Spirulina biscuit formulation with coconut cream substitution and its shelf life estimation

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Abstract. The Spirulina is a cyanobacterium group of microscopic blue-green algae, which contains protein, lipid, carbohydrate, and rich in minerals, vitamins, fibers, as well pigments. Spirulina has been applied for foods, such as biscuits. Biscuit can be modified with the addition of Spirulina and coconut cream to produce rich protein biscuit. The aims of this research were to obtain Spirulina biscuit formula, to compare the chemical composition of Spirulina biscuit with the commercial biscuit, and to estimate the shelf life of the Spirulina biscuit. The formula of Spirulina biscuit consisted of Spirulina powder, mocaf flour, coconut cream, margarine and sugar. The percentage of coconut cream in the biscuit was 7.19, 13.42 and 18.87% per dough. The selected biscuit formula was determined based on a sensory test, as well then determined the chemical composition and shelf life stored at room temperature. Based on the sensory test, the Spirulina biscuit with 7.19% (P1) of coconut cream contains protein (12.09%) higher than a commercial biscuit, while it has lipid (6.01%) lower than that of the commercial biscuit. Estimation of Spirulina biscuit shelf life stored at room temperature was 2.4 months.

Keywords: biscuit; chemical composition; cyanobacteria; microalgae; stability.

1. Introduction

Seafood Less energy protein is one of the problems which can be overcome by increasing protein consumption from various food resource including microalgae, in particularly Spirulina. Spirulina contains high nutrition and active compounds so it is good for food fortification such as biscuits. Spirulina has also been recognized as an excellent source of vitamins and proteins, and to be a source of fine chemicals, renewable fuel and bioactive compounds. Spirulina is a blue-green algae containing carotenoid, essential fatty acids, a vitamin complex B, a vitamin E, Mn, Cu, Mg, Fe, Se, Zn [1]. It also contains protein of 55-70%, lipid of 6-9%, carbohydrate of 15-20%, and mineral, vitamins, fiber, and pigments [2][3]. S. platensis contains unsaturated fatty acids such as palmitoleic acid (Omega 6), oleic acid (Omega 6), linoleic acid (Omega 6), and gamma linolenic acid (Omega 6) [4].

Other than good nutrition, Spirulina is also known to produce several of bioactive compounds (secondary metabolites) with diverse biological activities. Spirulina has fungicidal activity, antihyperglycemic activity [3], antimalarial activity [5], antioxidant and hepatoprotective activity, anticancer activity [6].
The other material for making biscuit is mocaf. Mocaf or modified cassava flour is cassava flour fermented using microorganism. They have protein content and physicochemical better than cassava flour [7]. The use of mocaf in this biscuit was to optimize the usage of local resources.

Beside Spirulina and mocaf, biscuit in this study was made using coconut cream as an ingredient. Coconut cream is a protein obtained from coconut oil processing; it can be used to fortify baby foods, biscuits or substitute of margarine which are useful as flavor [8].

The commercial biscuit usually is low protein, in order to increase the protein content of Spirulina biscuit was fortified with coconut cream. There was no research yet on this related innovation product. Additionally the biscuit ingredient affects its shelf life. The purposes of this study were to obtain mocaf Spirulina biscuit formula, to compare the chemical composition of mocaf Spirulina biscuit and the commercial biscuit, and to estimate the shelf life of the mocaf Spirulina biscuit.

2. Material and Methods
Mocaf was purchased from PT Bina Pangan Cibubur, Jakarta Timur, Indonesia, Spirulina derived from Brackishwater Aquaculture Development Centre in Jepara, Indonesia and cultured at the laboratory of Marine Biotechnology, Faculty of Fisheries and Marine Science of IPB University, Indonesia, as for the coconut cream obtained from the local market in Indonesia. All the chemicals derived from Merck and Plate Dextrose Agar/PDA purchased from Oxoid.

2.1. Formulation of biscuit
In this study, four formulas were used. All Spirulina biscuit formula were without coconut cream (P0), added 10 g of coconut cream per dough (7.19%, P1), 20 g per dough (13.42%, P2) or 30 g per dough (18.87%, P3). The biscuit formula is presented in Table 1. The biscuit was made with thickness of 3 mm and diameter of 3 cm. Roasting was done using an oven at 120 °C for 20-25 minutes.

Table 1. Formulation of mocaf Spirulina biscuits.

| Materials         | P0   | P1   | P2   | P3   |
|-------------------|------|------|------|------|
| Coconut cream (g)*| 0    | 10   | 20   | 30   |
| Rice flour (g)    | 20   | 20   | 20   | 20   |
| Baking soda (g)   | 0.5  | 0.5  | 0.5  | 0.5  |
| Sucrose (g)       | 8    | 8    | 8    | 8    |
| Yeast (g)         | 1    | 1    | 1    | 1    |
| Vanilla (g)       | 0.5  | 0.5  | 0.5  | 0.5  |
| Salt (g)          | 1    | 1    | 1    | 1    |
| Water (mL)        | 40   | 40   | 40   | 40   |
| Spirulina (g)     | 9    | 9    | 9    | 9    |

*Treatment.

2.2. Determining of selected biscuit
The biscuits were selected base on sensory tests referred to NSAI [9] and Lim [10]. This subjective test was conducted to measure the preference level of panelists using hedonic measurement with 30 semi-trained panelists. Parameters assessed include performance, color, taste, texture, and aroma. The 9-point hedonic scale used such as like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely.

2.3. Characterization and shelf life estimation of biscuit
The selected Spirulina biscuit was analyzed for it protein, lipid, water referred to AOAC [11] and carbohydrate measured by different), texture profile referred to Subarna et al. [12] and antioxidant activity [13]. The shelf life estimation carried out using Arrhenius approach by two equality namely ordo zero and ordo one. The products were kept on 25 °C, 35 °C, and 45 °C. The parameter assessed
was mold total referred to [9]. In the end storage, the biscuit was analyzed aw referred to Labuza et al. [14] and Thiobarbituric Acid/TBA referred to Andarwulan et al. [15].

2.4. Chemical composition analysis
The Spirulina biscuit is analyzed chemical composition included protein, lipid, ash, and water content referred to AOAC (2005). Carbohydrate content was calculated by different method referred to Andarwulan et al. [15]. Water activity of the products analyzed using aw meter Novasina ms 1.

2.5. Data Analysis
The experimental design included a T-test, complete randomized design, also linier model (ordo zero) and exponential model (ordo one). The chemical composition, antioxidant activity, and texture of biscuit were analyzed with T-Test. Complete randomized design is used to water activity (aw) and Thiobarbituric acid (TBA). Linier model (ordo zero) and exponential model (ordo one) are used to estimate shelf life of biscuit.

3. Result

3.1. Sensory value
Sensory value of mocaf Spirulina biscuit with coconut cream substitute was presented on Figure 1. Adding coconut cream provide significant effect (p<0.05) on color, texture and taste of biscuits, while performance and aroma were not significantly different.

3.2. Chemical composition of biscuit
Characterization of biscuit was determined based on chemical composition and physically. In this study, Spirulina biscuit with 7.19% coconut cream (P1) was compared with biscuit without coconut cream (P0) (Table 2). The addition of coconut cream has no significant different (p>0.05) to ash and protein content of the biscuit, while lipid, carbohydrate content, fragility and hardness has significant different (p<0.05).

3.3. Antioxidant activity of biscuit
The Spirulina biscuit fortified coconut cream (P1) has IC_{50} value lower than biscuit without added with coconut cream (P0), which mean that antioxidant activity of P1 was higher than P0 (Figure 2).
Table 2. Chemical and physical characteristic of Spirulina biscuit.

| Parameter                        | Characteristic | P0      | P1      | P0      | P1      |
|----------------------------------|----------------|---------|---------|---------|---------|
| Ash (%)                          |                | 3.51±0.17 | 3.63±0.13 | 10.55±0.52 | 12.09±0.52 |
| Protein (%)                      |                | 1.30±0.13 | 6.01±0.76 | 84.64±0.35 | 78.27±0.14 |
| Lipid (%)                        |                |          |          |          |          |
| Carbohydrate/by difference (%)   |                |          |          |          |          |
| Fragility (gf)                   |                | 326.9±0.95 | 408.5±4.12 |          |          |
| Violence (gf)                    |                | 2679.5±48.58 | 1088.5±43.66 |          |          |

Letter a and b show significant different effect on treatment (P<0.05).

Figure 2. Antioxidant activity (IC\textsubscript{50} value) of biscuit.

3.4. Shelf life estimation of biscuit

Estimation of shelf life was carried out based on microbiological damage, i.e. calculation of mold total presented on Table 3. It showed that total mold during storage at temperature 45°C has exceeded maximum limit mold in the biscuits which is 1 x 10\textsuperscript{2} (SNI 7388:2009).

Table 3. Mold total of Spirulina biscuit during storage.

| Temperature 25°C | Temperature 35°C | Temperature 45°C |
|------------------|------------------|------------------|
| Days             | cfu/g            | Days             | cfu/g            | Days             | cfu/g            |
| 7                | 1.6 x 10\textsuperscript{1} | 5                | 1.7 x 10\textsuperscript{1} | 3                | 1.9 x 10\textsuperscript{1} |
| 14               | 5.1 x 10\textsuperscript{1} | 10               | 5.3 x 10\textsuperscript{1} | 6                | 6.9 x 10\textsuperscript{1} |
| 21               | 1.1 x 10\textsuperscript{2} | 15               | 1.2 x 10\textsuperscript{2} | 9                | 1.3 x 10\textsuperscript{2} |
| 28               | 1.2 x 10\textsuperscript{2} | 20               | 1.3 x 10\textsuperscript{2} | 12               | 1.4 x 10\textsuperscript{2} |
| 35               | 1.3 x 10\textsuperscript{2} | 25               | 1.4 x 10\textsuperscript{2} | 15               | 1.5 x 10\textsuperscript{2} |

3.5. Accelerated Shelf Life Testing (ASLT)

The correlation of the coefficient value in the shelf life estimation calculation of the biscuits was represented on Table 4, while the mold changing rate on storage temperature of 25°C, 35°C and 45°C was represented on Figure 3.
Table 4. Correlation coefficient value in the shelf life estimation calculation of biscuits.

| Storage temperature (°C) | 1/T | R² | K Ordo zero | K Ordo one | Ln k Ordo zero | Ln k Ordo one |
|--------------------------|-----|----|-------------|------------|----------------|---------------|
| 25                       | 298 | 0.0034 | 0.5441 | 0.9714 | 370.8 | 0.1467 | 5.9157 | -1.9194 |
| 35                       | 308 | 0.0032 | 0.5469 | 0.9749 | 562.8 | 0.2086 | 6.3329 | -1.5673 |
| 45                       | 318 | 0.0031 | 0.5458 | 0.9768 | 1173 | 0.3597 | 7.0673 | -1.0225 |

Note:
- K = storage temperature (Kelvin);
- 1/T = 1/storage temperature;
- K = reaction rate;
- R² = correlation coefficient between 1/T and ln k on each storage temperature.

Figure 3. The mold total enhancement rate on Spirulina biscuit.

Table 5. Storage temperature and self life of Spirulina biscuit.

| Storage temperature (°C) | ln k  | K      | Date | Month | Year |
|--------------------------|-------|--------|------|-------|------|
| 25                       | -2.526802158 | 0.0799142 | 72.28 | 2.41  | 0.2  |
| 30                       | -2.347962898 | 0.0955636 | 60.45 | 2.01  | 0.17 |
| 35                       | -2.175333333 | 0.1135703 | 50.86 | 1.70  | 0.14 |
| 40                       | -2.008595563 | 0.134177  | 43.05 | 1.44  | 0.12 |
| 45                       | -1.84745302  | 0.1576382 | 36.64 | 1.22  | 0.10 |
| 50                       | -1.691628713 | 0.1842192 | 31.36 | 1.045 | 0.09 |

3.6. Water activity (Aw) of biscuits on the end storage
The range of the water activity value of biscuits on the end storage was between 0.49-0.61 (Figure 4). Temperature has a significant effect on the water activity value (p<0.005).

3.7. Thiobarbituric Acid (TBA) of biscuit on the end storage
The TBA value of biscuits which kept on 25, 35 and 45 °C are presented on Figure 5.
Figure 4. Water activity value of biscuit. The letters a and b show different effects between treatments (p<0.05).

Figure 5. The storage temperature were not significantly different to TBA value at P>0.05.

4. Discussion

4.1. Sensory value and chemical composition of biscuit

Formula P1 (coconut cream 7.19%) was the selected biscuit formula based on the hedonic analysis. Therefore next step such as characterization and estimation of shelf life used biscuit formula P1.

Protein of biscuit comes from ingredients in biscuit formula including Spirulina, mocaf flour, and rice flour. According to Seo et al. [2] Spirulina contains protein of 55-70%, with protein content of mocaf flour as much as 0.98% and rice flour of 9.59%. [16]. Based on its protein content, this biscuit is in accordance with the SNI 2973:2011 of biscuit quality requirement, which regulate that the protein content is 5% [9].

Ash content of mocaf Spirulina biscuit with 7.19% coconut cream (10 g per dough) was 3.63%. The ash content shows mineral content in the material. The ash content of this biscuit was higher than the quality requirement SNI 2973:2011 that is 1.5%. With that, it is restricted to consume it to avoid side effect. Darmono [17] stated that mineral can be toxic if consumed excessively. The ash content can be reduced by improving the biscuit formulation. Moreover, Spirulina as ingredient in biscuits also contributed to ash content in biscuits. Mineral contained in *S. platensis* includes Ca, F, Mg, Fe, Na, I, K, Zn, Mn, and Se [18].

On the lipid content, biscuit with coconut cream (P1) was higher than without coconut cream (P0). This shows that adding of coconut cream increased lipid content in biscuit. Coconut cream is an
emulsifier that can decrease violence of biscuit. Manley [19] stated that lipid can improve physical structures such as softness, texture, and aroma. Coconut cream contains relatively high lipid which 40%. Lipid has a shortening effect on baked goods such as biscuits, pastries and bread [20].

Carbohydrate content of Spirulina biscuit with coconut cream was higher than without coconut cream. Carbohydrate content in this biscuit has fulfilled the SNI requirement, which is 70% minimum [9].

Fragility and violence of mocaf Spirulina biscuit fortified coconut cream (P1) were better than biscuit without coconut cream (P0). Panelist also prefer texture of biscuit P1 than others (Fig.1).Coconut cream in the biscuit contribute to its crispiness. Kusnandar [21] stated lipid in the ingredient contribute to texture and sensory of product. Adding coconut cream to Spirulina biscuit influence significantly to IC_{50} for antioxidant activity (p<0.05).The results showed that antioxidant activity of the two biscuits were different. The sources of antioxidant activity in biscuits were Spirulina and coconut cream. Palungkun [22] stated that antioxidant substance of coconut include vitamin A 0-0.1 IU, thiamin 0-0.5 mg, vitamin C 2-4 mg.

4.2. Shelf life estimation of biscuit
The principle of the Accelerated Shelf Life Testing (ASLT) method is to accelerate the physicochemical damage of products with mathematical calculations. The food product that can be determined of shelf life with Arrhenius method are canned food, formula milk powder, snack/chip products including biscuits, instant noodle, frozen meat [23]. The number of mold increase on storage temperature of 25 °C is slower than 35°C and 45°C. In conclusion, shelf life of mocaf Spirulina biscuit was 2.41 months.

The aw value of 0.60 is a critical point that has the potential for microbial growth if the water content increases. Growth of fungi is generally found in the range of aw values from 0.60 to 0.75 [14]. Some molds have a minimum aw for clenching spores of 0.60 [24]. On the end storage at 45 °C, total biscuit mold of 1.5 x 10^2 cfu/g, Which means that higher the temperature storage, the shorter the self-life (15 days).

The TBA value of biscuit was lower than standard based on SNI 2973-2011,which maximum TBA value in fisheries and farm were 2 malonaldehida mg/kg [9]. One of the factors that influence the value of TBA is oxygen. The availability of oxygen in the product is influenced by packaging used. Other than that, rancidity is caused by hydrolysis and oxidation reaction. The reaction raises food off-flavor. This oxidation reaction could be caused by oxygen contained in the air, peroxidase, metal and others oxidizer. Overall, the biscuit has not been damaged and rancid yet.

5. Conclusion
The biscuit containing coconut cream 10 g (7.19%), Spirulina 9 g and mocaf flour 50 g in 100 g dough is the selected Spirulina biscuit. This biscuit contains protein of 12.69%, lipid of 6.01%, ash 3.63% and carbohydrate of 78.87%. The shelf life of mocaf Spirulina biscuits with Arrhenius method approach stored at room temperature (25°C) was 2.41 months.

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