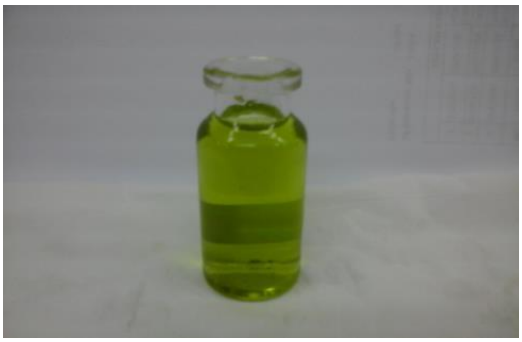

Figure 2: Lipid extraction

Extraction lipid was analyzed using a UV-VIS to determine the levels of DHA and EPA at a particular wavelength. The results of the analysis of DHA and EPA on Chlorella vulgaris could
be seen in Figure 3.

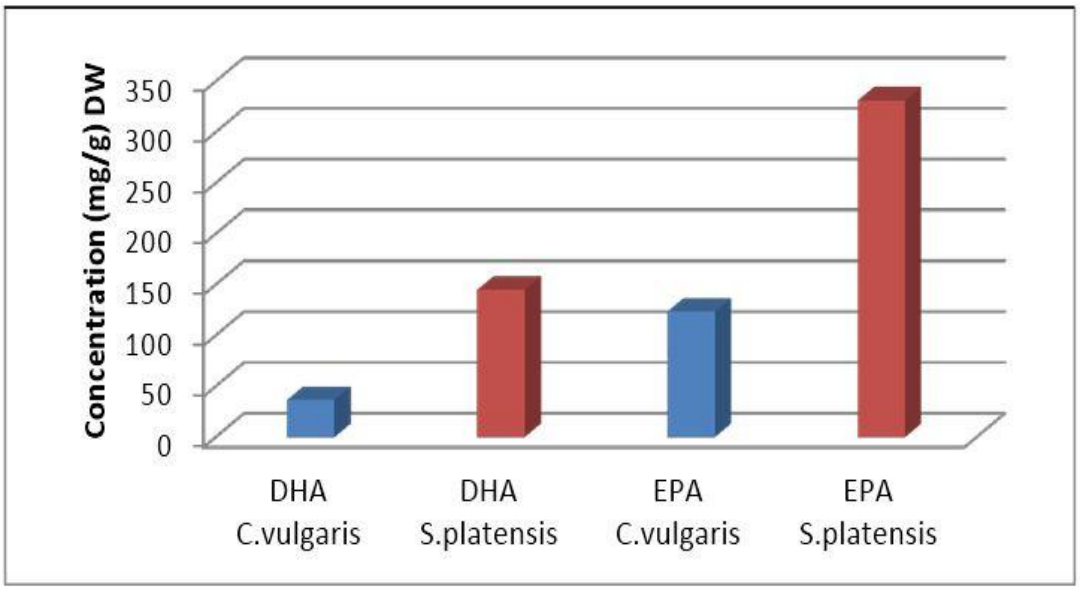

Figure 3: The concentration of DHA and EPA in *C.vulgaris* and *S.platensis*

Concentration of DHA and EPA on *C.vulgaris* and *S.platensis* crude extract using a wavelength of 272 nm to 310 nm for DHA and EPA. The results of the standard solution of DHA and EPA derived calibration curve y = 0.0003x + 0.0108 with a correlation coefficient (r) = 0.9998, whereas for the EPA maximum wavelength of 310 nm is obtained calibration curve equation was y = 0,00053x + 0, 00 813 with a correlation coefficient (r) = 0.99692. The coefficient correlation value of each calibration curve was determinate of levels of DHA and EPA correlation coefficient closed to 1.

The results of the analysis of the content of DHA and EPA on phytoplankton types of *C.vulgaris* shows the value of the EPA on phytoplankton production amounted to 123.46 mg/g DW DHA while production was less than the EPA as many as 36.53 mg/g DW. While the results of analysis content of DHA and EPA on phytoplankton types of *S.platensis* values obtained DHA was of 144.87 mg/g and EPA was 331.07 mg/g DW were presented on an existing chart in Figure 3. The result showed that the concentration DHA and EPA in *S.platensis* were higher than in *C.vulgaris*. Encapsulation process had been done to dry biomass using maltodextrin coating. Maltodextrin chosen because it was a good and inexpensive coating. In addition maltodextrin also hasdadvantages not sweet, and easily soluble in water. Conducting encapsulation composition could be seen in Table 3. After drying, they were observed using Scanning Electron Microscopy (SEM) at 1200 times magnification to see the morphology of the capsule and determine the best coating composition for the manufacture of baby biscuits. Observations using SEM 1200 times magnification could be seen in Figure 4 for *C.vulgaris* and Figure 5 for *S.platensis*.

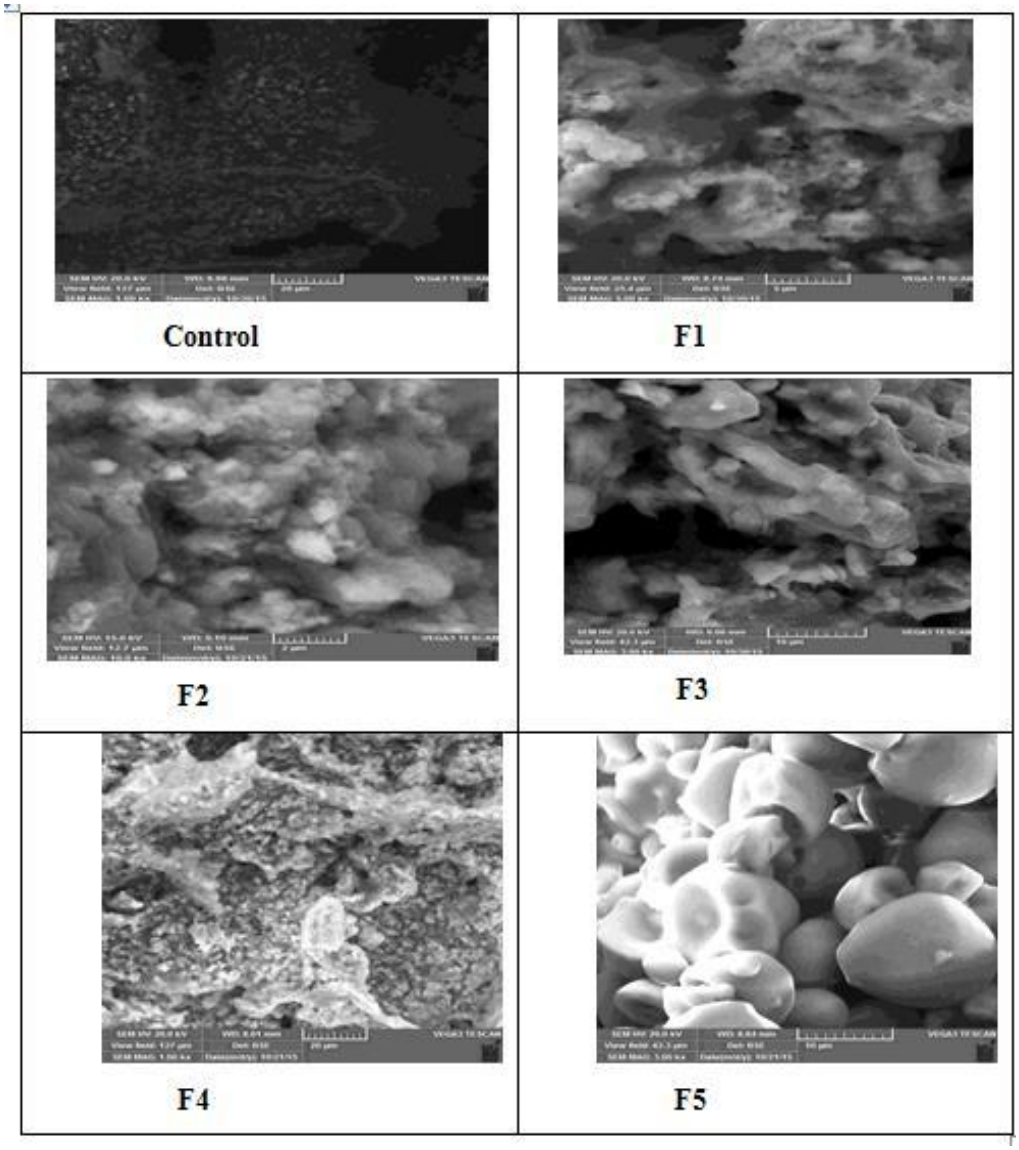

Figure 4. *Chlorella vulgaris* Results of Scanning Electron Microscopy (SEM) at a magnification of 1200 times

Observations using SEM magnification for 1200 times. Visible differences between controls, F1, F2, F3, F4 and F5. Controls; F1, F2, F3 and F4 still looks rough and not well coated. This was because the composition of maltodextrin was insufficient, so the formulas were not coated with the good and mutually cohere. While at F5 showed spheres, this was because *Chlorella vulgaris* was round, the mixture of maltodextrin and water could cover the entire surface of the phytoplankton and microcapsules.

Based on the results by SEM, observation using F5 was the best microencapsulated composition with formulations 1 gram of biomass *C.vulgaris*, 20 mL of water and 0.3 grams of maltodextrin. Coated microcapsules would completely protect the main compound in the phytoplankton. The microcapsules best fortified in the manufacture

of biscuits. While microencapsulation in the way of making the same *S.platensis* with microencapsulation in *C.vulgaris*. The observation of the encapsulation performed on Spirulina platensis analyzed using SEM 1200 times magnification could be seen in Figure 5.

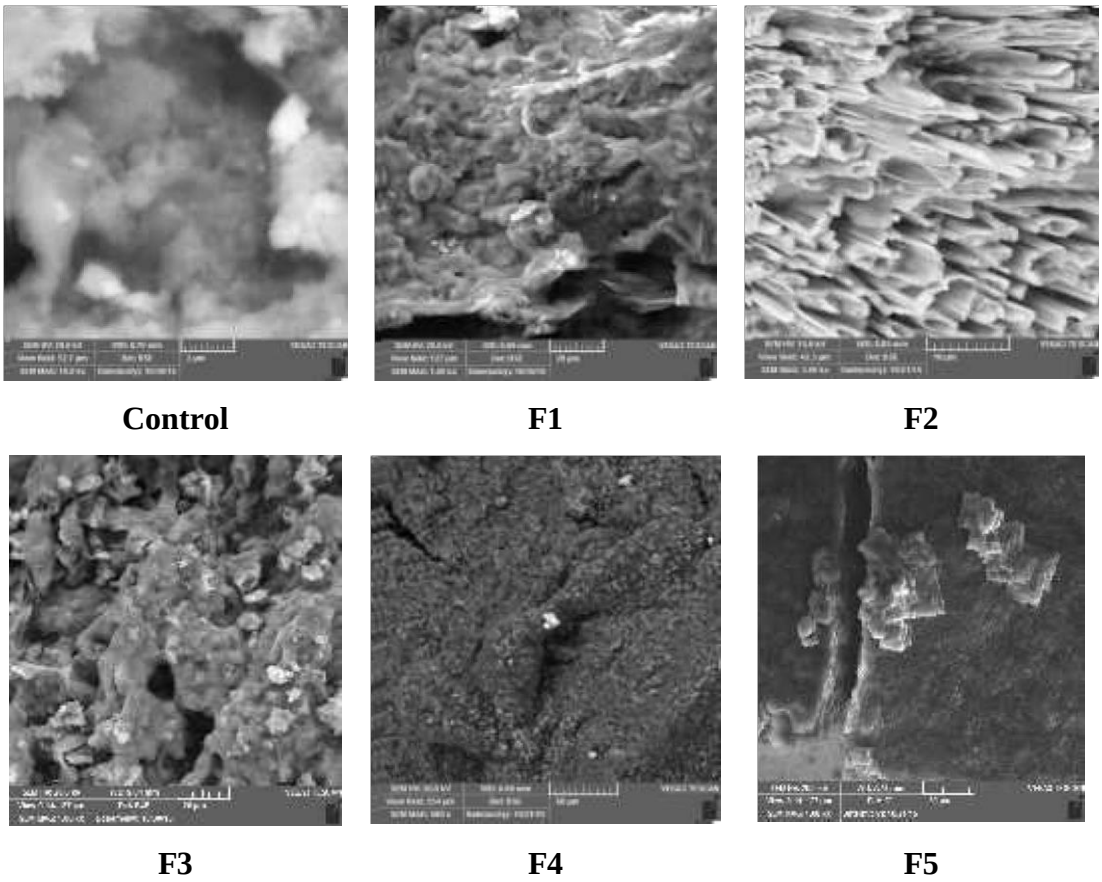

Figure 5. *Spirulina platensis* Results of Scanning Electron Microscopy (SEM) at a magnification of 1200 times

Based on the results of SEM in Figure 5, it appeared that the control and F1 did not form a capsule and structures on the surface look rough, very likely to be maltodextrin to Formula 1 could not be coating perfectly because the concentration was less, so the cells of *S.plantesis* with cells that others coherent. Formula 3, 4 and 5 were not as well formed as because maltodextrin-agglomerated formed. It had happened because the amount of excess made it difficult to form the capsule perfectly. Formula 2 showed that the *S.platensis* microalgae encapsulated well as cell shape was clearly visible encased by maltodextrin. The composisition of fomula could be seen in Table. The perfect microencapsulation shaped contained and made the coating substance would be longer lasting kept.

CONCLUSSION

Concentration of protein in crude extract of *C.vulgaris* was 13,64% and *S.platensis* was 55,98 %. The result showed that *S.platensis* was a spesies of phytoplankton had higher protein than *C.vulgaris*. DHA and EPA in *Chlorella vulgaris* were 36.53 and 123.46 mg /g DW, respectively. The concentration of DHA and EPA in *Spirulina platensis* was higher than *Chlorella vulgaris* neamly 72,345 mg/g dan 331,07 mg/g DW.

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