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RESEARCH STUDY
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Effect of Tempeh Substitution, Addition of Carrot Puree and Moringa Leaf Puree on Protein, Fiber, Iron Content, and Iron Bioaccessibility of Beef Sausage

Pengaruh Substitusi Tempe, Penambahan Puree *Wortel dan* Puree *Daun Kelor terhadap Kandungan Protein, Serat Pangan, Zat Besi, dan Bioassesibilitas Zat Besi Sosis Sapi*

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Sausage, Tempeh, Carrot Puree, Moringa Leaf Puree, Iron Bioaccessibility

ABSTRACT

Background: Cases of iron deficiency anemia among adolescent girls in Indonesia remain high. To address this issue, there is a need to formulate an inexpensive sausage product with relatively high protein, iron, and fiber content.
Objectives: This research was conducted to analyze the effect of tempeh substitution, carrot puree addition, and moringa leaf puree addition on the protein content, dietary fiber, iron, and Fe bioaccessibility in beef sausages.
Methods: This study employed a true experimental design to develop sausage formulations and analyze their protein content, dietary fiber, iron content, and iron bioaccessibility. A 3-factor factorial design was used, with factors including tempeh substitution, carrot puree addition, and moringa leaf puree addition.
Results: The protein content in the sausages was not influenced by any individual factor or interaction between factors, with values ranging from 6.27% to 6.91%. Fiber content was only affected by tempeh substitution, ranging from 11.89 to 13.84 mg/100 g. Iron bioaccessibility was influenced by both tempeh substitution and moringa leaf puree addition, with bioaccessibility values ranging from 84.3% to 87.1%. The highest bioaccessibility was observed with 40% tempeh substitution, 20% carrot puree addition, and 6% moringa leaf puree addition.
Conclusions: Tempeh substitution significantly affected fiber content, iron content, and iron bioaccessibility. Carrot puree addition had no effect on any parameter, while moringa leaf puree addition influenced only iron bioaccessibility.

INTRODUCTION

Adolescent girls are particularly vulnerable to anemia because of their menstrual cycles, which increase the body's need for iron to compensate for iron lost during menstruation[1]. Adolescent girls between the ages of 13 and 18 require approximately 15 mg of iron per day[2].

The prevalence of iron deficiency anemia among adolescent girls in Indonesia remains high[2]. To overcome this problem, an inexpensive sausage product that has relatively high levels of protein, iron, and fiber needs to be formulated.

Sausages are processed meats that are ground and preserved for further use in various dishes. They have a longer shelf life due to the preservation process involving salting[3]. The protein content and saturated fatty acid levels in animal sausages are generally relatively high[4]. Excessive consumption of animal sausages can lead to coronary heart disease and other health issues due to their high saturated fat content, which can be around 30%[5]. Additionally, animal sausages tend to be relatively expensive. Therefore, solutions must be initiated to substitute animal ingredients with vegetable ingredients, which are generally more affordable but can provide equivalent nutritional content. One such vegetable product is tempeh. Substituting tempeh and adding carrot puree can increase the energy and protein content while decreasing the fat and carbohydrate content of the produced beef sausages[6].

Tempe is a nutritious native Indonesian food made from fermented soybeans. During the fermentation process, there are changes in nutritional

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compounds, such as the content of tannins and phytates[7]. his is due to β-glycosidase produced by bacteria, which can release micronutrients and antioxidants from their conjugated bonds[7]. Tempe is highly sought after by the public for its savory and delicious taste, as well as its rich nutrient content[8]. Indonesia is the largest producer of tempeh in Asia, with soybean consumption being 50% for tempeh, 40% for tofu, and 10% for other products (such as tauco, soy sauce, and others)[9].

In addition to the risk of iron deficiency anemia, adolescent girls tend to consume fewer vegetables. The fiber consumption from fruits and vegetables among 95.5% of Indonesians aged 5 years and older is less than 5 servings per day[10]. Dietary fiber is a component of plants that cannot be digested or absorbed by the body. Sources of fiber include vegetables, fruits, legumes, grains, and wheat products[11]. One vegetable that is high in dietary fiber and is relatively preferred by adolescents is carrots.

The carrot plant (*Daucus carota*) is a widely produced vegetable in Indonesia. Carrots are a biennial vegetable with a harvest period of less than one year and are rich in various vitamins, mineral salts, and other nutrients[12]. They contain relatively high levels of beta-carotene and flavonoids, both of which act as antioxidants beneficial to the body[13].

To increase the iron content in sausage products, ingredients with relatively high iron levels, such as moringa leaves, can be added. Indonesia is also abundant in various types of moringa plants (*Moringa oleifera*). According to previous studies[14], in addition to its edible young pods and leaves, moringa is highly beneficial for human health due to its wealth of both macro- and micronutrients. Sausages formulated with moringa leaf and seed extract have been found to contain significantly higher levels of protein, fat, calcium, and iron compared to control groups[15].

This study formulates a sausage product that is more affordable than conventional meat sausages yet provides a comparable protein quality. Additionally, it contains relatively high levels of iron and dietary fiber. A sausage product with these qualities—affordable, nutritionally comparable in protein quality to commercial sausages, and enriched in iron and dietary fiber—could serve as a healthy snack alternative, especially for adolescent girls. The objective of this research is to analyze the effect of substituting tempeh and adding carrot and moringa leaf *puree* on the protein content, dietary fiber, iron, and Bioaccessibility of Fe in beef sausage.

METHODS

This study was a true experimental research project focused on developing a sausage formula and analyzing the protein content, dietary fiber, iron, and Fe bioaccessibility in beef sausage. Iron content refers to the total iron present in the product, while iron bioaccessibility was the percentage of iron available after digestion through the mouth, stomach, and small intestine, indicating the potential for absorption by the intestinal wall. The research design employs a factorial design with three factors: tempeh substitution (30% and 40%), addition of carrot *puree* (10% and 20%), and addition of moringa leaf *puree* (4% and 6%).

Table 1. Research Experiment Design: Tempeh Substitution, Addition of Carrot *Puree* and Moringa Leaf *Puree*

Intervention	W1		W2	
	K1	K2	K1	K2
T1	T1W1K1 (F1)	T1W1K2 (F2)	T1W2K1 (F3)	T1W2K2 (F4)
T2	T2W1K1 (F5)	T2W1K2 (F6)	T2W2K1 (F7)	T2W2K2 (F8)

F=formula, T=tempeh substitution, W=carrot puree addition, K=moringa leaf *puree* addition, T1=30%, T2=40%, W1=10%, W2=20%, K1=4%, K2=6%. Ingredients percentage was calculated from the total weight of beef and tempeh.

The ingredients for each sausage formula include beef, tempeh, carrot *puree*, moringa leaf *puree*, tapioca flour, skim milk powder, pepper, garlic, NaCl, granulated sugar, ice, and cooking oil. The weight of beef without tempeh substitution is 250 grams, with beef weight reduced by the amount of tempeh used for substitution. The formulations of Beef Sausage with Tempeh Substitution, Carrot *Puree*, and Moringa Leaf *Puree* are presented in Table 2.

Table 2. Beef sausages formulation with Tempeh Substitution, Addition of Carrot *Puree* and Moringa Leaf *Puree*

Bahan	Formula							
	F1	F2	F3	F4	F5	F6	F7	F8
Beef (g)	175	175	175	175	150	150	150	150
Tempeh (g)	75	75	75	75	100	100	100	100
Carrot *Puree* (g)	25	25	50	50	25	25	50	50
Moringa Leaf *Puree* (g)	10	15	10	15	10	15	10	15
Tapioca Flour (g)	40	40	40	40	40	40	40	40
Skim Milk Powder (g)	30	30	30	30	30	30	30	30
Pepper (g)	3	3	3	3	3	3	3	3
Garlic (g)	15	15	15	15	15	15	15	15
NaCl (g)	10	10	10	10	10	10	10	10
Granulated sugar (g)	7	7	7	7	7	7	7	7
Ice Cubes (g)	50	50	50	50	50	50	50	50
Cooking oil (g)	20	20	20	20	20	20	20	20

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g=gram, F=formula, F1-F8=Treatment according to experimental design

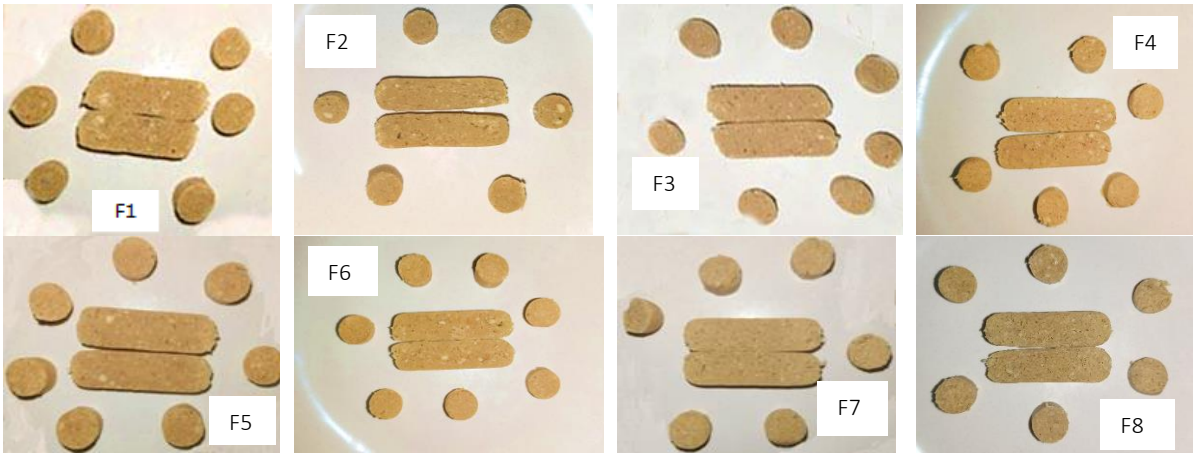
The stages of this study are as follows: 1) formulation and production of sausage; 2) protein analysis (macro Kjeldahl method); 3) dietary fiber analysis (enzymatic-gravimetric method); 4) iron analysis (Atomic Absorption Spectroscopy, AAS); and 5) iron bioaccessibility analysis (AAS). The materials used in this study are those required for sausage production. The primary ingredients for sausage preparation include beef, tempeh, and carrots sourced from the Wonokromo market in Surabaya, as well as moringa leaves obtained from Bojonegoro or Sumenep. Other ingredients for sausage preparation were also purchased from the Wonokromo market in Surabaya. The equipment used for sausage production includes a food processor, sausage stuffer, digital scale, mixing bowls, blender, steamer, trays, stockpot, ladle, strainer, cutting board, spatula, stove, knife, plates, tablespoons, teaspoons, refrigerator, and freezer.

Materials for analysis include H_2SO_4, $HClO_4$, HNO_3, deionized water, pepsin enzyme, pancreatin, bile extract (Sigma B-8631), PIPES buffer [piperazine-NN'-bis (2-ethane-sulfonic acid)] disodium salt (Sigma P-3768), HCl, $NaHCO_3$, KOH, and Bidwell-Sterling distilled water. Analytical instruments include a shaking water bath (Gesellschaft fur Labortechnik Type 1083), analytical balance (Precisa XT-220A), Spectra/Por® I dialysis tubes with a molecular weight cut-off of 6000-8000 (Spectrum, USA), dialysis tubes with a 5 cm diameter, chromameter (Minolta Chromameter CR-310), Atomic Absorption Spectrophotometer (Solaar MS), glassware for analysis, macro Kjeldahl apparatus, and UV-Visible Spectrophotometer. All collected data, including sensory test data and results of protein, dietary fiber, iron, and iron bioaccessibility analyses, were statistically analyzed using three-factor Analysis of Variance (ANOVA).

RESULTS AND DISCUSSIONS

Sausages Product

The sausage formulas were developed using tempeh substitution, carrot *puree*, and moringa leaf *puree*. The resulting sausage had a reddish-brown color due to the brown hue of the substituted tempeh, and the texture was chewy. The following image shows the cross-section (slice) and cut of the sausage.



Tempeh Substitution, Addition of Carrot *Puree* and Moringa Leaf *Puree*

F1=30%, 10%, 4%	F2=30%, 10%, 6%	F3=30%, 20%, 4%	F4=30%, 20%, 6%
F5=40%, 10%, 4%	F6=40%, 10%, 6%	F7=40%, 20%, 4%	F8=40%, 20%, 6%

Figure 1. Sausage produced with Tempeh Substitution, Addition of Carrot *Puree* and Moringa Leaf *Puree*

Table 3. Protein content, fiber, iron, and iron bioaccessibility

Treatment	Protein (%bb)	Fiber (%bb)	Fe (mg)	Bio Ass Fe (%)
F1	16.77±0.25^{Aax}	6.27±0.064^{Aax}	13.84±0.25^{Bax}	84.35±0.61^{Aax}
F2	16.26±1.05^{Aax}	6.39±0.11 ^{Aax}	13.03±0.46^{Bax}	85.50±0.87^{Aax}
F3	16.80±0.52^{Aax}	6.55±0.41^{Aax}	12.88±0.14^{Bax}	85.11±1.48^{Aax}
F4	16.84±0.06^{Aax}	6.33±0.48^{Aax}	12.39±0.46^{Aax}	85.83±0.21^{Bay}
F5	17.03±0.14^{Aax}	6.46±0.56^{Aax}	12.22±0.39^{Aax}	86.07±0.74^{Bay}
F6	17.10±0.06^{Aax}	6.90±0.18^{Bax}	12.52±0.94^{Aax}	86.89±0.18^{Bay}
F7	17.12±0.46^{Aax}	6.91±0.09^{Bax}	11.89±1.48^{Aax}	86.97±0.08^{Bay}
F8	16.69±0.44^{Aax}	6.79±0.04^{Bax}	12.67±0.25^{Aax}	87.10±0.35^{Bay}

%bb = wet weight percent, mg = milligrams, $^{A-B}$) Factor A namely tempeh substitution, $^{a-b}$) Factor B namely carrot *puree* addition, $^{x-y}$) Factor C namely moringa leaf *puree* addition. $^{A, B, a, b, x, y}$ = identical letter notations within the same column suggest that there are no substantial differences among the groups (p-value<0.05).

Protein Content

The protein content of the sausages was not influenced by any of the research factors or their interactions, indicating that tempeh substitution did not affect the protein content of the beef sausages. These results suggest that tempeh substitution does not decrease the protein content of beef sausages. This finding is consistent with the observations of Azizi et al.

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[6,16] and Estiningtyas[16], who reported that tempeh substitution enhances the protein content of sausages. Additionally, Rahmayanti et al.[17] noted that increasing the addition of moringa leaf powder leads to higher protein content in tempeh sausages. The protein content of the beef sausages ranged from 16.26% to 17.12%, which means that the produced sausages met the SNI 01-3820-1995 standard, which sets the minimum protein content for beef sausages at 13%[18]. The protein content of the best-performing product was 16.62%, providing 33.24% of the Recommended Dietary Allowance (RDA) for children aged 10 to 12 years[6].

Fiber Content

The dietary fiber content of the sausages was significantly influenced only by tempeh substitution, while the addition of carrot *puree*, moringa leaf *puree*, and interactions among factors showed no significant effect. This finding is likely attributed to the relatively high fiber content of tempeh. Data analysis indicated that the dietary fiber content in beef sausages substituted with 40% tempeh was higher compared to those with 30% substitution. The dietary fiber content of the beef sausages ranged from 6.27% to 6.91%. The relatively high fiber levels are suspected to be due to the carrot and moringa leaf *puree*s, which are known to be high in dietary fiber, although statistically, these two ingredients did not significantly impact the fiber content. In a related study, beef sausages substituted with banana heart and prepared by steaming contained 7.81% fiber, while those prepared by boiling contained 7.77% fiber[19]. These results contrast with the findings of Prayitno et al., who reported that increasing the substitution of pumpkin flour led to higher fiber content. Furthermore, Rahmayanti et al. observed that the fiber content of tempeh sausages increased with higher levels of moringa leaf powder addition[20,17].

Iron Content

The iron content of the sausages was significantly influenced only by tempeh substitution, while the addition of carrot *puree*, moringa leaf *puree*, and interactions among factors showed no significant effect. Interestingly, increased tempeh substitution resulted in a decrease in iron content, likely due to the relatively lower iron content in tempeh compared to beef. The iron content of the beef sausages ranged from 11.89 to 13.84 mg/100 g. Sausages with 60% tempeh flour substitution contained 23.24% protein, 2.14 mg/100 g of iron, and 1.25 mg/100 g of β-carotene[17].

Iron Bioaccessibility

The bioaccessibility of iron in the sausages was influenced by tempeh substitution and the addition of moringa leaf *puree*, while the addition of carrot *puree* and interactions among factors had no significant effect. Increasing tempeh substitution and adding moringa leaf *puree* enhanced iron bioaccessibility. The high iron bioaccessibility in tempeh is attributed to enzymes produced by bacteria (β-glucosidase) that release micronutrients and antioxidants from their conjugated bonds[7]. Overall, fermentation can serve as a valuable technique to enhance the bioaccessibility of calcium and iron in various food sources[21,22,23-25]. In the case of moringa leaves, the matrix binding iron may not be very strong, allowing for easier release of iron during the *puree*-making process. Fermentation methods may be beneficial for producing functional foods with increased bioavailability of essential micronutrients, which can help alleviate micronutrient deficiencies[26]. The iron bioaccessibility of the beef sausages ranged from 84.35% to 87.10%, with the highest average value observed in the treatment with 40% tempeh substitution, 20% carrot *puree* addition, and 6% moringa leaf *puree* addition.

Advantages and Disadvantages of Research

The sausage product produced contains relatively high protein, dietary fiber and iron with high iron bioaccessibility. This product is suitable as an alternative nutritious snack for teenage girls who suffer from anemia. In this study, only iron bioaccessibility testing was carried out which does not guarantee that the digested iron can also be absorbed properly, therefore it is necessary to continue to test the bioavailability of iron.

CONCLUSIONS

Tempeh substitution had an effect on fiber content, iron content, and iron bioaccessibility. The addition of carrot puree had no effect on any of the parameters. Moringa leaf puree only influenced iron bioaccessibility. Additionally, the interactions among the factors in this study had no effect on any of the parameters.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare that there are no conflicts of interest, and this research was conducted independently. This research article was not influenced by any conflicts of interest on the part of the authors. The study was entirely funded by the first author.

AUTHOR CONTRIBUTIONS

ECA: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, validation, visualization, original draft writing, review & editing; RR & SAM: supervision, reviewing, and mentoring of the article writing process.

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