

## DEVELOPMENT OF RED SPINACH AND SORGHUM-BASED COOKIES AS A FUNCTIONAL HIGH-IRON SNACK FOR ANEMIC ADOLESCENT GIRLS

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### ABSTRACT

**Background:** Utilization of red spinach which is rich in iron and vitamin C and utilization of sorghum flour which is rich in protein and iron can be used in making cookies as an alternative food for anemic adolescent girls. The purpose of this study was to determine the organoleptic quality and nutritional content (moisture content, ash, protein, fat, carbohydrates and iron) of cookies made from red spinach leaves and sorghum flour. **Methods:** This study was an experimental study using a completely randomized design (CRD) with two repetitions. This study has three formulas with a ratio of red spinach leaves and sorghum flour, namely F1 (10% : 90%), F2 (20% : 80%), and F3 (30% : 70%). Organoleptic test was analyzed using Kruskal Wallis and nutrient content was analyzed using One Way Anova and continued with Duncan's test. **Results:** The results of this study indicated that the nutritional contents of F1, F2, and F3 were as follows : water (5.01%, 2.66%, 5.94%), ash (1.53%, 1.59%, 1.60%), fat (11.82%, 13.19%, 12.68%), protein (8.46%, 8.27%, 9.53%), carbohydrates (73.17%, 73.90%, 70.23%), and iron (8.97%, 7.44%, 7.87%). **Conclusion:** The selected formulation (F3) has a nutritional content per serving of 75 g (5 pieces) of 325 kcal energy, 9.51 g fat, 7.14 g protein, 52.67 g carbohydrates, and 5.90 mg iron. Formulation F3 exhibits a brown color, a fragrant aroma, a [bitter/sharp] taste, a slightly hard texture, a medium aftertaste, and a firm mouthfeel. Furthermore, F3 meets the RDA for adolescents aged 16–18 years and qualifies for a 'high-iron' claim under general nutrition label reference standards (35.77%).

**Key words :** Adolescence girls, anemia, cookies, red spinach, sorghum flour.

### INTRODUCTION

Iron deficiency anemia remains a major public health problem worldwide, particularly in developing countries such as Indonesia (Mariana & Nur, 2013). Adolescent girls are considered anemic when their hemoglobin levels fall below 12 mg/dL (Ministry of Health, Republic of Indonesia, 2011). The prevalence of anemia in Indonesia remains high. Based on the 2023 Indonesia Health Survey, the prevalence of anemia among adolescents aged 15–24 years is

15.5%. The data indicate that the prevalence of anemia among female adolescents is 18%, while among male adolescents it is 14.4% (Ministry of Health of the Republic of Indonesia, 2023).

Several factors contribute to anemia among adolescent girls, including frequent skipping of breakfast, poor and monotonous dietary patterns, and inadequate intake of animal protein and iron (Putera et al., 2020). Menstruation is another

contributing factor, as substantial blood loss occurs during the menstrual cycle. Adolescent girls typically have limited iron stores and low iron absorption, making it difficult to replace the iron lost during menstruation (Prastika, 2011).

Anemia in adolescent girls can lead to symptoms such as dizziness, blurred vision, and pallor in the eyelids, lips, tongue, skin, and palms. If a woman experienced anemia during adolescence, it may negatively affect both her and her baby during pregnancy, as nutritional needs are significantly higher during gestation (Sandra, 2017). The consequences of anemia during pregnancy include low birth weight (LBW), preterm birth, fetal death, and postnatal infant mortality (Farhan & Davieka, 2021).

One preventive approach for anemia in adolescent girls is to increase the consumption of iron-rich foods such as red spinach and sorghum flour. Food products made from red spinach leaves and sorghum flour can be utilized as high-iron alternatives, such as cookies.

Cookies are a popular type of snack among adolescents due to their sweet flavor and crispy texture. However, most commercially available cookies are high in calories and sugar. Therefore, innovative cookie formulations that are not only energy-dense but also rich in essential nutrients are needed (Novitaroh et al., 2022). Incorporating red spinach and sorghum flour in cookie production is expected to improve the nutritional value, particularly in terms of iron content (Wahyani & Rahmawati, 2021).

Red spinach (*Amaranthus tricolor L.*) contains various nutrients such as protein, fat, carbohydrates, potassium, calcium, manganese, phosphorus, iron, purines, vitamins (A, B1, B2, B3, and C), carotene, chlorophyll, and saponins

(Faralia, 2012). Every 100 grams of red spinach contains approximately 7.0 mg of iron (Ministry of Health RI, 2018a). Its iron and folic acid content contributes to red blood cell formation, anemia prevention, and increased hemoglobin levels (Karina, 2012).

Sorghum (*Sorghum bicolor L.*) is a gluten-free cereal rich in protein, fiber, and iron. It typically has lower gluten and glycemic index values. Per 100 grams, sorghum flour contains 10.6 g of protein, 3.4 g of fat, and 5.4 mg of iron, which is higher than cassava, rice, or corn flour (USDA, 2015). However, the utilization of sorghum in Indonesia remains limited, mostly restricted to traditional dishes that are boiled or steamed (Wahyani & Rahmawati, 2021).

Therefore, this study aims to assess the organoleptic properties (hedonic and hedonic quality tests) and the nutritional content (moisture, ash, protein, fat, carbohydrate, and iron) of cookies formulated as high-iron alternative food products for anemic adolescent girls.

## METHOD

This study utilized a quantitative approach with an experimental design to develop cookies based on red spinach leaves and sorghum flour. The research was conducted using a Completely Randomized Design (CRD) with three treatment groups: F1 (10% red spinach leaves : 90% sorghum flour), F2 (20% : 80%), and F3 (30% : 70%).

The formulation and organoleptic evaluation were carried out in the Nutrition Laboratory at Kusuma Husada University, Surakarta. A total of 30 semi-trained panelists participated in the sensory evaluation, which included hedonic and hedonic quality testing. The nutritional analysis including

moisture, ash, protein, fat, carbohydrate, and iron contents was conducted at the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta.

The ingredients used in cookies production included red spinach leaves, sorghum flour, egg yolks, ovalet (emulsifier), powdered milk, icing sugar, margarine, cornstarch, and chocolate chips. For nutrient analysis, chemical reagents such as hydrochloric acid (8 N and 0.1 N), sulfuric acid, petroleum ether, potassium sulfate, mercury oxide or copper sulfate, sodium hydroxide, boric acid, and a mixed indicator solution were used.

Equipment for cookies production included an electric oven, hand mixer, spatula, knife, aluminum baking trays, bowls, saucepans, cookie molds, a blender, digital scale, and measuring cups. Organoleptic testing utilized scoring sheets, writing instruments, sample cups, drinking water, and clip-sealed plastic bags. Nutrient analysis required various tools such as analytical balances, porcelain and platinum crucibles, Soxhlet extractors, Kjeldahl equipment, desiccators, furnaces, and glassware appropriate for proximate, spectrophotometers and iron content measurements.

The research procedure involved three main stages. First, three different cookies formulations (F1, F2, and F3) were prepared based on the designated proportion of red spinach puree and sorghum flour, following the process registered under Patent No. HKI 000483833 (Niswa *et al.*, 2023). Second, organoleptic evaluation was carried out to assess six sensory attributes: color, aroma, taste, texture, aftertaste, and mouthfeel. Third, the nutritional content of each formulation

was analyzed. Moisture and ash content were measured using gravimetric methods (AOAC 2005), protein content was assessed using the Kjeldahl method, fat by Soxhlet extraction, carbohydrates by difference, and iron content using Atomic Absorption Spectrophotometry (AAS, AOAC 968.08). Each analysis was repeated twice.

The selected formulation was determined using a weighted scoring system combining organoleptic and nutritional evaluations. A total of 50% of the score was derived from organoleptic tests (hedonic and hedonic quality, including color, aroma, texture, taste, aftertaste, and mouthfeel), while the remaining 50% was based on nutritional parameters (moisture, ash, fat, protein, carbohydrate, and iron content).

This approach ensures a balanced assessment between consumer acceptability and nutritional value. The formulation with the highest total combined score was selected as the best formulation (Parwati *et al.*, 2023).

All statistical analyses were performed using IBM SPSS Statistics software. Statistical analysis included normality testing using the Shapiro-Wilk test and homogeneity testing using the Levene test. As the organoleptic data were non-normal and non-homogeneous, the Kruskal-Wallis test was used for analysis. Meanwhile, nutrient content data, which were normal and homogeneous, were analyzed using one-way ANOVA, followed by Duncan analysis to indicate the difference between each formula. Ash content did not show statistical significance and was therefore not subjected to post hoc analysis.

Table 1. Formulation Development of Red Spinach and Sorghum-Based Cookies

Ingredient	F1 (10%:90%)	F2 (20%:80%)	F3 (30%:70%)
Red spinach leaves (g)	15	30	45
Sorghum flour (g)	135	120	105
Egg yolk (g)	15	15	15
Sugar (g)	35	35	35
Ovalet (g)	5	5	5
Milk powder (g)	20	20	20
Margarine (g)	20	20	20
Cornstarch (g)	20	20	20
Chocochips (g)	0.01	0.01	0.01
Total	265.01	265.01	265.01

Notes:

F1: 10% red spinach leaves, 90% sorghum flour

F2: 20% red spinach leaves, 80% sorghum flour

F3: 30% red spinach leaves, 70% sorghum flour

## RESULTS AND DISCUSSION

### Organoleptic Test

Organoleptic testing, including hedonic testing, was conducted on cookies to determine the panelists' level of preference for cookie formulations F1, F2, and F3, covering six sensory attributes, namely color, aroma, taste, texture, aftertaste, and mouthfeel. The average scores from the organoleptic tests, including hedonic and hedonic quality tests, are presented in the Table 2 and 3.

### Color

Table 2 presents the mean hedonic scores for the color attribute of cookies in formulations F1 (2.87), F2 (3.00), and F3 (3.23). The highest color score was found in F3 (3.23: moderately liked), while the lowest was in F1 (2.87: disliked). These findings indicate that the color of cookies made with red spinach leaves and sorghum flour fell within a hedonic range of 2.87–3.23 (moderately liked). The overall average color score was 3.03 (moderately liked).

Table 3 shows the descriptive hedonic scores for cookie color, with average values in F1 (2.40: brown), F2 (2.43: brown), and F3 (2.40: brown). Among the treatment groups, F2 had the

highest average score, while F1 had the lowest; however, all formulations were rated as brown. The use of sorghum flour significantly influenced the color of the cookies. Sorghum contains tannins, which are polyphenolic compounds characterized by aromatic rings with one or two hydroxyl groups (Eka & Nur, 2019). The red color in sorghum grains is attributed to the presence of the testa, a thin cell layer beneath the pericarp, which contains tannins (Schons et al., 2012). An increased amount of sorghum flour leads to a darker cookie color (Syafitri et al., 2021).

In addition to sorghum flour, the use of red spinach leaves also affected the cookie color. A higher proportion of red spinach leaves in the formulation resulted in a deeper brown color (Meirina et al., 2021). In this study, the brown color was partly due to the red-brown hue of the red spinach leaf puree used in the formulation. Red spinach contains various nutrients including vitamins A, C, and E, protein, carbohydrates, minerals, iron, calcium, and anthocyanin pigments (Handayani, 2017). Anthocyanins are widely distributed plant pigments that are

water-soluble and appear in colors such as pink, red, purple, blue, and yellow (Adam, 2015).

In addition to the influence of sorghum flour and red spinach, the color of the cookies was also affected by the Maillard reaction occurring during baking (Syafitri, 2021). In this study, baking was conducted at 160°C for 15–20 minutes, which contributed to the brown color of the cookies. The color resulted from the Maillard reaction, a

browning process involving carbohydrates and proteins present in the ingredients (Viani, 2017). Other ingredients, such as chocolate chips, also contributed to the brown appearance, as chocolate chips are inherently brown due to their raw materials (Taufik et al., 2019). Sugar also plays a role in cookie coloration through caramelization during baking (Saragih et al., 2017).

Table 2. Mean Scores of the Hedonic Test for Cookies

Atribute	F1 (Mean±SD)	F2 (Mean±SD)	F3 (Mean±SD)	P-value
Color	2.87 ± 0.973 <sup>a</sup>	3.00 ± 1.145 <sup>a</sup>	3.23 ± 0.935 <sup>a</sup>	0.426
Aroma	3.03 ± 0.850 <sup>a</sup>	3.17 ± 0.874 <sup>a</sup>	3.00 ± 0.743 <sup>a</sup>	0.808
Taste	3.03 ± 0.890 <sup>a</sup>	3.33 ± 0.994 <sup>a</sup>	3.23 ± 0.971 <sup>a</sup>	0.550
Texture	3.01 ± 1.037 <sup>a</sup>	3.33 ± 1.061 <sup>a</sup>	3.40 ± 1.003 <sup>a</sup>	0.966
Aftertaste	2.93 ± 0.980 <sup>a</sup>	2.83 ± 0.950 <sup>a</sup>	3.07 ± 0.868 <sup>a</sup>	0.666
Mouthfeel	3.20 ± 1.064 <sup>a</sup>	3.30 ± 1.088 <sup>a</sup>	3.27 ± 0.740 <sup>a</sup>	0.858

\*\* : test results are significantly different (p<0.05)

<sup>a, b</sup> different letters in the same row indicate a significant difference in value (p < 0.05)

Table 3. Mean Scores of the Hedonic Quality Test for Cookies

Atribute	F1 (Mean±SD)	F2 (Mean±SD)	F3 (Mean±SD)	P-value
Color	2.40 ± 1.476 <sup>a</sup>	2.43 ± 1.357 <sup>a</sup>	2.40 ± 1.163 <sup>a</sup>	0.946
Aroma	2.70 ± 0.877 <sup>a</sup>	2.87 ± 0.819 <sup>a</sup>	3.03 ± 0.718 <sup>a</sup>	0.204
Taste	3.07 ± 0.828 <sup>a</sup>	2.97 ± 0.928 <sup>a</sup>	2.93 ± 0.785 <sup>a</sup>	0.725
Texture	2.87 ± 0.973 <sup>a</sup>	3.33 ± 0.922 <sup>a</sup>	3.37 ± 0.809 <sup>a</sup>	0.073
Aftertaste	3.07 ± 0.868 <sup>a</sup>	3.00 ± 0.947 <sup>a</sup>	3.30 ± 0.702 <sup>a</sup>	0.416
Mouthfeel	3.00 ± 0.910 <sup>a</sup>	2.97 ± 1.033 <sup>a</sup>	2.77 ± 0.858 <sup>a</sup>	0.58

\*\* : test results are significantly different (p<0.05)

<sup>a, b</sup> different letters in the same row indicate a significant difference in value (p < 0.05)

### Aroma

Table 2 presents the mean hedonic scores for the aroma attribute of cookies in formulations F1 (3.03), F2 (3.17), and F3 (3.00). The highest score was observed in F2 (3.17: moderately liked), while the lowest was in F3 (3.00: moderately liked). These findings suggest that the aroma of cookies made with red spinach leaves and sorghum flour ranged from 3.00 to 3.17, indicating a moderate preference by the

panelists. The overall mean aroma score was 3.06 (moderately liked).

Table 3 shows the descriptive hedonic scores for aroma, with average values for F1 (2.70: slightly unpleasant), F2 (2.87: slightly unpleasant), and F3 (3.03: moderately pleasant). F3 received the highest average aroma score, while F1 received the lowest. Aroma plays a critical role in determining the flavor of food. It serves as a strong sensory stimulus,

appealing to the olfactory sense and enhancing appetite. Aroma in food is generated by the formation of volatile aromatic compounds (Putri, 2017). Moreover, these aroma compounds play an important role in the development of flavor enhancers commonly used in the food service industry to improve taste and increase overall product appeal (Antara & Wartini, 2014).

The mean hedonic aroma ratings of cookies made from red spinach leaves and sorghum flour showed no statistically significant differences among the three formulations ( $p = 0.808$ ). F2 was the most preferred formulation in terms of aroma, with a mean score of 3.17 (moderately liked), while F3 was the least preferred, with a score of 3.00 (moderately liked). Similarly, the descriptive hedonic test also showed no significant differences in aroma scores among the formulations ( $p = 0.204$ ), with values of 2.70 for F1 (slightly unpleasant), 2.87 for F2 (slightly unpleasant), and 3.03 for F3 (moderately pleasant).

The improvement in aroma across formulations can be attributed to the reduced use of sorghum flour in F3 compared to F1 and F2. The less pleasant aroma in F1 and F2 may be linked to the higher sorghum flour content. Sorghum flour contains an intermediate level of amylose (22.73%), which can influence the aroma quality of the dough (Suprijadi, 2012). Ingredients with intermediate amylose levels tend to produce more favorable aroma profiles than high-amylose ingredients, as their softer gel consistency has lower binding affinity for volatile aromatic compounds (Syafitri *et al.*, 2021).

In addition to sorghum flour, the use of red spinach also contributed to the aroma profile of the cookies. Red spinach has a characteristic earthy or

grassy smell, which may affect consumer preference. Higher amounts of red spinach tend to reduce panelists' preference for the aroma of red spinach-based dry noodles (Suwita *et al.*, 2012). However, in this study, the use of red spinach in F3 contributed to a moderately pleasant aroma, supported by the presence of fats such as margarine, powdered milk, and eggs in the formulation. Fat is a crucial component in cookie production, enhancing the overall aroma (Rahmat *et al.*, 2020). The addition of powdered milk helps develop aroma, bind water, act as a filler, and strengthen the structure of the dough due to the presence of casein protein (Viani, 2017). Eggs also serve as a binding agent and contribute to a more aromatic cookie (Sutomo, 2008).

### **Flavor**

Table 2 shows the mean hedonic scores for the flavor attribute of cookies in formulations F1 (3.03), F2 (3.33), and F3 (3.23). The highest score was found in F2 (3.33: moderately liked), while the lowest was in F1 (3.03: moderately liked). These results indicate that the flavor of cookies made from red spinach leaves and sorghum flour ranged from 3.03 to 3.33, which falls within the "moderately liked" category. The overall mean flavor score was 3.19 (moderately liked).

According to Table 3, the descriptive hedonic scores for flavor were F1 (3.07: moderately pleasant), F2 (2.97: slightly unpleasant), and F3 (2.93: slightly unpleasant). F1 received the highest average score, whereas F3 had the lowest. Flavor is a sensory parameter that may produce different sensations among panelists due to varying sensitivity of sensory organs, individual familiarity with specific tastes, and personal preferences (Setyaningsih *et al.*, 2015). Flavor is a

key determinant of food preference (Holinesi & Meri, 2021) and is influenced by factors such as temperature, chemical compounds, concentration, and interactions with other flavor components (Kurota, 2015).

The mean hedonic flavor ratings of cookies formulated with red spinach leaves and sorghum flour showed no statistically significant differences across all treatments ( $p = 0.725$ ). F2 was the most preferred formulation with a mean score of 3.33 (moderately liked), while F1 received the lowest score at 3.03 (moderately liked). Similarly, the descriptive hedonic evaluation also indicated no significant differences between treatments ( $p = 0.204$ ), with average scores of 3.07 for F1 (moderately pleasant), 2.97 for F2 (slightly unpleasant), and 2.93 for F3 (slightly unpleasant). Overall, the flavor scores decreased across formulations.

The decrease in flavor quality in F2 and F3 may be attributed to the higher proportion of red spinach used compared to F1. Higher levels of red spinach addition significantly influence flavor, where increased bitterness is associated with decreased panelist preference (Aksamina, 2019). The characteristic bitterness of spinach leaves intensifies with greater addition, negatively affecting flavor (Indraswari *et al.*, 2017). Moreover, a higher amount of sorghum flour may also contribute to reduced flavor acceptability due to the presence of phenolic and tannin compounds, which can impart a slightly bitter and astringent taste (Wisnu *et al.*, 2018).

Interestingly, although F1 contained more sorghum than F2 and F3, it was rated as the most pleasant in flavor. This could be explained by the role of other ingredients such as sugar, powdered milk, margarine, and eggs.

Sugar acts as a sweetening agent that enhances the overall flavor of cookies (Viani, 2017). Powdered milk contributes to flavor and structure by binding water, serving as a filler, and providing protein (casein) that enhances texture (Viani, 2017). Margarine enhances flavor by imparting a savory and rich taste (Anni, 2008). Eggs influence not only flavor but also color and texture, especially due to the emulsifying properties of lecithin found in the yolk (Rakhman, 2012).

### **Texture**

Table 2 shows the mean hedonic scores for the texture attribute of cookies in formulations F1 (3.01), F2 (3.33), and F3 (3.40). The highest score was observed in F3 (3.40: moderately liked), and the lowest in F1 (3.01: moderately liked). These findings indicate that the texture of cookies made from red spinach leaves and sorghum flour ranged from 3.01 to 3.40, categorized as "moderately liked." The overall mean score for texture was 3.24 (moderately liked).

Table 3 presents the descriptive hedonic scores for texture, with mean values of F1 (2.87: not hard), F2 (3.33: slightly hard), and F3 (3.37: slightly hard). Among the formulations, F3 had the highest average score, while F1 had the lowest. Texture is a complex attribute related to the structural composition of food and includes mechanical elements (hardness, elasticity), geometrical properties (grittiness, crumbliness), and mouthfeel (oiliness, moisture) (Setyaningsih *et al.*, 2010). It is defined as the sensory perception of force detected by the mouth during biting, chewing, swallowing, or by touch (Mardesci, 2018). Crispiness or texture is a key determinant of biscuit quality and is closely related to consumer acceptance (Syafitri *et al.*, 2021). Texture is a

characteristic influenced by the physical properties of the material—such as size, shape, quantity, and structural components—perceived through tactile and sensory organs, including the mouth and vision (Midayanto & Yuwono, 2014).

The mean hedonic scores for the texture of red spinach and sorghum cookies showed no statistically significant differences across treatments ( $p = 0.966$ ). F3 was the most preferred formulation with a mean score of 3.40 (moderately liked), while F1 was the least preferred at 3.01 (moderately liked). Similarly, the descriptive hedonic texture scores also showed no significant differences ( $p = 0.073$ ), with average scores of 2.87 for F1 (not hard), 3.33 for F2 (slightly hard), and 3.37 for F3 (slightly hard). The texture across formulations showed a general trend toward increased hardness. This may be attributed to the decreasing proportion of sorghum flour. As sorghum content was reduced, cookies became less hard. Sorghum flour affects cookie texture due to its low gluten content, which limits dough expansion and pore formation, resulting in harder cookies (Kusuma *et al.*, 2017).

Furthermore, the increasing amount of red spinach added influenced the crispness of the product (Mughtar, 2017). The use of red spinach in puree form also impacted cookie texture; the moisture content in the final product did not meet the standard specified in SNI 2913:2022. In this study, the baking process influenced the resulting texture. Cookies in F3 became slightly harder due to a longer baking time (20 minutes), compared to F1 and F2 (15 minutes), as the dough in F3 was slightly softer. According to Rahmiyati *et al.* (2021), baking temperature and duration significantly affect the characteristics and doneness of the final

product. Prolonged baking may result in increased hardness of the food.

The texture of cookies is also influenced by the ingredients used. Sugar contributes to hardness; the higher the sugar content, the harder the texture (Anni, 2008). Egg yolks, on the other hand, soften texture due to the emulsifying properties of lecithin (Rakhmah, 2012). This study also used cornstarch, which has a higher amylopectin content compared to sorghum flour, contributing to a firmer biscuit texture.

#### **Aftertaste**

Table 2 presents the mean hedonic scores for the aftertaste attribute of cookies in formulations F1 (2.93), F2 (2.83), and F3 (3.07). The highest score was observed in F3 (3.07: moderately liked), while the lowest was in F2 (2.83: disliked). These results suggest that the aftertaste of cookies made with red spinach leaves and sorghum flour ranged from 2.83 to 3.07, indicating a range from "disliked" to "moderately liked." The overall mean aftertaste score was 2.94 (disliked).

According to Table 3, the descriptive hedonic scores for aftertaste were 3.07 for F1 (moderate), 3.00 for F2 (moderate), and 3.30 for F3 (moderate), with F3 receiving the highest average score and F2 the lowest. Spinach, including red spinach, inherently has a bitter taste that tends to persist in food products regardless of the quantity used. Even small additions can impart a noticeable bitterness (Octaviyanti, 2017). The aftertaste of red spinach intensifies as its proportion in the product increases, resulting in a more pronounced and lingering flavor in cookies (Suriani *et al.*, 2022).

Sorghum flour contains relatively high levels of tannins, particularly in unpolished (whole grain) forms, which can contribute to an

astringent or puckering sensation (Syafitri et al., 2021). However, the addition of margarine and eggs can influence the aftertaste of cookies. The fat and protein content in the dough helps improve the overall aftertaste quality of the final product (Yudhistira et al., 2019).

### Mouthfeel

Table 2 shows the average hedonic scores for the mouthfeel attribute of the cookies in formulations F1 (3.20), F2 (3.30), and F3 (3.27). The highest mouthfeel score was observed in F2 (3.30: moderately liked) and the lowest in F1 (3.20: moderately liked). Thus, the mouthfeel scores for cookies made with red spinach leaves and sorghum flour ranged from 3.20 to 3.30, indicating a moderate liking by the panelists. The overall average hedonic score for mouthfeel was 3.25 (moderately liked)

According to Table 3, the descriptive hedonic evaluation for mouthfeel showed average scores of 3.00 for F1 (slightly hard), 2.97 for F2 (not hard), and 2.77 for F3 (not hard). Among the formulations, F1 had the highest average score (slightly hard), while F3 had the lowest (not hard).

Products formulated with non-gluten flours tend to result in a less crispy mouthfeel (Rosniar, 2016). Suarni (2016) reported that the use of sorghum flour leads to cookies with a crumbly and less cohesive mouthfeel. Sorghum flour lacks gluten proteins, which play a crucial role in forming the structure and mouthfeel of cookies (Rahmawati et al., 2020). The addition of red spinach also affects cookie crispness; increasing its proportion tends to reduce crispiness (Muhtar, 2017). Moreover, the mouthfeel of cookies can be improved by the inclusion of binding agents such as cornstarch (maizena), which provides a desirable texture due to its carbohydrate content (Utomo, 2019).

### Nutritional Composition

Red spinach leaves and sorghum flour, as the main ingredients in cookie formulation, each contribute specific nutritional advantages. The cookies formulated with red spinach leaves and sorghum flour were prepared in three different formulations: F1, F2, and F3. The nutritional content analysis of these cookies included proximate analysis—covering moisture, ash, protein, fat, and carbohydrate content—as well as iron content. The results of the nutritional analysis are presented in the Table 4.

Table 4. Nutrient Content of Cookies

Attribute	F1 (Mean±SD)	F2 (Mean±SD)	F3 (Mean±SD)	P value	SNI 2022
Moisture (%)	5.01 ± 0.04 <sup>a</sup>	2.66 ± 0.05 <sup>b</sup>	5.94 ± 0.370 <sup>c</sup>	0.000	max. 5
Ash (%)	1.53 ± 0.75 <sup>a</sup>	1.59 ± 0.01 <sup>a</sup>	1.60 ± 0.15 <sup>a</sup>	0.649 <sup>a</sup>	max. 0.1
Fat (g/100 g)	11.82 ± 0.65 <sup>a</sup>	13.19 ± 0.07 <sup>b</sup>	12.68 ± 0.00 <sup>c</sup>	0.000	
Protein (g/100 g)	8.46 ± 0.33 <sup>a</sup>	8.27 ± 0.12 <sup>a</sup>	9.53 ± 0.28 <sup>b</sup>	0.002	min. 4.5; min. 4.1 <sup>1)</sup> ; min. 2.7 <sup>2)</sup>
Carbohydrates (g/100 g)	73.17 ± 0.23 <sup>a</sup>	73.90 ± 0.15 <sup>b</sup>	70.23 ± 0.24 <sup>c</sup>	0.000	
Iron (mg/100 g)	8.97 ± 0.023 <sup>a</sup>	7.44 ± 0.23 <sup>b</sup>	7.87 ± 0.04 <sup>c</sup>	0.000	

\*\* : test results are significantly different (p<0.05)

<sup>a, b</sup> different letters in the same row indicate a significant difference in value (p < 0.05)

### Ash Content

Based on Table 4, the results of the One-Way ANOVA analysis showed no significant effect on the ash content of the cookies ( $p = 0.649$ ). The mean ash content for each formulation was F1 (1.53%), F2 (1.59%), and F3 (1.60%), with the highest mean value observed in F3 and the lowest in F1. The results indicate that the ash content of the cookies increased slightly across all formulations: F1 (1.53%), F2 (1.59%), and F3 (1.60%). However, the addition of red amaranth leaves and sorghum flour did not cause a statistically significant difference in ash content. Furthermore, the ash content values exceeded the maximum limit set by the Indonesian National Standard (SNI 2973:2022), which is 0.1 g.

The increase in ash content is likely due to the incremental addition of red amaranth leaves, which are known to contain substantial mineral levels, including calcium (520 mg/100 g), phosphorus (80 mg/100 g), iron (7.0 mg/100 g), and zinc (0.8 mg/100 g) (Ministry of Health RI, 2018a). Different food ingredients contain varying amounts of ash, as ash is composed of various minerals inherent in the raw materials used (Andarwulan *et al.*, 2011). This finding is consistent with research by Handayani (2017), which reported that higher use of red amaranth increased ash content in wet noodles. Minerals, classified as inorganic substances, are referred to as ash elements in food. When food is heated, the remaining organic matter constitutes mineral elements, which contribute to increased ash levels as the concentration of red amaranth rises (Shiddiq *et al.*, 2019). The rise in ash content with increasing levels of sorghum flour may also be attributed to its mineral content, including magnesium (171 mg/100 g), calcium

(2.5 mg/100 g), zinc (0.44 mg/100 g), and manganese (1.15 mg/100 g) (Susila, 2012). This aligns with findings from Sofyaningsih and Arumsari (2020), who stated that ash content tends to increase with higher amounts of added ingredients due to the accompanying increase in total mineral content.

### Moisture Content

According to Table 4, the results of the One-Way ANOVA test showed that the use of red amaranth leaves and sorghum flour in the cookies had a significant effect on moisture content ( $p = 0.000$ ). Duncan's multiple range test revealed that the moisture content differed significantly across all treatments. The moisture content in formulations F1 and F3 was 5.01% and 5.94%, respectively—both of which did not meet the quality standard requirement of SNI 2973:2022 (minimum 5%). Meanwhile, F2 with 2.66% moisture content complied with the SNI standard.

In this study, the moisture content decreased from F1 to F2. The compliance of F2 with the SNI standard may be due to tighter plastic clip sealing compared to F1 and F3, limiting exposure to ambient air and moisture. The increase in F3's moisture content was likely due to the higher proportion of red amaranth leaf purée, which naturally contains more water than powder form (Handayani, 2016). In addition, sorghum flour used in this research has a relatively low water absorption capacity of 1.51% per 100 grams. This absorption characteristic relates to its amylose content, which ranges from 23–28%, with the remainder being amylopectin. A lower amylose content reduces water absorption and volume expansion, whereas higher amylose content results

in greater water uptake and expansion (Wisnu *et al.*, 2018).

Moisture content is also affected by drying time and temperature. The higher the drying temperature, the faster the evaporation, resulting in lower moisture levels in the material. Likewise, the longer the drying contact time with heat, the more moisture is lost (Gita, 2021). In this study, baking was conducted at 160°C for 15–20 minutes, which also influenced the moisture content of the cookies. However, elevated moisture content could also stem from suboptimal storage practices, especially in the drying phase. Factors affecting drying efficiency include surface area, thickness of the material layer, drying temperature, and inappropriate processing treatments (Nuraeni, Yudi, & Iyan, 2018). In this study, the increased moisture content may have been due to prolonged storage in the laboratory and insufficiently sealed storage in clip-lock plastic bags and containers.

Moisture content in food is closely related to texture and shelf life (Lubis, 2018). Water reduction through drying or the addition of other substances serves to preserve food products by preventing chemical and microbiological spoilage. Water activity is a key factor that influences the stability of dried foods during storage (Normilawati *et al.*, 2019). Moisture content determines product freshness and shelf life. Excessively high moisture levels can promote the growth of bacteria, yeasts, and molds, accelerating food spoilage (Normilawati *et al.*, 2019). The gravimetric method, which was used to determine moisture content, involves heating the sample to evaporate the water and weighing it until a constant mass is reached. A drawback of this method is that other volatile substances

besides water may also evaporate, leading to overestimation of moisture loss.

### **Fat Content**

Based on Table 3, the results of the One-Way ANOVA analysis showed a significant effect on the fat content of the cookies ( $p = 0.000$ ). The average fat content in treatments F1, F2, and F3 was 11.82 g, 13.19 g, and 12.68 g, respectively. The highest mean was found in F2, and the lowest in F1. Duncan's post hoc test revealed that all treatments (F1, F2, and F3) showed significant differences.

The fat content increased from F1 (11.82 g) to F2 (13.19 g), which may have been influenced by other factors, such as the excessive application of margarine to the baking tray during the F2 formulation compared to F1. The fat content decreased from F2 (13.19 g) to F3 (12.68 g), which was likely due to a reduction in sorghum flour used in F3, as sorghum flour contains relatively low fat (3.4 g/100 g) (USDA, 2015). This result contradicts the findings of Loaloka *et al.* (2021), who reported that the increasing addition of red spinach in each formulation led to a decrease in fat content. Red spinach contains low fat, at only 0.8 g/100 g (Kemenkes RI, 2018a).

Other ingredients such as margarine, powdered milk, and egg yolk also contribute to the fat content of cookies. Each formulation used 20 g of margarine (providing 0.06 g fat/100 g), 15 g of powdered milk (providing 3.33 g fat/100 g), and 15 g of egg yolk (providing 2.72 g fat/100 g) (Kemenkes RI, 2018a).

Fat serves as the most energy-dense macronutrient and is essential for various physiological functions. It provides essential fatty acids, including linoleic and linolenic acid, which play a role in growth processes. Additionally, fat aids in the transport and absorption

of fat-soluble vitamins such as vitamins A, D, E, and K (Sunita, 2009).

### **Protein Content**

Based on Table 3, the One-Way ANOVA analysis showed a significant effect on the protein content of the cookies ( $p = 0.002$ ). The average protein content of treatments F1, F2, and F3 was 8.46 g, 8.27 g, and 9.53 g, respectively. The highest value was recorded in F3 and the lowest in F2. Duncan's post hoc test showed significant differences among all treatments.

The higher protein content in F1 compared to F2 is likely due to the greater proportion of sorghum flour in F1. Sorghum flour is a good source of protein, containing 10.6 g/100 g (USDA, 2015). Higher concentrations of sorghum flour increase the protein content in fish nugget formulations (Wisnu *et al.*, 2018).

The increase in protein from F2 to F3 was due to the higher amount of red spinach used in F3. Red spinach contributes 2.2 g of protein per 100 g, a higher value compared to green spinach (Kemenkes RI, 2018a). The addition of red spinach in squid nugget formulations increased the protein content (Rasyid *et al.* 2020).

The protein content in all cookie formulations met the quality standard specified by SNI 2973:2022, which requires a minimum of 4.5% protein. However, it should be noted that protein degradation can occur during baking. High-temperature processing can reduce protein levels in food (Yulianti, 2016). Baking at high temperatures can denature proteins, altering their structure (Sundari *et al.*, 2015). In this study, cookies were baked at 160°C for 15–20 minutes, which may have affected the final protein content (Novia *et al.*, 2011). Additional protein sources in the formulation include eggs (12.4 g

protein/100 g) and powdered milk (24.6 g protein/100 g) (Kemenkes RI, 2018a).

### **Iron**

Based on Table 3, the results of the One-way ANOVA analysis showed that there was a significant effect on the iron content of the cookies, with a  $p$ -value of 0.000. The average iron content in each formulation was F1 (8.97 mg), F2 (7.44 mg), and F3 (7.87 mg), with the highest value found in F1 and the lowest in F2. Duncan's post-hoc test indicated that all treatments (F1, F2, and F3) were significantly different from one another.

According to the 2019 Indonesian Recommended Dietary Allowance (RDA), adolescent girls require 15 mg of iron per day. This high requirement is due to the loss of iron during menstruation (Astrika *et al.*, 2020). Iron is found in all body cells and is primarily stored as ferritin in the liver, spleen, and bone marrow, especially within the reticuloendothelial system (Yuniastuti, 2014). The total amount of iron in the body varies based on age, sex, nutritional status, and iron intake (Darawati, 2014).

Iron plays a crucial role for adolescent girls due to regular blood loss during menstruation. It functions as a cofactor in neurotransmitter activity related to cognition, in hemoglobin synthesis, and in oxygen transport. Iron also supports metabolic processes and contributes to growth and development during adolescence (Martha *et al.*, 2022).

From the iron content analysis, F1 had the highest value (8.97 mg), followed by F3 (7.87 mg), and F2 had the lowest (7.44 mg). The decrease in iron content from F1 to F2 is likely due to the reduced amount of sorghum flour in F2. Since F1 used a higher proportion of sorghum flour, it resulted in greater iron content, as sorghum flour is known

to be rich in iron. Sorghum contains approximately 5.4 mg of iron per 100 grams (USDA, 2015; Wahyani & Rahmawati, 2021).

The slight increase in iron content from F2 to F3 is attributed to the increased amount of red amaranth leaves used in F3. A higher concentration of red amaranth contributes more iron, as this vegetable contains 7.0 mg of iron per 100 grams, which is higher than that of green amaranth (Kemenkes RI, 2018a; Syafitri *et al.*, 2021).

Besides red amaranth and sorghum flour, other ingredients such as powdered milk also contributed to the iron content. Powdered milk contains approximately 3.0 mg of iron per 100 grams (Kemenkes RI, 2018a).

### Selected Formulation

The selection of the preferred cookies formulation was based on the average scores of organoleptic tests and nutritional composition analysis. The final determination was made by combining the percentage scores of sensory evaluation (hedonic and

hedonic quality tests) and nutrient content (moisture, ash, fat, protein, carbohydrates, and iron). A weighting ratio of 50:50 was applied in accordance with the quality standard for cookies as stated in SNI 2973:2022 (Parwati *et al.*, 2023). Based on this scoring system, the formulation that achieved the highest overall score and was selected as the best formulation was F3.

The hedonic test results for formulation F3 indicated good acceptance in terms of color, aroma, taste, texture, aftertaste, and mouthfeel. The F3 cookies had a brown color, moderately pleasant aroma, fairly good taste, slightly hard texture, moderate aftertaste, and a firm mouthfeel.

The nutritional content per 100 grams of cookies from formulation F3 was as follows: energy 433.16 kcal, fat 12.68 grams, protein 9.53 grams, carbohydrates 70.23 grams, and iron 7.87 mg. However, the proximate analysis showed that the moisture content (5.95%) and ash content (1.60%) did not meet the requirements of SNI 2973:2022.

### Contribution of the Selected Formulation to RDA and Nutrient Reference Value (NRV)

Table 5. Nutritional Content Contribution per Serving (75 g / 5 Pieces) Compared to the Recommended Dietary Allowance (RDA) for Female Adolescents Aged 16–18 Years

Nutrient	RDA for Female Adolescents (16–18 years)	Amount of Nutrient Content per Serving Size (75 g / 5 pieces)	% RDA (10–15% Recommended Range)
Energy (kcal)	2100	325	15.47%
Fat (g)	70	9.51	13.58%
Protein (g)	65	7.14	10.98%
Carbohydrates (g)	300	52.67	17.55%
Iron (mg)	15	5.9	39.33%

Table 6. Nutritional Content Contribution Compared to the Nutrient Reference Value (NRV)

Nutrient	Nutrient Reference Value (NRV)	Amount of Nutrient Content per 100 g	% NRV	Nutrition Claim
Energy (kcal)	2150	433.16	20.14%	-
Fat (g)	67	12.68	18.92%	-
Protein (g)	60	9.53	15.88%	-
Carbohydrates (g)	325	70.23	21.60%	-
Iron (mg)	22	7.87	35.77%	High

Table 4 presents the contribution of the selected red spinach and sorghum-based cookies to the Recommended Dietary Allowance (RDA) for adolescent girls aged 16–18 years, based on a serving size of 75 grams (equivalent to five cookies). The serving size was determined to reflect approximately 10–15% of the daily nutritional requirements for this age group.

Based on the results, one 75-gram serving provides 15.47% of the daily energy requirement, 13.58% of total fat, 10.98% of protein, 17.55% of carbohydrates, and 39.33% of the daily iron requirement for adolescent girls aged 16–18 years. These values indicate that the red spinach and sorghum-based cookies meet the nutritional needs of adolescent girls and can serve as a functional food to support iron intake during adolescence.

Table 5 shows the percentage of the Nutrient Reference Value (NRV) for a 100 g serving of the selected cookie formulation. The results demonstrate that the formulation contributes 20.14% of daily energy needs, 18.92% of fat, 15.88% of protein, 21.60% of carbohydrates, and 35.77% of iron based on Indonesian nutrition labeling standards.

These results support the potential of the product to carry a “high in iron” nutrition claim. According to national regulations, a food product can be labeled as “high in iron” if it provides at least 30% of the daily reference value per serving. Therefore, the selected cookie formulation fulfills this criterion, indicating its suitability as an iron-rich functional snack, particularly for adolescent girls who are at higher risk of iron deficiency anemia.

The development of this product is grounded in the growing need for functional foods that not only provide basic nutrition but also address specific micronutrient deficiencies. Iron deficiency remains one of the most prevalent nutritional problems among adolescents, and conventional supplementation programs often face challenges related to compliance and acceptability. Incorporating iron into a widely accepted snack format such as cookies offers a practical and appealing alternative to improve iron intake.

Compared to commercially available cookies, which are generally high in energy, sugar, and fat but low in micronutrients, the developed formulation demonstrates a clear nutritional advantage. Most market cookies do not significantly contribute to daily iron intake and are not designed

with specific health functions in mind. In contrast, this product is specifically formulated to deliver a meaningful amount of iron while maintaining acceptable sensory qualities, thereby positioning it as a value-added functional food.

This innovation highlights the potential of food-based approaches to complement existing nutrition interventions by providing convenient, acceptable, and nutrient-dense snack options that align with consumer preferences while addressing public health concerns.

## CONCLUSIONS AND SUGGESTIONS

The results of this study demonstrated that the nutritional composition of the three cookie formulations (F1, F2, and F3) were as follows: moisture (5.01%, 2.66%, 5.94%), ash (1.53%, 1.59%, 1.60%), fat (11.82%, 13.19%, 12.68%), protein (8.46%, 8.27%, 9.53%), carbohydrates (73.17%, 73.90%, 70.23%), and iron (8.97 mg, 7.44 mg, 7.87 mg), respectively. The analysis indicated that the combination of red spinach leaves and sorghum flour significantly influenced the nutritional content of the cookies, particularly in terms of moisture, protein, fat, carbohydrates, and iron.

The selected formulation (F3), containing 30% red spinach leaves and 70% sorghum flour, yielded a nutritional profile per 75-gram serving (equivalent to five cookies) of 325 kcal energy, 9.51 g fat, 7.14 g protein, 52.67 g carbohydrates, and 5.90 mg iron. Organoleptically, F3 cookies were characterized by a brown color, moderately pleasant aroma, slightly undesirable taste, firm texture, moderate aftertaste, and a hard

mouthfeel. These findings confirm that the variation in red spinach and sorghum flour proportions significantly affected the sensory quality of the cookies.

Moreover, the F3 formulation met the recommended dietary allowance (RDA) percentages for adolescent girls aged 16–18 years and provided 35.77% of the Nutrient Reference Value (NRV) for iron, thereby qualifying for a “high in iron” claim under general labeling regulations. Thus, cookies based on red spinach and sorghum flour, particularly formulation F3, have the potential to be developed as functional food products aimed at reducing the risk of iron deficiency anemia among adolescent girls.

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