

# Modification of Microencapsulate Protein Crude Extract Formula from *Chlorella vulgaris* and *Spirulina platensis* for Baby Biscuit

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| TIME SUBMITTED | 12-FEB-2020 01:33PM (UTC+0700)                           | CHARACTER COUNT | 16888 |
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To cite this article: E Mayasari *et al* 2019 *J. Phys.: Conf. Ser.* **1341** 032046

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## Modification of Microencapsulate Protein Crude Extract Formula from *Chlorella vulgaris* and *Spirulina platensis* for Baby Biscuit

E Mayasari<sup>1</sup>, I Raya<sup>1\*</sup>, Z Dwyan<sup>2</sup>, A M Anshar<sup>1</sup> and A R Arif<sup>1</sup>

<sup>1</sup>Department of Chemistry, Hasanuddin University, Makassar, Sulawesi Selatan, Indonesia

<sup>2</sup>Department of Biology, Hasanuddin University, Makassar, Sulawesi Selatan, Indonesia

\* E-mail: [indharya05@gmail.com](mailto:indharya05@gmail.com)

**Abstract.** Modification of microencapsulate protein crude extract from *Chlorella vulgaris* and *Spirulina platensis* were conducted to get the best formula for baby biscuit application to offer a solution to malnutrition. Analysis method were carried out by the ultrasonic extraction for short and cheap lyses of phytoplankton biomasses; proximate test to determine protein concentration from phytoplankton crude extract and Spectroscopy Electron Micrograph (SEM) to show shape of microencapsulated. The result showed for the best microencapsulated composition were Formula 3 for *Chlorella vulgaris* and Formula 4 *Spirulina platensis*, while ratio maltodextrin and crude extract protein were 1.75:1 and 2:1 respectively. High protein concentration crude extract for *Chlorella vulgaris* addition was 47.45% DW, therefore *Spirulina platensis* addition was 78.025 % DW. Baby biscuit *S.platensis* addition was very potential application which average weight of mice in six week was 36.56 gram than *C.vulgaris* and control biscuit. Overall data showed that crude extract protein of *S.platensis* microencapsulate was the best material as ingredients in baby biscuit.

Keywords: *Chlorella vulgaris*, *Spirulina platensis*, Microencapsulation, Maltodextrin

### 1. Introduction

Nutrition and healthy for young generation of millions children in Indonesia were very important factor for country sustainability [1]. In other hand, many problems increased due to malnutrition to the children, one of them was stunting [2]. Children are defined as stunting if their height-for-age is more than two standard deviation below the WHO Child Growth Standard median. Stunting is the impaired growth and development that children experience from poor nutrition, repeated infection and inadequate physicochemical stimulation [3]. In 2017, around 150.8 million (22.2 %) toddler in the world, experience stunting. However, this number has decreased by compared with stunting rates in 2000 of 32.6 %. More than half stunting toddler in the world originating from Asia (55%) and according to data of WHO 2018, the second largest proportion comes from South-east Asia. Indonesia became the third country that big portion of stunting toddler is 36.4 % for twelve years (2005-2017) [4].

This is a challenge for researchers to provide solutions for stunting problems especially in Indonesia. One of the most promising alternatives is the fortification of biscuits using microalgae as a complementary food for breast milk to overcome chronic energy and protein deficiencies [5]. These interventions include feeding supplementary activities (PMT) in pregnant women to address chronic energy and protein deficiencies [2].



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Microalgae is a natural material that holds enormous potential so that it is predicted to become a source of super materials in the future and may wish an enhance the nutritional and functional quality of foods [3-6]. Microalgae applications include the food industry, food supplements, protein sources, biofuels, absorbents, food coloring, the pharmaceutical industry, and animal feed; whether in the form of gel or powder as a basic composition. This is caused by the content of the compounds contained in it [6-9]. Among them are DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid), Astaxanthin (a green pigment), beta-carotene, bioactive pigments, natural d<sub>10</sub>, functional compounds, polysaccharides, antioxidants and extracts from these microalgae [1, 5-10]. Spirulina and Chlorella are types of microalgae that have long been used, especially Spirulina [8]. This happens because Spirulina is easily grown outdoors with conditions of pH 9-11 at high carbonation levels, and temperatures from 35 to 37 °C. In addition, high protein in Spirulina and Chlorella is 55.8-71; 51-58, respectively [8]. Spirulina, one of the most popular microalgae, has been described by the World Health Organization as one of the greatest super foods on earth serving as an example of the potential of microalgae [10-16].

Meanwhile, according to [17] stated that *C.vulgaris* in protein and fatty acids were high enough even so it had huge potential as a source of DHA and EPA [6].

Therefore, the crude extract protein content in microencapsulated by maltodextrin from *S.platensis* and *C.vulgaris* to keep the quality of protein and other nutrition was conducted in this study to application in baby biscuit.

## 2. Material and Methods

### 2.1 Material

*Chlorella vulgaris* and *Spirulina platensis* (from Jepara Jogjakarta, Indonesia), Stock A, Stock B and Stock C to make Conwy medium [17] filter paper, aluminum foil, tissue, maltodextrin aluminum foil, paper Whatman 40 and 42. Material for baby biscuit dough were flour, vegetable oil, eggs, sugar and crude protein extract microencapsulation from phytoplankton.

Glassware, hemocytometer-Neubauer, Handing counter, microscopes, Salinometer, oven, centrifugation, freeze drying method, digital balance NO AP 110 Ohaus, Ultrasonic with 42 kHz at room temperature for 6 hours and SEM Tescan Vega 3SB.

### 2.2. Methods

#### 2.2.1 Culture and preparation *S.platensis* and *C.vulgaris*

Research methods were Culture and preparation *S.platensis* and *C.vulgaris*, extract crude protein from *S.platensis* and *C.vulgaris* with sonication method according to methods in [17].

#### 2.2.2 Microencapsulating Biomass of *C.vulgaris* and *S.platensis* by Freezing Dryer

Microencapsulating formula (F<sub>1</sub>-F<sub>4</sub>) and formula as control (F<sub>0</sub>) that showed in Table 1. Formula was done by freezing drying equipment for 48 hours, and morphological observation were carried out with Scanning Electron Microscope (SEM) instrument as basis for selecting the best microencapsulated formula.

**Table 1.** Composition of Microencapsulation <sup>5</sup>Formula

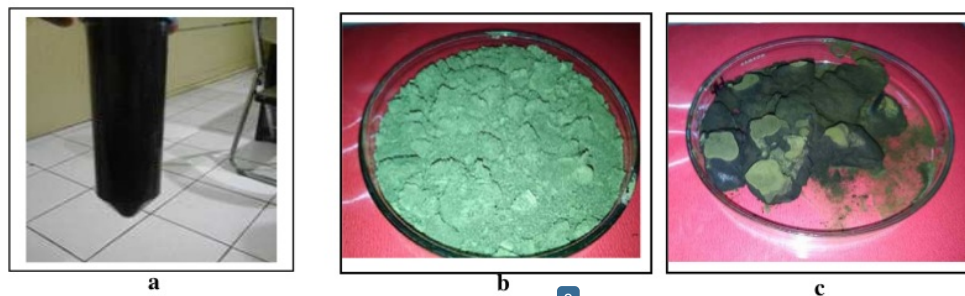
| Material                         | Formula 0<br>(F <sub>0</sub> )<br>Control | Formula 1<br>(F <sub>1</sub> ) | Formula 2<br>(F <sub>2</sub> ) | Formula 3<br>(F <sub>3</sub> ) | Formula 4<br>(F <sub>4</sub> ) |
|----------------------------------|---|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Dry Biomass of phytoplankton (g) | 0,5                                       | 0,5                            | 0,5                            | 0,5                            | 0,5                            |
| Maltodextrin (g)                 | -   | 0,625                          | 0,75                           | 0,875                          | 1                              |
| Distilled water (mL)             | 10  | 10                             | 10                             | 10                             | 10                             |

**2.2.3. Producing Baby Biscuit with *C.vulgaris* and *S.platensis* Microcapsule Fortification Addition**  
Biscuits were made by mixing the basic ingredients of making biscuits (flour, eggs, sugar, water) by adding *S.platensis* and *C.vulgaris* microcapsulated of various additional variations formula, respectively.

**2.2.4. Proximate Analysis of Baby Biscuit *C.vulgaris* and *S.platensis* Fortification Addition**  
It was according to previous studies which have been published in the journal [17].

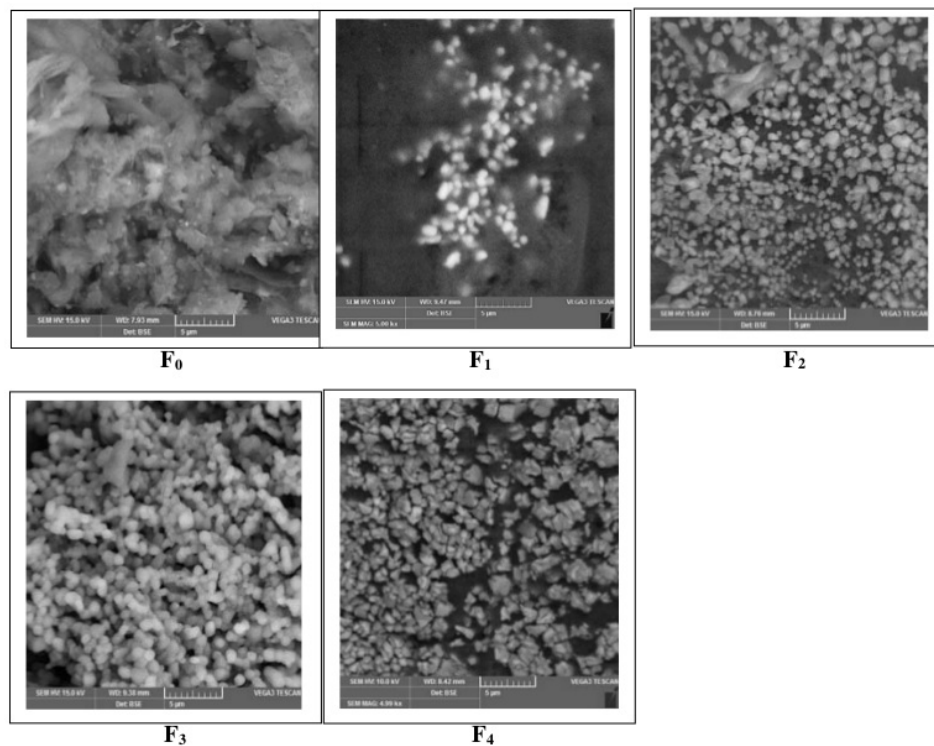
### 3. Result and Discussion

**3.1. Producing biomass of *C.vulgaris* and *S.platensis*** has been carried out in previous studies contained in [17]. Figure 1 showed wet and dry biomass of *C.vulgaris* and *S.platensis*. *S.platensis* was greener than *C.vulgaris*. This was influenced by the pigment contain in it. *S.platensis* was contained natural dye blue-green dye from phycocyanin [14-18].



**Figure 1.** Biomass of phytoplankton. Wet Biomass (a); Dry biomass of *C.vulgaris* (b); Dry biomass of *S.platensis* (c)

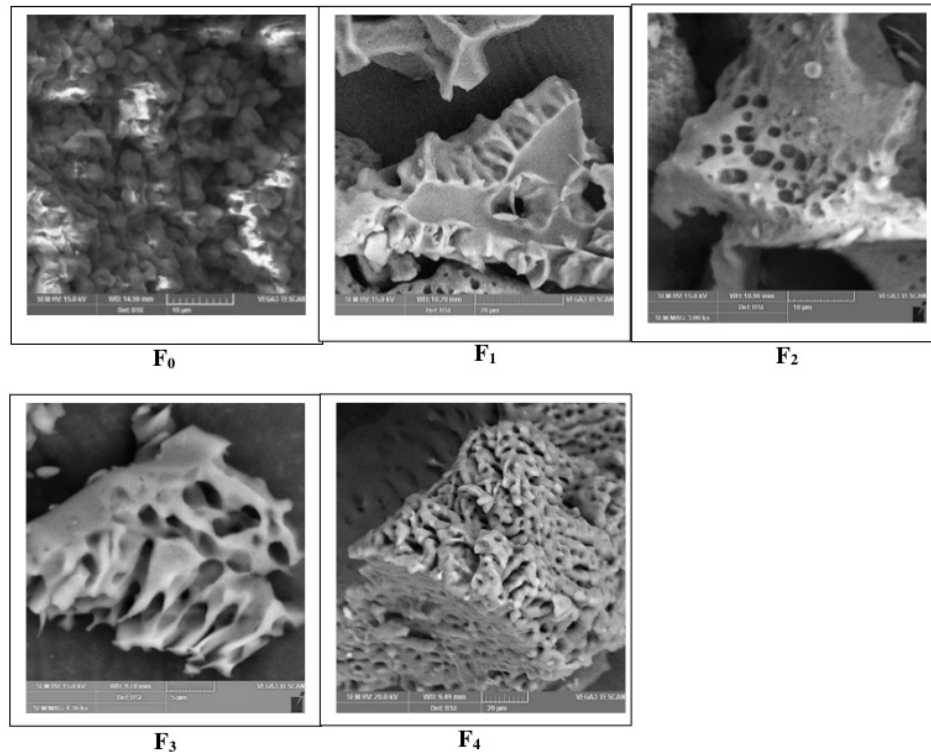
3.2. Analysis of Protein from Crude extract of *C.vulgaris* and *S.platensis* and Microencapsulation method  
It was according to previous studies which have been published in the journal [17].



**Figure 2.** *Chlorella vulgaris* Results of Scanning Electron Microscopy (SEM-1200 times of magnification)

The observation show differences between control as F<sub>0</sub>, and F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub> as *C.vulgaris* microencapsulation. The SEM picture in F<sub>1</sub>, F<sub>2</sub>, and F<sub>4</sub> 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 F<sub>3</sub> 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.

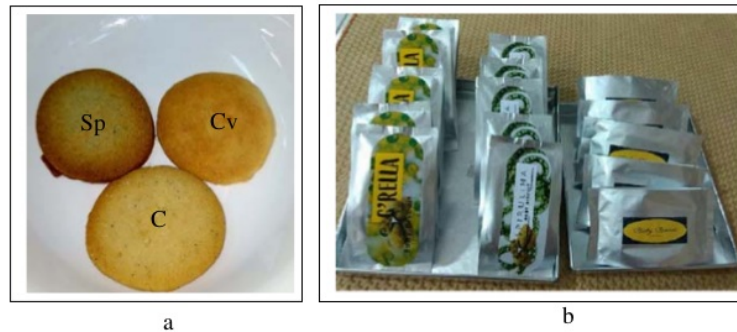
Based on the instrument result, observation using F<sub>3</sub> 0.5 gram dry biomass of *C.vulgaris* was the best microencapsulated composition with The Third formulations were 0.5 gram of biomass *C.vulgaris*, 10 mL of water and 0,875 g grams of maltodextrin (dry biomass 1:1.75 of maltodextrin). The main compound as crude extract for protein in formula 3 would completely protected. Meanwhile, the observations of *S.platensis* formula using SEM 1200 times magnification could be seen in Figure 3.



**Figure 3.** *Spirulina platensis* Results of Scanning Electron Microscopy (SEM)

Based on the results of SEM in Figure 3, Formula 4 (F<sub>4</sub>) was the best formed. The F<sub>4</sub>, contained dry biomass 1:2 of maltodextrin, showed that the *S.platensis* microencapsulated well as cell shape was clearly visible encased by maltodextrin (The composition of formula in Table 1). The best formula microencapsulated would affect the shelf life of food, the coating substance would be longer lasting kept. The formula F<sub>2</sub>, and F<sub>3</sub> of capsule formed and structures appeared maltodextrin-agglomerated. It was shown from the surface look rough and cell encapsulated incompletely. The best formula (F<sub>3</sub> and F<sub>4</sub>) used to make baby biscuit as fortification baby biscuit with *C.vulgaris* and *S.platensis*, respectively.

The Figure 4 confirmed that baby biscuit prototype control (C) without phytoplankton crude extract protein addition, *C.vulgaris* (Cv) and *S.platensis* (Sp) addition. *S.platensis* showed greener than *C.vulgaris* addition, because of *S.platensis* crude extract protein contained more concentrated dyes by the presence of phycocyanin compound and spread evenly in biscuit dough, whereas in crude extract protein of *C.vulgaris* presence astaxanthin did not blend well in it. The prototype that had been made repeatedly to get the best formula of biscuit dough could be seen in figure 4 below.



**Figure 4.** Baby biscuit: Control (C), *C.vulgaris* (Cv), *S.platensis* (Sp) (a); Prototype packing (b)

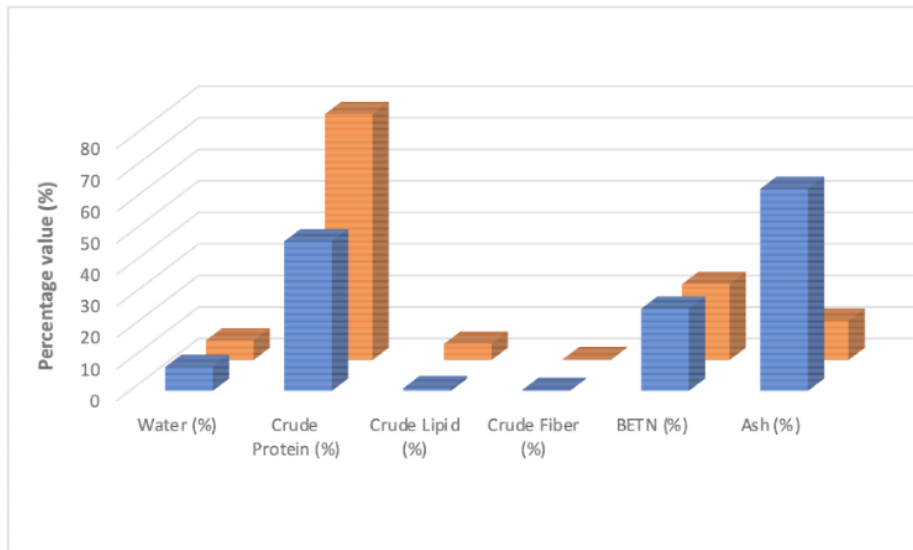
**3.4. Proximate Analysis Result of Baby Biscuit with *C. vulgaris* and *S. platensis* Addition**

Proximate analyzing of baby biscuit had showed in Figure 5. The chart showed the proportion of proximate analyzing data. Crude protein in *S.platensis* biscuit was higher than *C.vulgaris*, 78.025 and 47.45 % DW respectively. It proved that *S.platensis* addition the best for baby biscuit while babies need protein for their growth cell.

The water content of baby biscuits was still slightly above the quality requirements of biscuits based on the Indonesia Standard (SNI 01-2973-1992 standard) which states that the maximum water content found on biscuits was 5%.

**Table 3.** Proximate Analyzing Data of Baby Biscuit *C.vurgaris* dan *S.platensis* Addition

| Phytoplankton              | Water (%) | Crude Protein (%) | Crude Lipid (%) | Crude Fiber (%) | BETN (%) | Ash (%) |
|----------------------------|-----------|-------------------|-----------------|-----------------|----------|---------|
| <i>Chlorella vulgaris</i>  | 7.58      | 47.45             | 0.845           | 0.28            | 26.08    | 64.065  |
| <i>Spirulina platensis</i> | 6.24      | 78.025            | 5.13            | 0.28            | 24.06    | 12.505  |



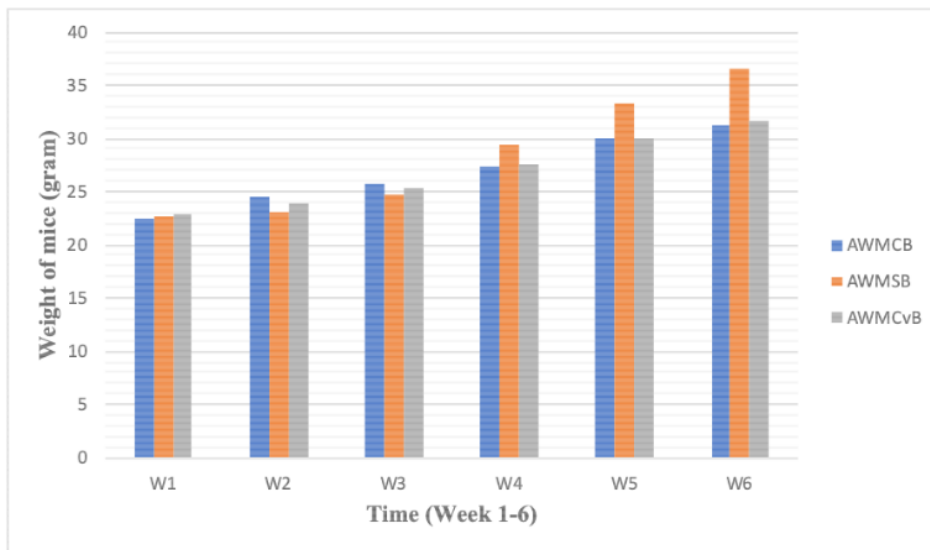
**Figure 5.** Chart of Proximate Analyzing of Baby Biscuit *C.vurgaris* dan *S.platensis* Addition

### 3.5. In-Vivo Testing

The research used mice in In-Vivo test, where mice were given biscuit feed that had been produced and then observed the growth of mice.

**Table 3. In-Vivo Test Data in Six Weeks to Mice Weight**

|               | Weight in Week (1-6) (gram) |       |       |       |       |       |
|---------------|-----------------------------|-------|-------|-------|-------|-------|
|               | W1                          | W2    | W3    | W4    | W5    | W6    |
| <b>AWMCB</b>  | 22.39                       | 24.42 | 25.65 | 27.45 | 29.96 | 31.33 |
| <b>AWMSB</b>  | 22.60                       | 23.09 | 24.75 | 29.33 | 33.22 | 36.56 |
| <b>AWMCvB</b> | 22.81                       | 23.90 | 25.35 | 27.60 | 30.02 | 31.71 |



Note. AWMCB : Average Weight Mice with Control Biscuit  
AWMSB : Average Weight Mice with *S.platensis* Biscuit  
AWMCvB : Average Weight Mice with *C.vulgaris* Biscuit  
W (1-6) : week (1-6)

**Figure 6.** In-Vivo Test Results against Control Biscuits, *C.vulgaris* and *S.platensis*

Based on Figure 6, it was seen that mice fed biscuits without the addition of phytoplankton (*S.platensis* and *C.vulgaris*) grew from the beginning to the 6th week. This was indicated by the increasing weight of mice from the first week to the sixth week (AWMCB). Whereas for mice fed phytoplankton biscuits they were seen that in the second and third weeks there was a slight increase in body weight and were below the average weight of mice with control biscuits. After the third week until the sixth week, the data were seen that the mice's weight gain were so rapid. The decrease in weight and very little increase in body weight were likely caused by mice that are not familiar with the new environment and feed made from biscuits with the addition of phytoplankton. However, after the third week until the sixth week, the increase in body weight were very rapid and even the average body weight of mice fed on phytoplankton biscuits of the type of *S.platensis* (AWMSB) was the best than those of *C.vulgaris* (AWMCvB) and control (AWMCB). The average weight of *S.platensis*, *C.vulgaris* and control biscuits were 36.56; 31.71 and 31.33 gram. The rate of growth in mice fed phytoplankton biscuits were caused by the high content of protein and other nutrients, especially in *S.platensis* biscuits.

## CONCLUSION

Concentration of protein in crude extract of baby biscuit *C.vulgaris* addition was 47.45 and *S.platensis* was 78.025 % DW. The result showed that *S.platensis* was a species of phytoplankton had higher protein than *C.vulgaris*. While the quality of the resulting microcapsules for *Chlorella vulgaris* by using maltodextrin as a coating that is Formula 3 (F<sub>3</sub>) with ratio 1:1.75 of maltodextrin and Formula 4 (F<sub>4</sub>) 1: 2

of maltodextrin for *Spirulina platensis* and the average weight of mice with baby biscuit *S.platensis* addition was 36.56 gram in the last week. For all that baby biscuit *S.platensis* addition was very potential than other.

#### ACKNOWLEDGEMENT

The authors would like to say thank to the Ministry of Research and Technology of Indonesia was supported by Masterplan Percepatan dan Perluasan Pembangunan Ekonomi Indonesia (MP3EI), Hasanuddin University for the facilities and Koperasi Thaybah Ma'had 'Aly al-Wahdah as mitra.

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