Nutrient Enrichment of Artemia salina Using the Bioencapsulation Method with Single Cell Protein Extract from Chlorella Vulgaris

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Abstract

Single Cell Protein (SCP) is a biotechnological product designed to enhance biomass or extract proteins and lipids from a given material. One of the methods to utilize single-cell protein is bioencapsulation. Bioencapsulation is a nutrient enrichment technique that involves adding specific substances to natural feed to improve its quality and quantity, thus enhancing its overall nutritional value. The single-cell protein used in this study is Chlorella vulgaris (C. vulgaris), while the bioencapsulation material or observed subject is Artemia salina (A. salina). This study aims to analyze the nutritional content based on the retention or absorption capacity of A. salina using the SCP bioencapsulation method from C. vulgaris and to determine the optimal SCP dosage for bioencapsulation in A. salina. The tested dosages in this study were 100 mg/L, 200 mg/L, 300 mg/L, and 0 mg/L (without SCP administration as a control). The results showed that the SCP retention value in A. salina was significantly different ($P<0.05$) across the tested dosage treatments. The highest retention value was observed at a dosage of 300 mg/L, with the highest retention of soluble protein and fat recorded at 85.65% and 0.27%, respectively. However, the overall results indicate that as the administered SCP dosage increases, the retention or absorption of SCP nutrients in A. salina also increases.

Keywords: *Artemia salina, Bioencapsulation, Chlorella vulgaris, Retention, Single Cell Protein.*

A. INTRODUCTION

Single Cell Protein (SCP) is a biotechnological product aimed at increasing biomass or extracting protein that can be used as a food supplement (Bharti et al., 2014). In addition to protein, SCP also contains lipids in the form of fatty acids, which can reach up to 35.13% (Kurnia et al., 2018). SCP has been successfully developed from various types of microalgae, one of which is Spirulina sp. (Sharma et al., 2019). However, the use of Spirulina sp. as an animal feed supplement is considered less effective due to high production costs and limited availability of raw materials. Therefore, an alternative microalga that can be utilized is Chlorella vulgaris. This microalga is easier to cultivate and can grow on agricultural waste, making its production costs more affordable with sufficient raw material availability.

Research on the use of SCP for the survival of various fish and shrimp larvae has been conducted, including tilapia larvae with a survival rate of 87% (Hussein et al., 2013), zebra fish larvae at 90% (Sisman et al., 2013), and vannamei shrimp larvae at 77.6% (Hamidoghli et al., 2019). However, to date, no information has been found regarding the application of SCP in barramundi larvae. Therefore, the utilization of SCP in barramundi larvae can be carried out using the bioencapsulation method.

Bioencapsulation is a nutritional enrichment method by adding specific substances to natural feed to improve its quality and nutrient content (Sarmudianto et al., 2015). One of the organisms that can be used as a bioencapsulation medium is Artemia salina. This zooplankton has a size that matches the mouth opening of larvae and acts as a non-selective filter feeder, meaning its nutritional content is highly influenced by the type of feed it consumes (Toi et al., 2013). Therefore, feeding A. salina with Single Cell Protein (SCP) at specific doses can serve as a strategy for transferring nutrients from SCP to cultivated fish or shrimp.

Based on this background, this study is designed to analyze the nutrient composition based on the retention or absorption capacity of A. salina using the SCP bioencapsulation method from C. vulgaris and to determine the optimal SCP dosage for A. salina bioencapsulation. The findings of this study are expected to contribute to the development of efficient natural feed products for fish and shrimp.

B. MATERIALS AND METHODS

1. Preparation and Production of Single Cell Protein (Chlorella vulgaris)

The microalga Chlorella vulgaris or C. vulgaris was selected as the SCP material in this study. Its ability to be produced in large quantities and its availability were the primary factors in its selection, in addition to its high nutritional content. The C. vulgaris starter culture was obtained from the Brackish Water Aquaculture Center in Takalar and then mass-cultured independently. Substrate used for SCP production was wastewater from tofu processing industries, which was collected from Karanganyar Village, Mamajang District, Makassar City. The production process of Single Cell Protein (SCP) consists of several stages, as outlined below:

a. Substrate Preparation

The medium used for the preparation of the single cell protein substrate is a mixture of seawater and tofu wastewater obtained from a tofu processing industry in the Karang Anyar area, Makassar (Figure 1):

Figure 1. Tofu Production Waste from Karang Anyar (Production House "Pak Slamet")

Before mixing procces, medium was sterilized. Seawater is sterilized at a temperature of 100°C for 15 minutes, while tofu wastewater is sterilized at 70°C for 15 minutes using an autoclave (Putri et al., 2018). After sterilization, both media are mixed in a ratio of 30% tofu wastewater and 70% seawater with a salinity of 41 ppt. The composition of the tofu wastewater mixture as a substrate, according to the study by Putri et al. (2018), is as follows:

Table 1. Comparison of C. vulgaris Composition Using Tofu Wastewater Substrate and Without Tofu Wastewater Substrate as a Culture Medium

Culture Medium	Cell Produced (cells/mL)	Protein total (%)
Without Tofu Wastewater Substrate	17,67 x 10 ± 2,25	31,80% ± 3,10
C. vulgaris Composition Using Tofu Wastewater	42,52 x 106 ± 2,80	52,32% ± 3,31

b. Maintenance of C. vulgaris Culture

The C. vulgaris culture was carried out by adding a starter to the substrate medium at a ratio of 1:3. A total of 3 liters of substrate medium was used. The culture maintenance was conducted for 4 days or until the cell density reached the exponential phase (Prayitno, 2016). Maintenance process was modified by setting the temperature at 21–25°C with strong aeration, adjusting salinity to 30–31 ppt, and maintaining the pH between 7.5 and 8.5 (Figure 4). The dissolved oxygen (DO) level in the medium was regulated at 5–7 mg/L (Putri et al., 2018).

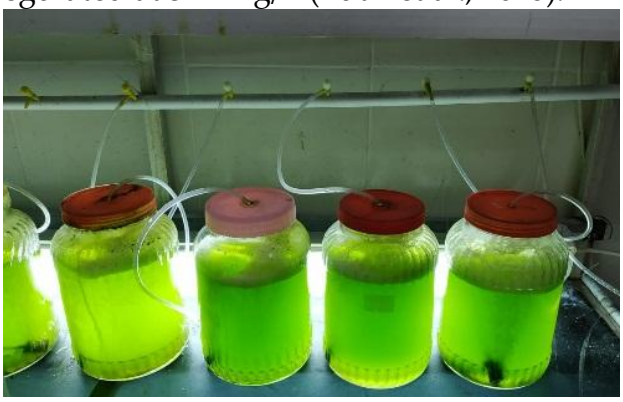


Figure 2. C. vulgaris Culture Process

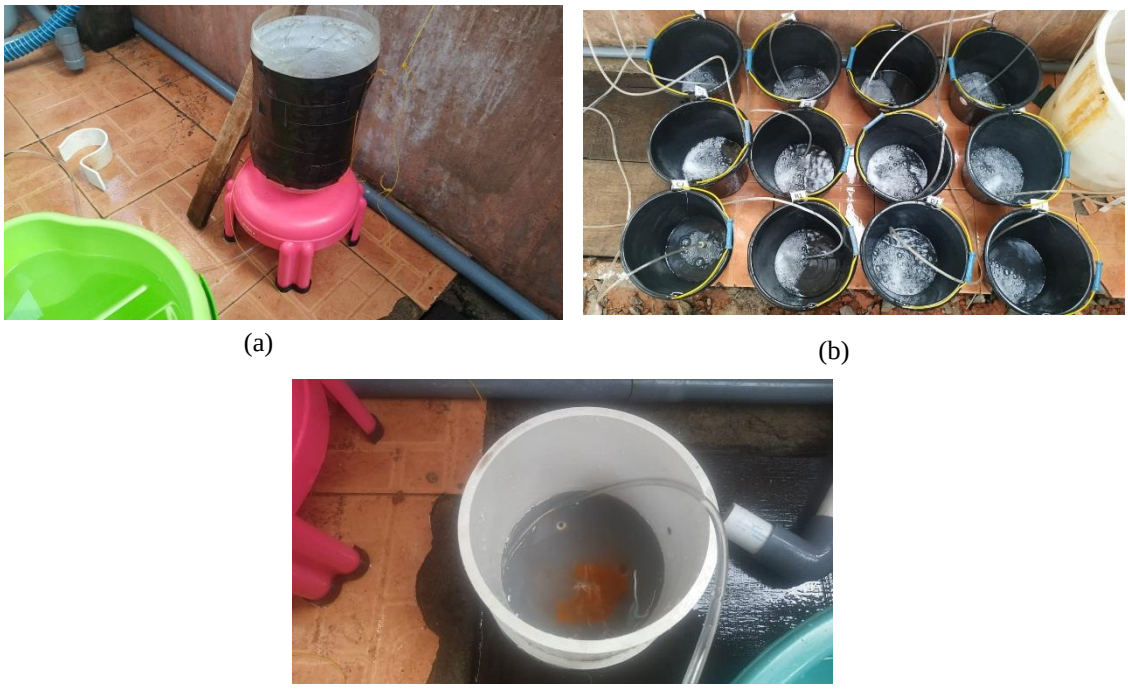
c. Single Cell Protein Extraction

Biomass harvesting was carried out by allowing the SCP culture to settle in a closed container for 24 hours (1 day). Once sedimentation occurred, the precipitate was collected and then heated in an oven to remove moisture and obtain a dry solid form of SCP biomass. The incubator was set at a temperature of 60°C for 35 hours (Darsi et al., 2012). Afterward, the resulting SCP biomass was ground into a fine powder using a mortar.

Figure 3. Processing of C. vulgaris into SCP Products

d. Application of Bioencapsulation in Artemia Salina

The bioencapsulation material used in this study was A. salina nauplii, with the commercial brand Mackay Marine. The bioencapsulation process began with hatching 1 gram of A. salina cysts in seawater with a salinity of 31 ppt under strong aeration (Figure 6a). The Artemia were then harvested after 24 hours. Bioencapsulation of A. salina was conducted at the instar 2 stage using a container filled with 1 liter of seawater with a salinity of 31 ppt (Figure 6b). The population of A. salina was concentrated at a density of approximately 25,000 individuals per liter in each container (Figure 6b). The A. salina culture was supplemented with SCP according to the designated treatment dosage. The bioencapsulation process lasted for 5 hours (Suyanto et al., 2019). After the treatment, A. salina nauplii were harvested using a plankton net (Figure 6c).



(a) (b)

(c)

Figure 4. (a) A. salina culture container. (b) A. salina bioencapsulation container. (c) Harvesting of bioencapsulated A. salina.

e. Treatment and Experimental Design

Based on related studies, the determination of microalgae dosage as an enrichment ingredient in fish/shrimp feed, as conducted by Suyanto et al. (2019) and Pamungkas & Khasani (2006), can serve as a reference. This study utilized a Completely Randomized Design (CRD) consisting of four treatments, each with three replications. The treatments applied were as follows:

A = No Single Cell Protein (SCP) Extract Supplementation (Control)
B = SCP Extract Supplementation at a Dose of 100 mg/L
C = SCP Extract Supplementation at a Dose of 200 mg/L
D = SCP Extract Supplementation at a Dose of 300 mg/L.

2. Research Variables

The research included several variables, as detailed below:

a. Chemical Composition Analysis of SCP Products

The chemical composition analysis included crude protein content, crude fat, nitrogen-free extract (NFE), crude fiber, ash, and moisture content of the produced SCP. The analysis was conducted according to the standard operating procedures (SOPs) used in the Animal Feed Chemistry Laboratory, Faculty of Animal Science, Hasanuddin University.

b. Analysis of Nutrient Retention for Soluble Protein and Fat Content in Bioencapsulated A. salina using

The analysis of soluble protein and fat content in bioencapsulated Artemia was conducted by comparing the retention values obtained from the soluble protein and fat content in Artemia before and after bioencapsulation, as well as the SCP feed provided. The retention of soluble protein and fat content in A. salina was calculated using a modified formula from Simon et al. (2019):

$$REa = 100 \times ((B - A))/R$$

Description:
REa = Nutrient retention in A. salina (%)
A = Nutrient value (soluble protein/fat) in Artemia before bioencapsulation (%)
B = Nutrient value (soluble protein/fat) in Artemia after bioencapsulation (%)
R = Average total nutrient (soluble protein/fat) in the substituted SCP (%).

3. Data Analysis

The test data obtained, including the retention analysis of soluble protein and fat content in bioencapsulated Artemia, were analyzed using the Analysis of Variance (ANOVA) method. If significant effects were found, the results were further analyzed using the W-Tuckey Test. The statistical analysis was performed using the R-Studio software package

C. RESULTS AND DISCUSSION

1. Proximate Analysis of Single Cell Protein

The results of the proximate analysis of SCP extracted from Chlorella vulgaris are presented in Table 2:

Table 2. Proximate Analysis Results of SCP Extracted from Chlorella vulgaris Microalgae

Ingredients	composition (%)
Air	20.86
Protein	8.55
Lemak	0.87
Serat	0.86
BETN	12.82
Abu	76.91

Note: Except for moisture content, all fractions are expressed in dry matter

Based on the data in Table 3, there are differences in the composition of the produced SCP. The ash content in SCP is relatively high, reaching 79.91% per gram of

SCP. In addition to ash, the SCP contains nitrogen-free extract (NFE) at 12.38% and protein at 8.55%, which are the dominant components in the total nutritional content of SCP.

The proximate analysis results show that three main components make up the single-cell protein: ash content, NFE, and protein. The protein content obtained in this study is 8.55% or 0.085 g/L, which is significantly lower than the protein content in single-cell protein derived from other microalgae sources, such as Scenedesmus dimorphus, which can produce a maximum protein content of 0.2 g/L (Wang et al., 2013). However, SCP from Chlorella vulgaris still has a higher protein yield compared to single-cell protein derived from the fungus Penicillium expansum, which was cultured on an orange peel substrate and resulted in a protein content of 9.89% in 0.31 g of biomass, or when converted, it yields 0.03 g/L of protein (Khan & Dahot, 2010).

The relatively high ash content in SCP may be attributed to the ability of C. vulgaris to bind metal ions through its cell walls (Yulita, 2014). The process of metal ion absorption in C. vulgaris occurs through biosorption (passive uptake), where the C. vulgaris cells transfer the bound metal ions from the cell wall to deeper organelles (bioaccumulation/absorption). This mechanism occurs in conjunction with the consumption and accumulation of metal ions for cell growth (Purnamawati et al., 2014). As a result, a higher cell density in C. vulgaris cultures may lead to an increased content of metal ions or other minerals.

Ash content serves as an indicator of essential minerals found in food, which play a crucial role in biological functions (Fendjalang, 2018). However, not all minerals can be absorbed by the body; only certain types are effectively utilized (Goff, 2018). Despite this, the use of microalgae, including C. vulgaris, presents a promising alternative for single-cell protein production. In addition to its high protein content (Saeed et al., 2016), C. vulgaris is known to support animal growth and positively influence organismal physiology (Xie et al., 2017). SCP derived from C. vulgaris can enhance protein content in fish and shrimp, thereby improving the quality of their meat for human consumption.

The potential use of SCP in aquaculture could offer a cleaner alternative to conventional feeds, which may be contaminated with heavy metals or other harmful chemicals. Consequently, fish and shrimp raised on SCP-based feed may be safer for human consumption.

2. Analysis of Soluble Protein Retention in Artemia

The results of the soluble protein retention analysis in Artemia are presented in the following graph:

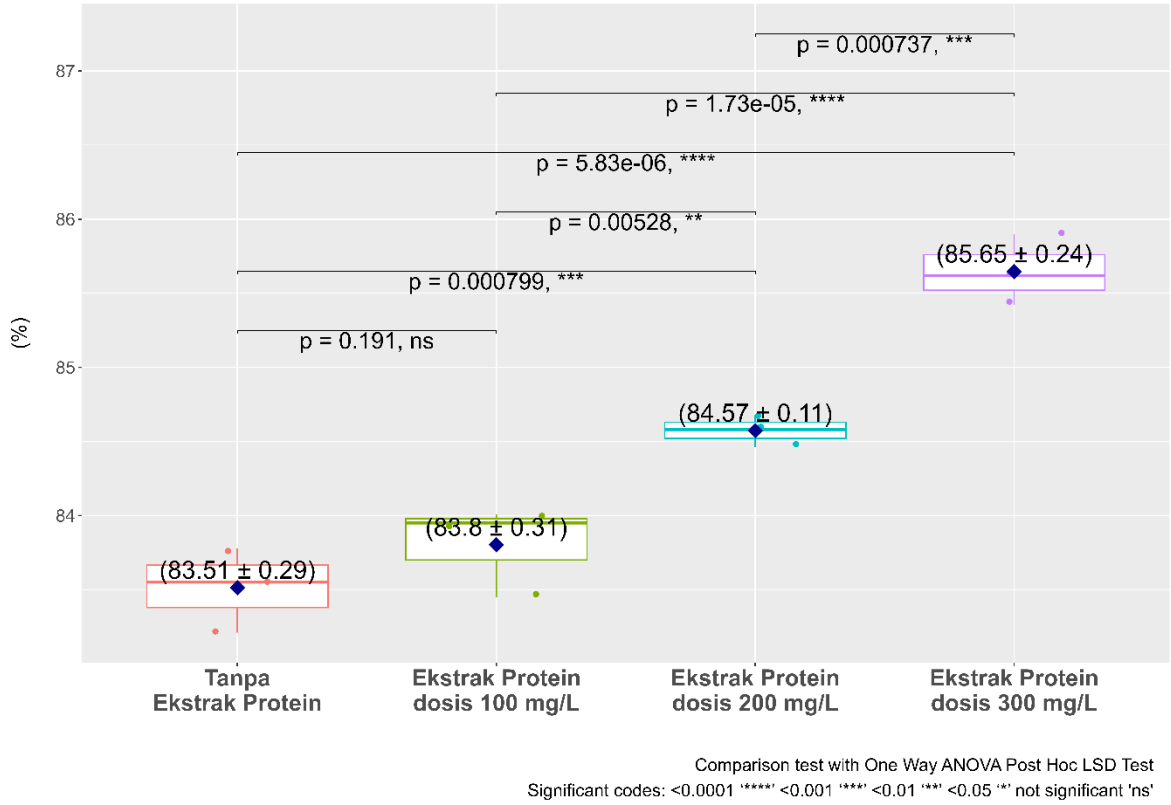


Figure 5. Soluble Protein Retention in A. salina

Based on the data in Figure 5, the bioencapsulation treatment using different doses of SCP in A. salina significantly affected the retention of soluble protein content across the tested doses (P<0.05). The treatment with a 300 mg/L dose showed the highest significance compared to other treatments. The highest fat retention value obtained from the enrichment of A. salina through the bioencapsulation process in this study was 85.65%.

The retention values for soluble protein ranged from 83.51% to 85.65%, indicating an increase in soluble protein content with higher SCP doses during the bioencapsulation process. Soluble protein is an important indicator of protein quality in a material, as this type of protein consists of short-chain oligopeptides that are easily absorbed by an organism's body (Mardhika et al., 2020).

According to related studies analyzing the total protein content of A. salina enriched with C. vulgaris for 6 hours, the protein content obtained was 0.12 mg/L (Lakshmanasenthil et al., 2013). The values obtained in this study showed a significant increase with higher SCP doses, with the best dose being 300 mg/L. However, overall, the use of higher doses than those tested in this study (100 mg/L–300 mg/L) could be recommended to achieve even higher soluble protein values.

3. Analysis of Fat Retention in Artemia

The results of the fat retention analysis in Artemia are presented in the following graph:

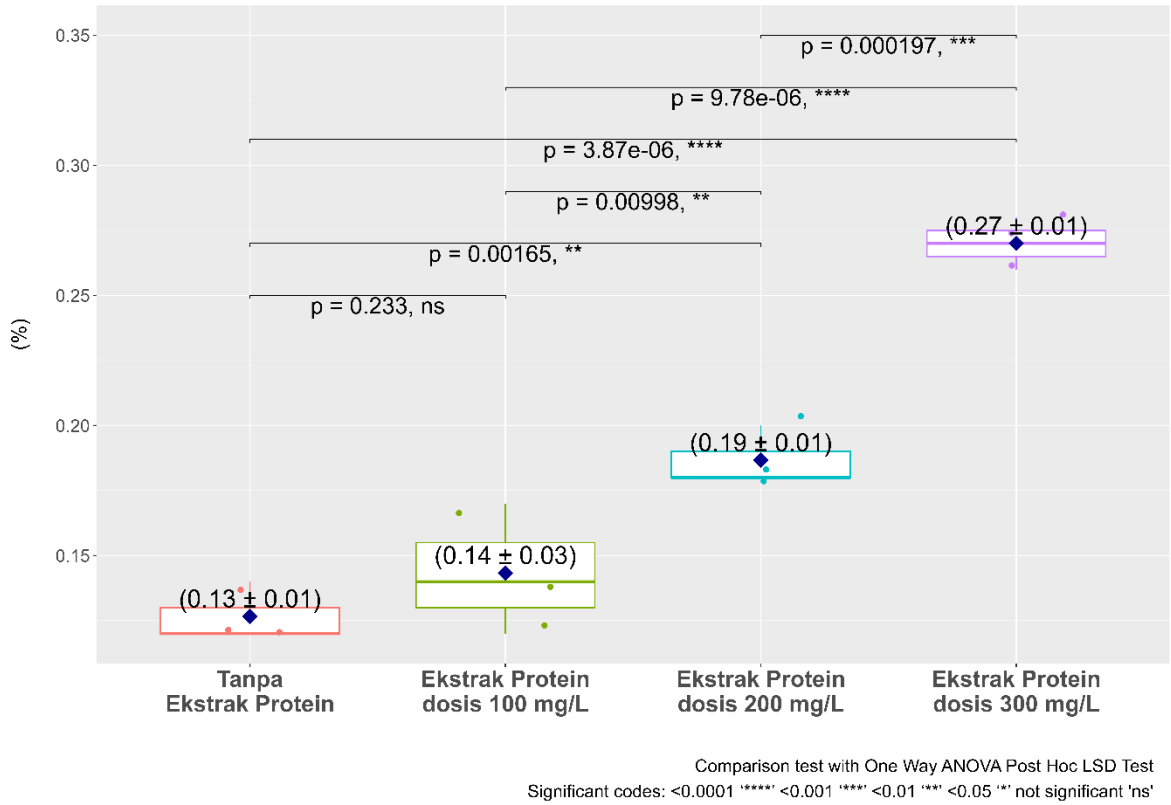


Figure 6. Fat Retention in A. salina

Based on the data in Figure 6, the bioencapsulation treatment using different doses of SCP in A. salina significantly affected the retention of fat content across the tested doses (P<0.05). The treatment with a 300 mg/L dose showed the highest significance compared to other treatments. The highest fat retention value obtained from the enrichment of A. salina through the bioencapsulation process in this study was 0.27%.

The fat retention values absorbed by A. salina through the bioencapsulation process in this study ranged from 0.19% to 0.27% (0.19 mg/L – 0.27 mg/L). According to related research on A. salina enriched with C. vulgaris over a 6-hour period, the fat content obtained was 0.38 mg/L (Lakshmanasenthil et al., 2013). Based on this data, the application of SCP at the tested doses has not yet achieved optimal fat levels. Statistically, the administration of SCP at the tested doses in this study did not produce a maximal effect on the fat content of A. salina. Therefore, higher SCP doses than those used in this study could be recommended to achieve more optimal fat levels.

According to related studies on nutrient retention, specifically fat content in A. salina, when fed with Spirulina and Scenedesmus powder, the fat retention values were 12.70% and 1.01%, respectively (Claus et al., 1979). These results indicate that using C. vulgaris as SCP provides better fat absorption potential compared to other microalgae species. However, in this study, the applied SCP doses were not yet optimal. Therefore, considering the fat retention values obtained, a higher SCP dose from C. vulgaris is recommended. The findings of this study suggest that increasing

the SCP dose leads to higher fat retention, with the recommended dose being 300 mg/L.

D. CONCLUSIONS

A higher SCP dose can enhance the retention of soluble protein and fat content in A. salina. The application of SCP using the bioencapsulation method in A. salina resulted in the best dose at 300 mg/L, achieving the highest retention values for soluble protein and fat at 85.65% and 0.27%, respectively.

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