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# The Addition Effect of Spirulina Platensis on the Physco-chemical and Hedonic Properties of Gluten-Free Cookies Based on Mocaf and Sorghum Composite Flour

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**A B S T R A C T**

Spirulina platensis has a high nutritional content as a source of protein and offers various health benefits. Spirulina provides an attractive natural green color, so it has the potential to be used as an additional ingredient in food processing, notably in cookie products. Mocaf and sorghum flour are local gluten-free foods that have the opportunity to be functional foods, so they can be utilized as substitutes for ingredients in cookie recipes. This research aims to determine the impact of adding spirulina on water content, water activity, protein content, texture and organoleptic of non-gluten cookies based on mocaf and sorghum composite flour. The method used in this research is using Completely Randomized Design (CRD) with 4 treatments and 5 repetitions, to obtain 20 experimental sample units. The test parameters observed were water content, water activity, protein content, texture, and organoleptic and hedonic quality. Statistical testing analysis uses the Analysis of Variance (ANOVA) parameter test with a significance level (P<0.05). Analysis of the data obtained was carried out using the SPSS for Windows 26.0 application. If the significant level has a real effect (p<0.05), then the Duncan Multiple Range Test (DMRT) is continued. Non-parametric data analysis, namely Kruskall Wallis. If there are differences data obtained, then proceed with the Mann-Whitney test. The results showed that the most favorable outcomes, along with the highest level of acceptance among panelists, are achieved at a concentration of 1% spirulina. Specifically, at this concentration, the cookies exhibit an aw value of 0.358, a protein content of 4.26%, and a color profile characterized by an L* value of 15.44 ± 0.089, an a* value of 0.86 ± 0.167, and a b* value of 2.64 ± 0.152.

Keywords: *Gluten-free cookies, Mocaf, Sorghum, Spirulina platensis*

## 1. Introduction

Cookies are commonly consumed by Indonesian people, known for their sweet and slightly savory taste. They are usually enjoyed alongside tea or coffee. Cookies are characterized by their small size, dense yet crunchy texture, and high sugar and fat content with low water content that less than 5% (Trihaditia *et al*., 2020). Cookies have a long shelf life, lasting from 1 to 6 months (Faisal, 2023). Cookies are a type of pâtisserie product that are crafted through baking or grilling techniques.

Cookies are made by baking a mixture of wheat flour or suitable substitutes, oil/fat, and with or without permitted food additives (SNI 2973:2011, 2011). The primary ingredients for making cookies are flour, water, fat (margarine), sugar and eggs. Wheat flour serves as the main component in making cookies, but its high gluten content renders foods containing significant levels of wheat flour unsuitable for individuals with gluten-intolerance (Simatupang, 2024).

The utilization of diverse flour types is expected to enhance food security at the national level, while also fostering diversity in food consumption by harnessing Indonesia's natural resources. Research focus has shifted towards utilizing local flours derived from tubers, nuts, and fruits in the development of bread and cake products (Wati *et al*., 2023). Mocaf and sorghum are types of flour that are currently being developed.

Mocaf flour is the result of cassava fermentation using lactic acid bacteria (SNI 7622:2011, 2011). In this study, mocaf flour was selected as a substitute for wheat flour in making cookies because it has lower fat content and higher fiber content than wheat flour. The high dietary fiber content and low glycemic index in mocaf flour make it suitable as a raw material for making cookies. In addition, the use of mocaf flour in food product development can help reduce blood glucose levels, so mocaf flour can be beneficial for individuals with hyperglycemia.

Sorghum is a local product commonly used as a source of carbohydrates. Sorghum contains 72% carbohydrates, 4% fat, 11% protein, and essential nutrients such as dietary fiber and iron. Sorghum can be processed into gluten-free flour, which can inhibit cancer development, treat diabetes and insulin resistance problems, and is more resistant to *Fusarium fungus* contamination. Sorghum's fiber and micronutrient levels are higher than wheat. According to Dhanasatya *et al*., (2020), sorghum contains slowly digested starch and dietary fiber, so it has the potential to control blood sugar levels and influence appetite hormones for diabetes sufferers. On the other hand, there is a source of raw material rich in protein, namely spirulina.

*Spirulina platensis* is a type of filamentous green algae and has been widely used as a food supplement, animal feed and in cosmetics. Spirulina has a protein content ranging from 63–68%, carbohydrate content of 18–20% and fat 2–3% (Suita *et al*., 2023). The use of spirulina in cookies not only provides additional nutritional value in the form of protein, but also offers extensive functional benefits. According to Lubis and Lubis (2023), spirulina exhibits immunomodulatory, anti-inflammatory, antioxidant, anti-cancer, anti-tumor, anti-viral, cholesterol-lowering, anti-rust, hepatitis-preventing, metal-chelating, blood-forming activities. Spirulina contains carotenoids and flavonoid phenolic compounds so it is an attractive choice to provide a natural green color to food products and natural antioxidants that beneficial for the body.

## 2. Material and methods

### *2.1 Material and Tools*

The materials used in this study were water, mocaf flour, sorghum flour, and spirulina. Additional ingredients included margarine (Blueband cake and cookie), eggs, skim milk, powdered sugar (Gulus), salt, and baking powder (R&W) obtained from Sumber Rejeki Cake and Bread Ingredients Store. Mocaf and sorghum flour were sourced from Jais Organic Store. Spirulina powder was obtained from Albitech Store.

The tools used were oven, baking pan, baking paper, knife, stirrer, gloves, cookie molds, analytical balance (Shimadzu), mixer (KitchenAid), erlenmeyer flask (Iwaki), graduated cylinder, porcelain cup, desiccator, $a_w$ meter, clamps, mortar, Kjeldahl flask, distillation equipment, titration equipment, fume hood, burette, pipettes, colorimeter, and black paper.

### *2.2 Cookies Production*

The cookies production method in this study refers to Dhanasatya *et al*., (2020) which has been modified. Cookies production begins by preparing 60 gr of margarine, 7.5 gr skim milk, 60 gr powdered sugar and 0.75 gr of salt, then mix at medium speed for 4 minutes until fluffy. Next, add 15 ml water and 25 gr of egg yolk and mix again for 1 minute. The next ingredients to mix are 112.5 gr of mocaf flour, 37.5 sorghum flour, and 4.5 gr baking soda. Then, add spirulina with formulations of 0 gr, 1 gr, 2 gr, 3 gr, and 4 gr. mix for 4 minutes until homogeneous. After mixing thoroughly, the dough is molded into small circles with a wet weight of 6 grams per piece. Place the cookies on a baking sheet, bake in the oven for 20 minutes at 150° Celsius. The biscuits obtained were then stored closed at room temperature with the addition of silica gel.

### *2.3 Water Activity*

The $a_w$ value is determined using an $a_w$ meter. The calibration standard (distilled water) is first used to calibrate the water activity meter until it shows the water activity of distilled water is 1.000±0.003. The sample is macerated until smooth and then 1 g of sample placed into a water activity measuring tube and then it is inserted into the $a_w$ meter. The screen of the $a_w$ meter displays the measurement progress. The measurement process continues until the $a_w$ value stabilize that indicated by a beep from the instrument. The sound signals from the $a_w$ meter indicates that the water activity measurement process has been completed. The value of water activity is displayed in the water activity meter (Aozora *et al*., 2022).

### *2.4 Protein Analysis*

The protein content is measured using the Kjedahl method using a Kjeldahl flask according to the AOAC (2005) standards. The testing process commenced with sample digestion in a flask. The distillation process is carried out on samples diluted with distilled water. The distillate are collected in an Erlenmeyer flask and an indicator is added to determine the end point of the titration process. Color change during titration indicates the reaction occurring in cookies containing protein levels. The protein content can be calculated using the formula:

$$Protein\ content\ (\%) = (\frac{(sample\ titer - blank\ titer) \times NHCl \times 14{,}008}{Sample\ weight\ (g) \times 1000} \times 100\%$$

The calculated result is multiplied by a conversion factor of 6.25.

### *2.5 Color Analysis*

Color analysis is performed using a colorimeter. The product is placed on black paper and then measured. Measurements produce L, a and b values. L value represents the brightness parameter (achromatic color, ranging from 0 for black to 100 for white). The chromatic color of red and green mixture is indicated by the value a+ = 0-100 for red, a- = 0-(-80) for green. The chromatic color of the blue and yellow mixture is indicated by the value b+ = 0-70 for yellow, b- = 0-(-70) for blue. Color assessment was conducted three times (Engelen, 2018)).

### *2.6 Hedonic Analysis*

The hedonic test is a test in organoleptic sensory analysis used to determine the difference in quality between several similar products by providing an assessment or score on certain properties of a product and to determine the level of liking (Jagat *et a*l., 2017). The samples are presented in identical containers and coded with 3-digit random numbers. The hedonic test is involved by 25 untrained panelists who rated the product based on a scale from 1 as the lowest value to 4 as the highest value. The results are then analyzed statistically to determine the level of liking and any significant differences between the samples.

### *2.7 Statistical Analysis*

Data analysis in this research utilized both parametric and non-parametric data analysis. Parametric data analysis involved protein, water, and color test results. Parametric data was tested statistically, including normality, homogeneity and Analysis of Variance (ANOVA) tests. ANOVA determine the real effect of treatment with a significance level of (P<5%). If the obtained data shows significant differences, then a subsequent Honest Significant Difference test is conducted. Non-parametric analysis was performed on the results of hedonic tests using the Kruskal-Wallis test to ascertain whether differences in treatment influenced the dependent variable, followed by the Mann-Whitney test.

## 3. Result and Discussions

### *3.1 Water Activity*

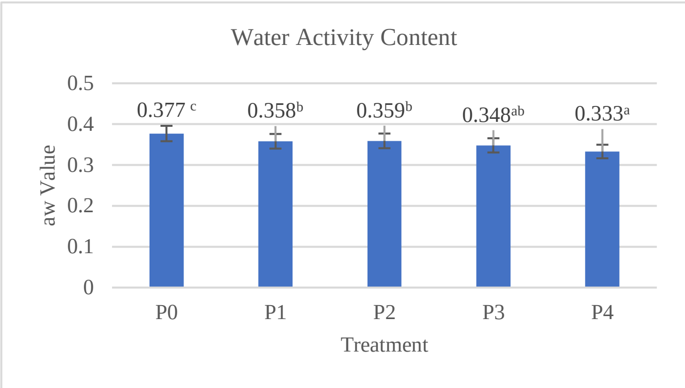


Fig. 1 - Chart of Water Activity Test Result of Spirulina Cookies

The addition of spirulina to sorghum and mocaf cookies has been shown to significantly reduce their water activity ($a_w$), which has critical factor in enhancing the shelf life and microbial stability of baked goods. Spirulina, a blue-green algae packed with proteins, vitamins, and minerals, possesses hygroscopic properties that enable it to bind water molecules more effectively than the base flours. Research conducted by Onacik-Gür, *et al.* (2018) suggests that sorghum cookies enriched with spirulina exhibit lower water activity compared to those without spirulina, indicating that the hydrophilic components of the algae play a pivotal role in this reduction. This binding of water molecules reduces the availability of free water, thereby inhibiting microbial growth and slowing down spoilage processes.

Similarly, a study by Fitriani *et al.* (2022) discovered that the incorporation spirulina into cookies not only decreased water activity but also enhanced their nutritional content. The proteins and other bioactive compounds present in spirulina contribute to the formation of a gel matrix within the cookie structure, which traps water and further reduces its availability. This phenomenon results in a denser and more stable product that exhibit better resistance to microbial contamination and biochemical degradation. As a result, utilizing spirulina as an ingredient in sorghum and mocaf cookies not only enhances their health benefits but also significantly extends their shelf life by lowering their water activity.

### *3.2 Protein Content*

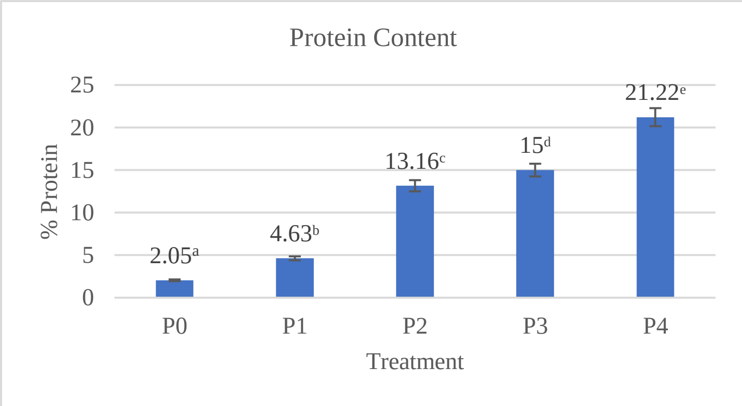


Fig. 2 - Chart of Protein Content Test Result of Spirulina Cookies

One of the important tests conducted to determine the quality of cookies is by analyzing their protein content. The protein content of spirulina cookies depicted in Figure 1. The results indicate a significant variance in protein content among spirulina cookies. The lowest protein content was obtained in treatment P0, or the control group (cookies without spirulina addition), with a value of 2.05 ± 0.44a%, while the highest value was observed in treatment P4, or cookies with the addition of 1.5% spirulina, with a protein content value of 21.22 ± 1.24e%. The obtained results are consistent with the findings of Shahbazizaadeh *et al*. (2015), who also observed an increase in protein content with increasing concentrations of spirulina added to biscuits. Spirulina is recognized as one of the ingredients commonly incorporated into food products to improve quality characteristics and enhance the nutritional value of food, particularly its abundant protein content. The addition of spirulina at 2.6% concentration in snacks resulted in increased protein (22.6%), minerals (46.4%), and lipids (28.1%) without significantly affecting their sensory and physiological characteristics (Koli *et al*., 2022). Spirulina-enriched snacks with up to 11.3% protein have been developed, offering a healthy alternative for consumers (Lucas *et al*., 2017).

The amount and method of Spirulina extract addition can be influenced by various factors such as the type of food. Spirulina extract can be used in a wide range of foods and beverages, such as flavored dairy products, cheese, dairy-based desserts, processed fruits and vegetables, baked goods, and beverages. The food type can influence the quantity and method of spirulina extract addition, which in turn affects the final Spirulina content in the product (AlFadhly *et al*., 2022). The other factor is manufacturing process. The production method of the food product can significantly impact the spirulina content. For example, in the development of functional pasta with microencapsulated Spirulina, the technological and sensorial effects of the process were studied. Similarly, a study on the use of spirulina in animal feed investigated the effect of cumulative Spirulina intake on broiler meat quality, nutritional, and health-related attributes was investigated in a study on the use of Spirulina in animal feed (Lafarga *et al*., 2020).

### *3.3 Color*

Table Result of Color Test of Spirulina Cookies

| Treatment | Color | L* | a* | b* | Appearance |
|---|---|---|---|---|---|
| Addition of *Spirulina 0%* | Light brown | $17.32 \pm 0.179^{c}$ | $1.72 \pm 0.837^{d}$ | $4.38 \pm 0.110^{d}$ |  |
| Addition of *Spirulina 1%* | Light brown | $15.44 \pm 0.089^{b}$ | $0.86 \pm 0.167^{c}$ | $2.64 \pm 0.152^{c}$ |  |
| Addition of *Spirulina 2%* | Light green | $15.22 \pm 0.045^{b}$ | $0.42 \pm 0.045^{b}$ | $2.52 \pm 0.110^{c}$ |  |
| Addition of *Spirulina 3%* | Faded green | $13.64 \pm 0.313^{a}$ | $0.36 \pm 0.182^{b}$ | $2.04 \pm 0.114^{b}$ |  |

| | | | | | |
|---|---|---|---|---|---|
| Addition of *Spirulina 4%* | Faded green | $13.50 \pm 0.707^{a}$ | $0.12 \pm 0.084^{a}$ | $1.76 \pm 0.089^{a}$ |  |

Data followed by different lowercase letters in the same column are significantly different (P<0.05)

The addition of spirulina to sorghum and mocaf cookies have significantly impact on their color properties, particularly regarding lightness (L* value) and the chromatic components a* (red-green) and b* (yellow-blue). Spirulina is known for its intense green-blue pigment due to its high content of phycocyanin and chlorophyll. When added spirulina to cookies, this pigment causes a noticeable reduction in lightness, making the cookies appear darker. A study by Sundari *et al*. (2023) demonstrated that sorghum cookies with spirulina showed a significant decrease in L* values compared to those without spirulina, indicating a darker appearance. This change is attributed to the strong color imparted by spirulina, which dominates the lighter tones of the base flours.

In addition to affecting lightness, spirulina also influences the a* and b* values of the cookies. The incorporation of spirulina typically shifts the a* value towards the green spectrum and the b* value towards the blue spectrum, altering the overall color balance. Research by Fitriani *et al.* (2022) on mocaf cookies highlighted that the addition of spirulina resulted in lower a* values, indicating a greener hue, and lower b* values, indicating a shift towards blue. These changes in chromaticity can enhance the visual appeal of the cookies, making them more attractive to health-conscious consumers looking for functional foods. Overall, spirulina not only enriches the nutritional profile of sorghum and mocaf cookies but also significantly modifies their color characteristics, which can influence consumer perception and acceptance.

### *3.4 Hedonic*

Table Result of Hedonic Test of Spirulina Cookies

| Treatment | Sensory Attributes | | | |
|---|---|---|---|---|
| | Color | Aroma | Texture | Taste |
| P0 | $3.64 \pm 0.700^{d}$ | $3.32 \pm 0.802^{c}$ | $3.32 \pm 0.802^{b}$ | $3.12 \pm 0.781^{b}$ |
| P1 | $3.00 \pm 0.577^{c}$ | $3.36 \pm 0.490^{c}$ | $3.32 \pm 0.627^{b}$ | $3.16 \pm 0.746^{b}$ |
| P2 | $2.52 \pm 0.714^{ab}$ | $2.68 \pm 0.690^{ab}$ | $2.48 \pm 0.653^{a}$ | $2.40 \pm 0.764^{a}$ |
| P3 | $2.72 \pm 0.614^{ac}$ | $2.68 \pm 0.557^{ab}$ | $3.12 \pm 0.526^{b}$ | $2.88 \pm 0.781^{b}$ |
| P4 | $2.16 \pm 0.746^{b}$ | $2.36 \pm 0.638^{a}$ | $3.00 \pm 0.764^{b}$ | $2.84 \pm 0.850^{b}$ |

Information:

Data ± standard deviation

Data followed by different lowercase letters in the same column are significantly different (P<0.05)

P0 = Cookies with the addition of *S. platensis* 0%

P1 = Cookies with the addition of *S. platensis* 1%

P2 = Cookies with the addition of *S. platensis* 2%

P3 = Cookies with the addition of *S. platensis* 3%

P4 = Cookies with the addition of *S. platensis* 4%

The appearance of a product is a critical component in the context of food, primarily because color can serve as a consumer attraction, influencing whether a product is liked or not. Additionally, color can also be used as a means of identification and a quality attribute. The appearance of cookies tends to decrease as the spirulina concentration increases. The value decline of color was caused by a greeny effect of spirulina pigment that contribute to biscuit color (Kumar *et al*., 2019). The value obtained ranged from 3.64 (P0) to 2.16 (P4) in 5 scale of hedonic tests. S. platensis has a distinctive and characteristic aroma. When this aroma is combined with the other ingredients of cookies such as flour and margarine it could mask the specific fishy smells of spirulina. The best results obtained by P1 with hedonic value of 3.36 and the lowest results were P4 with hedonic value of 2.36. The hedonic value decreases as a result of the addition of spirulina content spiral to the cookies. One way to mask spirulina aroma is to incorporate another strong aromatic ingredient to mask the off flavor produced by spirulina (El Nakib *et al*., 2019).

The taste of spirulina cookies is polarizing, with individuals either embracing or rejecting its distinctive flavor profile. The earthy, vegetal notes derived from spirulina may appeal to health-conscious consumers seeking alternative ingredients. However, mainstream acceptance may be hindered by the perceived "green" taste, which deviates from conventional cookie flavors. Preferred taste is specific to the product and should not be bitter or astringent.

The cookies with 1% addition of spirulina (P1) had a specific product taste and tended to be sweet rather than bitter, resulting in the highest acceptance value of 3.16 on 5 scale of hedonic test. Increasing the concentration of S. platensis led to a slightly bitter taste in the cookies. Bitterness intensified with greater concentrations of Spirulina platensis, driven by its innate bitter qualities. The presence of chlorophyll, known for its bitter taste, notably impacts the overall flavor profile (Shahbazizadeh *et al*., 2015). The taste of a product is shaped by its constituent elements, and manufacturing methods can also sway consumer preferences.

The texture of spirulina cookies is influenced by multiple factors, including recipe formulation, baking process, and spirulina concentration. Achieving an optimal texture requires careful consideration of these factors to balance chewiness, crispiness, and mouthfeel while ensuring uniformity and appeal. The acceptance values for the texture of the cookies ranged from 2.48 to 3.32 with little significance difference. The values recorded in this study differ from a result documented by Doiphode and Mane (2021), who recorded texture values ranging from 7.1 to 7.5 across concentrations of 2% to 6%. This increase in the texture parameter could associated with difference formula used on cookies production. Protein serves as a vital component for imitating certain gluten-like characteristics. Moreover, it is widely acknowledged that starch is indispensable and may significantly contribute to improving rheological and baking properties by virtue of its filling function. The disparities in texture values arise from the synergistic interplay between protein and gelatinized starch, facilitated by hydrogen bonding between the two (Song *et al*., 2007).

## 4. Conclusion

The research on the addition of spirulina to mocaf and sorghum cookies reveals significant improvements in several key parameters, including water activity ($a_w$), protein content, color, and sensory acceptance (hedonic value). The incorporation of spirulina effectively reduces the water activity of the cookies, enhancing their shelf life and microbial stability. Additionally, spirulina significantly boosts the protein content, providing a nutritional advantage. However, it also imparts a darker color and shifts the chromatic values, leading to a greener and bluer hue due to its natural pigments. Sensory evaluations indicate that while the distinctive color might affect initial visual appeal, the overall acceptance of the cookies remains high due to their enhanced nutritional benefits and unique flavor profile. Thus, spirulina-enriched mocaf and sorghum cookies present a promising functional food option with improved preservation, nutritional value, and favorable sensory properties.

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