Formulation of Black Soybean Tempeh Flour Cookies Fortified with Microencapsulated Iron for Adolescent Girls with Anemia

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ABSTRACT
Background:
Iron deficiency anemia in Indonesian adolescent girls remains high, with a prevalence of 21.7%. To address this, strategies include increasing iron intake per the Recommended Dietary Allowance (RDA). A promising approach is developing iron-rich food products with high acceptability among adolescents. Cookies are a potential vehicle, as they are widely consumed and in demand in Indonesia.
Objective: *This study aims to develop a formula of black soybean tempeh flour cookies with iron microencapsulated fortification which has the potential to prevent iron deficiency anemia in adolescent girls.*
Materials and methods: *This study employed an experimental design using a Completely Randomized Design (CRD) to evaluate the formulation of black soybean tempeh flour cookies fortified with microencapsulated iron. Four levels of substitution of wheat flour with tempeh flour were applied, namely 0%, 20%, 40%, and 60%.*
Results: *The results indicated that cookies formulated with 20% substitution of black soybean tempeh flour (F1) demonstrated the highest acceptability and overall quality among the tested formulations. One serving of the selected formulation contributed approximately 5% of the daily energy requirement for adolescent girls aged 13–18 years. In terms of nutritional value, the selected cookies contained 1.98 mg of iron and 2.31 g of protein per 100 g. These findings suggest that the product has potential as a functional snack, providing a source of iron and protein to support nutritional intake among adolescent girls with anemia.*
Conclusion: *F1 cookies have claim high in iron and a source of protein. Based on the content of iron and protein in 100 grams, each have 1,98 mg and 2,31 grams.*

Keywords: *Black soybean tempeh flour; cookies; fortification; iron*

BACKGROUND

Adolescence is a transitional stage towards adulthood marked by the development of all aspects or functions of the body.[1] Physical changes can affect health status and nutritional status. Nutritional problems can occur if there is an imbalance between nutrient intake and daily requirements. The most common nutritional problem among adolescents is anemia, especially among adolescent girls. The prevalence of anemia among adolescents in Indonesia is 21.7%, with 18.4% of this figure representing the 15-24 year age group.[2] Data from Riskesdas 2018 indicate an increase in the prevalence of anemia among women, reaching 27.2%, with 32% of this group consisting of women aged 15-24 years.[3]

Anemia is a condition that occurs when the number of red blood cells and the oxygen-carrying capacity in the body are insufficient to meet the body's needs.[4] According to the Basic Health Survey 2013 data, the most common type of anemia experienced by adolescent girls in Indonesia is iron deficiency anemia.[5] This can be influenced by both the body's ability to absorb iron and by low iron intake that is below normal requirements.[6] The government's efforts to address anemia among adolescents have so far been limited to providing iron supplementation. Another approach that can help prevent anemia is to provide foods made from ingredients with sufficient iron content. One such ingredient is tempeh.

Tempeh is a food made from fermentation using various types of molds (Rhizopus sp.). These molds produce enzymes that are capable of breaking down complex organic compounds into simpler forms, making them easier for the body to utilize.[7] Another chemical change that occurs during the tempeh fermentation process is the increase in iron solubility due to the formation of protein complexes that are more easily absorbed by the body.[8] Black soybeans (Glycine max L.) are one variety of soybean that serves as a source of plant-based protein, containing 35–40% protein. The anthocyanin isoflavone and iron contents in black soybeans are higher than those in yellow soybeans.[9] The use of black soybeans as a raw material for making tempeh is expected to enhance the nutritional potential of the product.

The development of cookies made with black soybean tempeh flour was chosen because cookies are a popular snack in Indonesia, with an average annual consumption of 0.4 kg/person.[10] Cookies are sweet, dry biscuits with small sizes. The processing of cookies involves baking, a process that can cause some minerals, including iron, to become oxidized and affect their absorption in the body.[11] Therefore, to reduce this risk, iron fortification can be carried out to increase the availability of iron in cookies. Iron microencapsulation fortification is an approach used to maximize the iron content in processed cookie products. Microencapsulation technology for iron is quite effective in reducing interactions between iron and other compounds in food, as well as during human digestion.[12] The microencapsulation process can enhance the stability of the ingredient so that it is less likely to be damaged under high temperatures. Therefore, it is hoped that the development of cookies based on black soybean tempeh flour fortified with microencapsulated iron can provide nutritional benefits as a supplementary food for adolescent girls. In addition, based on those background, the aims of this study was to develop a black soybean tempeh flour cookie formula with iron microencapsulation fortification that has the potential to prevent iron deficiency anemia in adolescent girls.

MATERIALS AND METHODS

Research Design, Time, and Place

This study used an experimental study design with a Completely Randomized Design (CRD). The research was conducted from February to April 2021. The production of black soybean tempeh and cookies was carried out in the Food Experiment Laboratory, while the organoleptic tests took place in the Organoleptic Laboratory, Department of Community Nutrition. The production of iron microcapsules was conducted at the SEAFAST Laboratory, IPB. Proximate analysis, including moisture, ash, fat, and protein content, was conducted at the Nutrient Analysis Laboratory, Department of Community Nutrition. Analysis of iron content was performed at the Laboratory of Education in Nutrition Science and Feed Technology.

Materials

The equipment used in this research is divided into four types: for making cookies, producing microencapsulates, conducting organoleptic tests, and for proximate and iron analyses, as well as another organoleptic test. The equipment required for making black soybean tempeh flour cookies includes baking trays, molds, whisk, oven, and other kitchen utensils such as spoons, bowls, basins, and so on. The equipment used for producing iron microencapsulates includes scales, a blender, and a spray dryer. The tools used during the organoleptic test are organoleptic test forms, pens, drinking water, and samples. The equipment for nutrient analysis includes aluminum dishes, porcelain dishes, ovens, furnaces, analytical balances, a set of protein analysis tools using the Kjeldahl method, and a set of fat analysis tools using the Soxhlet method.

The materials used in this research are divided into three types: for making cookies, iron microencapsulates, and nutrient analysis. The ingredients for making cookies consist of the main ingredients, which are black soybean tempeh flour and wheat flour. Other supplementary ingredients are eggs, cocoa powder, powdered sugar, powdered milk, margarine, and baking powder. The materials used for making iron microencapsulates are iron supplements, gum powder, maltodextrin, and distilled water. The materials required for proximate and iron content analysis include distilled water, H_2SO_4 96%, HCl 37%, selenium mix, H3BO3 10%, NaOH 40%, methyl red, hexane, and HNO_3 65%.

Research stages

The process of making black soybean tempeh refers to Nurhidajah 2010 and Briawan et al 2012.[13,14] The process begins with sorting and washing the soybeans under running water. The cleaned black soybeans are boiled in water at a ratio of 1:4 for about 1 hour in boiling water (100°C). The soybeans are then transferred into a container filled with clean water and soaked for 24 hours. After soaking, the soybean skins are removed and the beans are washed again, then steamed for 15 minutes at a temperature of 80–100°C. Next, the soybeans are dried on a dry tray and tempeh starter is sprinkled at a ratio of 1 gram of starter per 1 gram of black soybeans. The soybeans are then packaged using perforated plastic and fermented for 36 hours at room temperature (28–30°C).

After producing black soybean tempeh, the next step is processing it into tempeh flour. The flour-making process refers to Mustakim et al. with modifications.[15] This step begins with slicing the tempeh into pieces 0.5-1 cm thick and steaming them for about 15 minutes. The slices are then dried in an oven for 1-2 hours at 110°C. Finally, the tempeh is ground using a blender.

The microencapsulation process of iron uses two types of coating materials (gum powder and maltodextrin). The iron compound used is iron sulfate in the form of iron tablet powder. The encapsulation process is carried out using the spray drying technique.[16] The first step is to mix maltodextrin and gum powder in a ratio of 70:30, along with iron tablet powder amounting to 7.5% of the total coating material. The mixture is then dissolved in distilled water at a concentration of 40% and homogenized for 5-10 minutes then dried using a spray dryer. Subsequently, the Fe content in the microcapsules is analyzed using the AAS method. The formulation of cookies based on black soybean tempeh flour refers to Pitaloka with modifications.[17] The formulation is carried out by substituting wheat flour with tempeh flour at levels of 2%, 40%, and 60%. The formula for black soybean tempeh flour cookies fortified with iron is shown in Table 1.

Table 1. Modified black soybean tempe cookies formula by Pitaloka[17]

Ingredients	Amount (g)			
	F0 (0%)	F1 (20%)	F2 (40%)	F3 (60%)
Flour	100	80	60	40
Tempeh flour	0	20	40	60
Sugar	42	42	42	42
Skim milk	20	20	20	20
Egg yolk	20	20	20	20
Margarine	50	50	50	50
Cocoa powder	5	5	5	5
Baking powder	5	5	5	5
Microcapsule of Iron	15.3	15.3	15.3	15.3

The process of making cookies begins with beating sugar and egg yolks for 1 minute until the mixture becomes fluffy. Once fluffy, margarine and baking powder are added and mixed again until the color becomes paler, for about 1 minute. Next, the mixture is combined with the dry ingredients that had been previously mixed together. These dry ingredients include flour, black soybean tempeh flour, cocoa powder, powdered milk, and microencapsulated iron. After all the ingredients are well mixed, the dough is then shaped on a baking sheet. After shaping, the dough is baked in the oven at 130°C for 40 minutes.

The organoleptic test of the cookies consists of a hedonic (preference) test and a hedonic quality test. The organoleptic test was conducted by 30 semi-trained panelists from IPB students. The hedonic test was conducted to determine the panelists' level of preference for the cookie product. The scale used ranged from 1 (dislike very much) to 9 (like very much).[18] The hedonic quality test was carried out to determine the quality of the cookies based on the attributes of color, beany aroma, sweetness, tempeh flavor, bitterness, texture, mouthfeel, and aftertaste. The scale used for the hedonic quality test was a 9-point scale, from 1 (extremely weak) to 9 (extremely strong). Moreover, this study involved human participants in a sensory evaluation test. Although formal ethical approval was not obtained, all procedures were conducted in accordance with ethical principles. Informed consent was obtained from all participants prior to participation, and participants were screened for potential food allergies.

Nutritional content analysis of cookies refers to the Indonesian National Standard (SNI) number 01-2891-1992 for food and beverage testing methods.[19] The analyses conducted include moisture content using the gravimetric method, ash content using the gravimetric method, fat content using the Soxhlet method (AOAC 991.36 2005), protein content using a conversion factor of 6.25, carbohydrate content using the by-difference method, and iron content using the AAS (Atomic Absorption Spectrophotometry) method. The calculation of the nutrient contribution of the cookies is based on the Recommended Dietary Allowance (RDA) for adolescent girls aged 13–18 years. The RDA calculation includes energy, protein, fat, carbohydrate, and iron to assess the adequacy level in a single serving of the selected product. In addition, the calculation of the nutrient contribution to the Nutrition Facts Reference Value (ALG) for processed foods is also carried out. The determination of the estimated selling price for the selected product is done by considering the cost of raw materials, production costs such as electricity and labor, as well as profit. The estimated result is then compared to the selling price of similar cookie products.

Data Processing and Analysis

The data from organoleptic analysis and proximate analysis were tabulated and averaged using Ms Excel 2013, then further processed using the Statistical Package for the Social Science (SPSS) version 16 for Windows. The data were analyzed using analysis of variance (ANOVA) and a follow-up Duncan test.

RESULTS

Development of Black Soybean Tempeh Flour Cookies Products

Microencapsulation is a process of coating a core material with a wall-forming substance to protect the core material from external environmental influences that could interfere with its function.[20] The iron mineral used as the core of the microcapsule is ferrous sulfate compound in the form of Iberet Folic (IF) 500 tablets. The resulting Fe microcapsules have a bright green color. This differs from the microcapsules in previous studies, which stated that the resulting Fe microcapsules were white with a slight yellow tint.[16,21] The Fe microcapsule materials used consisted of 16.67 grams of IF500 powder, 66.64 grams of maltodextrin, and 155.49 grams of gum powder. The total weight of the mixture was 238.79 grams, with the final yield of Fe microcapsules being 157 grams. Based on the Fe mineral analysis results, the Fe content in the microcapsules was found to be 180.55 mg, resulting in a Fe concentration in the microcapsules of 1.2%. The following are the results of black soybean tempeh and black soybean tempeh flour, as shown in Figure 1 and 2. The results of producing black soybean tempeh and black soybean tempeh flour are presented in Table 2.

Table 2 shows that the yield of tempeh obtained was 147%. This increase occurred due to an increase in bulk density volume resulting from soaking and the growth of mold, which makes the structure denser and more compact.[22] The resulting tempeh flour had a yield of 42%. This reduction occurred because the water content in the tempeh was lost during the drying process using an oven.

Figure 1. Black soybean tempeh

Figure 2. Black soybean tempeh flour

Tabel 2. Results of black soybean tempeh and tempeh flour production

Black soybean tempeh		Black soybean tempeh flour	
Soybean weight (g)	500	Tempeh weight (g)	743
Tempeh weight (g)	734	Tempeh flour weight (g)	310
Yield of tempeh (%)	147	Yield of tempeh flour (%)	42

Organoleptic Test Characteristics

Hedonic testing is conducted to determine the acceptability of a food product based on the panelists' preferences for each test attribute. The higher the score given by the panelists, the more they like the product. The scale used in hedonic testing ranges from dislike extremely (1) to like extremely (9). The results of the hedonic test on black soybean tempeh cookies are presented in Table 3.

Tabel 3. Results of the hedonic test on black soybean tempeh flour cookies (mean ± SD)

Atribut	F0 (0%)	F1 (20%)	F2 (40%)	F3 (60%)
Colour	6.90 ± 0.80^{a}	6.97 ± 0.85^{a}	6.53 ± 1.07^{a}	6.63 ± 0.96^{a}
Aroma	6.03 ± 1.54^{a}	6.50 ± 1.11^{a}	6.53 ± 1.28^{a}	6.40 ± 1.35^{a}
Taste	5.67 ± 1.64^{a}	6.83 ± 1.06^{b}	5.83 ± 1.42^{a}	5.43 ± 1.19^{a}
Texture	5.60 ± 1.52^{a}	5.87 ± 1.25^{a}	5.93 ± 1.17^{a}	6.53 ± 1.36^{b}
Aftertaste	5.10 ± 1.54^{a}	6.37 ± 1.13^{b}	5.23 ± 1.41^{a}	4.90 ± 1.24^{a}
Overall	5.57 ± 1.47^{a}	6.57 ± 0.97^{b}	6.03 ± 1.14^{ab}	5.83 ± 0.97^{a}

Note:
a, b, c = Results of ANOVA and Duncan's post hoc test; different letters in the same row indicate significantly different values (p<0.05)
F0 = 0% black soybean tempeh flour, 10% wheat flour F2 = 40% black soybean tempeh flour, 6% wheat flour
F1 = 20% black soybean tempeh flour, 8% wheat flour F3 = 60% black soybean tempeh flour, 4% wheat flour

The results of the hedonic test showed an average acceptance score ranging from 5 (neutral) to 6 (somewhat like). The highest average acceptance score was found in F1. Statistical analysis results indicated that the color and aroma attributes did not differ significantly among the treatment levels given (p>0.05). However, for the taste, aftertaste, and overall attributes, significant differences were found in F1, which had the highest average compared to the other formulas (p<0.05).

The hedonic quality test was conducted to determine the panelists' responses to the organoleptic quality characteristics of several attributes. The hedonic quality attributes assessed included color, aroma, taste, texture, mouthfeel, and aftertaste. The results of the hedonic quality test on black soybean tempeh flour cookies are presented in Table 4.

Table 4. Results of the hedonic quality test of black soybean tempeh flour cookies (mean ± SD)

Atribut	F0 (0%)	F1 (20%)	F2 (40%)	F3 (60%)
Colour	6.23 ± 1.59^{a}	5.93 ± 1.31^{ab}	6.87 ± 1.20^{b}	6.83 ± 1.15^{b}
Unpleasant aroma	5.93 ± 2.42^{a}	6.10 ± 2.01^{a}	5.73 ± 2.18^{a}	6.50 ± 2.06^{a}
Sweet taste	5.17 ± 1.84^{a}	6.40 ± 0.93^{b}	5.23 ± 1.55^{a}	4.80 ± 1.40^{a}
Tempeh taste	4.07 ± 1.91^{a}	4.03 ± 2.01^{a}	4.60 ± 1.90^{a}	4.00 ± 1.97^{a}
Bitter taste	5.00 ± 2.38^{b}	3.70 ± 1.91^{a}	5.47 ± 2.10^{b}	6.63 ± 1.45^{c}
Texture	4.93 ± 2.80^{b}	5.23 ± 2.13^{b}	4.70 ± 2.34^{ab}	3.63 ± 2.20^{a}
Mouthfeel	5.27 ± 2.20^{a}	5.20 ± 1.88^{a}	5.23 ± 1.96^{a}	5.30 ± 1.97^{a}
Aftertaste	6.23 ± 2.08^{b}	4.67 ± 1.95^{a}	6.17 ± 2.04^{b}	6.70 ± 1.39^{b}

Note:
a, b, c = Results of ANOVA and Duncan's post hoc test; different letters in the same row indicate significantly different values (p<0.05)
F0 = 0% black soybean tempeh flour, 10% wheat flour F2 = 40% black soybean tempeh flour, 6% wheat flour
F1 = 20% black soybean tempeh flour, 8% wheat flour F3 = 60% black soybean tempeh flour, 4% wheat flour

The results of statistical analysis show that for the attributes of beany aroma, tempeh flavor, and mouthfeel, there were no significant differences among the treatment levels given (p>0.05).

For the color attribute, it was found that F was significantly different from F1, F2, and F3, indicating that the addition of tempeh flour made the cookies progressively darker in color (p<0.05). Meanwhile, for the attributes of sweetness, bitterness, and aftertaste, F1 was significantly different from the other formulas (p<0.05). For the texture attribute, a significant difference was observed in F3 compared to the other formulas (p<0.05).

Nutritional Content of Cookies Made from Black Soybean Tempeh Flour

The analysis of nutrients is divided into proximate analysis and mineral analysis. The proximate analysis conducted includes moisture content, ash, protein, fat, and carbohydrates. The mineral analysis performed is for iron content. Table 5 shows the amounts of nutrients in the four formulations of cookies made from black soybean tempeh flour.

Tabel 5. Results of the nutritional content analysis of iron-fortified cookies made from black soybean tempeh flour based on %w/w (mean ± SD)

Nutritional content	F0 (0%)	F1 (20%)	F2 (40%)	F3 (60%)	SNI 2973:2011[23]
Water (%)	1.87±0.03^{b}	2.12±0.23^{b}	1.84±0.22^{b}	1.38±0.11^{a}	Max 5
Ash (%)	3.30±0.20^{a}	3.36±0.12^{a}	3.63±0.17^{a}	3.65±0.27^{a}	
Protein (%)	8.87±0.36^{a}	12.32±0.19^{b}	17.03±1.06^{c}	20.82±1.26^{d}	
Fat (%)	20.68±0.90^{a}	22.04±0.72^{b}	23.55±0.30^{c}	25.86±0.56^{d}	Min 4.5
Carbohydrate (%)	65.28±0.63^{a}	60.17±0.55^{b}	53.95±0.93^{c}	48.21±0.97^{d}	
Iron (mg/100g)	8.62±0.25^{a}	9.07±0.92^{b}	10.01+0.12^{c}	10.02±0.12^{c}	

Note:
a, b, c = Results of ANOVA and Duncan's post hoc test; different letters in the same row indicate significantly different values (p<0.05)
F0 = 0% black soybean tempeh flour, 10% wheat flour F2 = 40% black soybean tempeh flour, 6% wheat flour
F1 = 20% black soybean tempeh flour, 8% wheat flour F3 = 60% black soybean tempeh flour, 4% wheat flour

DISCUSSION

The black soybean tempeh produced in the preliminary study had a physically bright appearance on the outside. The peeling of the soybean hull affects the exterior color of the tempeh, making black soybean tempeh brighter and more similar to yellow soybean tempeh. However, the interior of the black soybean tempeh is darker and has a very compact texture. This makes black soybean tempeh less acceptable to the public and difficult to find in the market. After producing the black soybean tempeh, the next stage is processing it into tempeh flour.

The initial formulation was based on research conducted by Pitaloka with modifications.[17] The modifications included substituting wheat flour with black soybean tempeh flour, adding cocoa powder, as well as fortifying with iron microcapsules in F0, F1, F2, and F3. The levels of wheat flour substitution with tempeh flour were determined through a trial process. Pitaloka stated that the acceptability of cookies made with black soybean tempeh flour only reached a ratio of 50% wheat flour and 50% tempeh flour.[17] Therefore, the initial substitution levels used in this study were 0% (F0), 15% (F1), 30% (F2), and 45% (F3). However, after conducting trials, it was found that even at a 60% tempeh flour substitution, the taste was still acceptable, so the substitution levels used were 0% (F0), 20% (F1), 40% (F2), and 60% (F3). This differs from the study by Pitaloka, as cocoa powder was added in this research.[17] The addition of cocoa powder in these amounts can increase the acceptability of products made with tempeh flour by reducing the beany odor and enhancing the flavor, which can be better accepted by panelists.[23] Another modification was the addition of iron microcapsule fortificant. The amount of iron fortificant added was 15.3 grams, containing 17.7 mg of Fe.

The preferred formula was determined based on the panelists' acceptability of the cookies that were most liked, as indicated by the hedonic test results, physical characteristics, and nutritional content. Panelist ratings on a scale of 5 to 9 show that the cookie products were acceptable to the panelists. Based on the overall hedonic test results, the black soybean tempeh flour cookie (F1) had the highest average score of 6.57 (somewhat like), and statistical analysis indicated that F1 was significantly different from the other formulas. The hedonic quality test results also showed that F1 had better physical properties or quality than the other three formulas. The nutritional content of F1, with 20% black soybean tempeh flour substitution, had higher protein and iron levels compared to F0. Therefore, based on these considerations, the selected formula for the black soybean tempeh flour cookies is F1.

The black soybean tempeh flour cookie products have a moisture content ranging from 1.38% to 2.12% (Table 5). These results are in accordance with the quality standards for cookies based on SNI 2973:2011. The analysis showed that the ash content in the products ranged from 3.30% to 3.65%. ANOVA results indicate that the substitution of tempeh flour for wheat flour did not have a significant effect on the ash content of the cookies (p>0.05).

The results of the protein content analysis show that the black soybean tempeh flour cookies ranged from 8.87% to 20.82%. The protein content of the resulting cookies meets the quality requirements of SNI 2973:2011, which specifies a minimum protein content of 4.5%. Table 5 shows that the lowest protein content was found in treatment level F, and the highest at treatment level F3. Substitution of tempeh flour for wheat flour had a significant effect on the protein content at each treatment level (F0, F1, F2, and F3) (p<0.5). The high protein content in tempeh flour is the result of the fermentation process during tempeh production. This fermentation process serves to break down the complex macromolecular compounds found in soybeans into simpler compounds such as amino acids, peptides, fatty acids, and monosaccharides.[24]

The results of the fat content analysis show that the fat content in the cookies ranged from 20.68% to 25.86%. The increase in fat content among the different formulation treatments was significant (p<0.05). This indicates that higher substitution levels of black soybean tempeh flour for wheat flour will further increase the fat content of the cookies. Carbohydrate content was determined by difference, which is based on the proportion of other nutritional components including water, ash, fat, and protein. Carbohydrate content in black soybean tempeh flour cookies ranges from 48% to 65%. The statistical analysis results for carbohydrate content shown in Table 5 indicate a significant difference among the four treatment levels (p>0.05).

Iron (Fe) is a micro mineral that is very important for the body because it functions in the formation of red blood cells and plays a role in the synthesis of hemoglobin.[25] The analysis results for Fe show that the iron content in 100 grams of cookies ranges from 8.62 mg to 10.02 mg. Statistical analysis using ANOVA indicates that the Fe levels in treatments F and F1 are significantly different from those in F2 and F3. The high iron content in the cookies comes from both the fortification with iron microcapsules and several food ingredients such as black soy tempeh flour and egg yolk. Tempeh flour has an iron content of 24.5 mg per 100 grams.[25] The amount of iron in the cookies gives the product a higher functional iron value compared to similar products. An analysis of iron bioavailability is needed to determine how much of the iron mineral present in the cookies and the fortificant can actually be absorbed by the body.

Contribution to RDA, Nutrition label, and Nutrition Claims

The nutritional content per serving size is used to determine the contribution of energy and nutrients from the cookies compared to the recommended dietary allowance (RDA) for adolescent girls aged 13–18 years. The contribution of black soybean tempeh flour cookies to the RDA for adolescent girls and the general recommended daily intake (ALG) is shown in Table 6.

Tabel 6. Contribution of selected iron-fortified black soybean tempeh flour cookies formulas to RDA and general RDI

Nutrient	Nutrient content (100 g)	Serving size (22g)	% of RDA (female adolescent)	General RDI	% of Nutrition label	Nutrition claims
Energy (kkal)	488	107	5	2150	23	-
Protein (g)	12.32	2.71	4	60	21	Source
Fat (g)	22.04	4.85	7	67	33	-
Carbohydrate (g)	60.17	13.24	4	325	19	-
Iron (mg)	9.02	1.98	13	22	41	High

One serving of cookies provides 5% of daily energy, 2.71% protein, and 13% iron. The iron contribution is expected to help meet the iron intake needs of adolescent girls. Black soybean tempeh flour cookies contain 12.3 grams of protein and 9.02 mg of iron per 100 grams, respectively. According to BPOM Regulation No. 13 of 2016 concerning Supervision of Claims on Labels and Advertisements of Processed Foods, black soybean tempeh flour cookies can be claimed as a source of protein and high in iron. This is based on the protein contribution of 21% and iron contribution of 41% in black soybean tempeh flour cookies.

CONCLUSION

Black soybean tempeh flour cookies were made with black soybean tempeh flour substitutions of %, 20%, 40%, and 60%. Each formulation was fortified with 15.3 grams of iron. The cookie formula with the highest acceptability and best organoleptic quality was at the 20% tempeh flour substitution level (F1). The nutritional content of F1 per 100 grams is: 488 kcal energy, 12.32 grams protein, 22.04 grams fat, 60.17 grams carbohydrates, and 9.07 mg iron. The number of cookies in one serving is 7 pieces, weighing 22 grams. The contribution of the cookies to the Recommended Dietary Allowance (RDA) for adolescent girls in one serving is 5% energy, 4% protein, 7% fat, 4% carbohydrates, and 13% iron

Cookies formulated with black soybean tempeh flour fortified with microencapsulated iron demonstrate potential as a functional food to support anemia prevention in adolescents. However, the overall acceptability score of 6.57 indicates moderate consumer preference, suggesting that further sensory optimization is required to enhance product competitiveness. The relatively lower acceptability may be associated with the characteristic beany flavor, darker color, and textural changes resulting from the incorporation of tempeh flour. Therefore, formulation improvements should not be limited to increasing energy density through the addition of high-calorie ingredients such as cheese or chocolate chips, but should also focus on enhancing sensory quality. Potential strategies include the use of natural flavor masking agents (e.g., vanilla or cocoa powder), optimization of fat composition to improve mouthfeel, and modification of baking conditions to achieve a more desirable texture and aroma. In addition, partial substitution with other composite flours or the use of emulsifiers may help improve structural properties and overall palatability. These approaches are expected to increase consumer acceptance and support the product's potential for wider application and commercialization.

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