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The Effect of Combining Pumpkin Seeds and Straw Mushroom with Lesser Yam as a Binder on the Proximate and Amino Acid Profile of a Meat Analog

Pengaruh Kombinasi Biji Labu Kuning dan Jamur Merang dengan Pengikat Umbi Gembili Terhadap Kadar Proksimat dan Profil Asam Amino Daging Analog

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Dyslipidemia, Meat Analog, Pumpkin Seeds, Straw Mushroom, Lesser Yam

ABSTRACT

Background: Dyslipidemia is a significant risk factor for cardiovascular disease. The high prevalence of dyslipidemia is caused by the habitual consumption of foods contain saturated fats, such as beef. Therefore, meat analog made from plant-based ingredients is a potential alternative due to its high protein content and low fat content, especially regarding saturated fats.
Objectives: To determine the effect of pumpkin seeds and straw mushroom combination with lesser yam as a binder on proximate and amino acid profile of meat analog.
Methods: This study used a completely randomized design with three formulations based on the proportions of pumpkin seed flour and straw mushroom flour, such as F1 (75%:25%), F2 (50%:50%), and F3 (25%:75%). Each formulation is added with 25 g lesser yam puree. Proximate analysis was conducted to determine moisture, ash, protein, fat, and carbohydrate, with analysis of amino acid profile of meat analog. Statistical analysis of the data was conducted using ANOVA and Duncan's Multiple Range (DMRT) test.
Results: The three formulations of meat analogue had moisture 42.19-42.29%, ash 2.81-4.34%, protein 15.50-16.90%, fat 3.86-14.08%, carbohydrate 24.01-33.98%, and total amino acids 11.97-14.60%. The results showed that the three formulations of pumpkin seed flour and straw mushroom flour significantly affected ash content (p-value=0.020), fat content (p-value<0.001), carbohydrate content (p-value=0.004), as well as the amino acids threonine (p-value=0.019) and lysine (p-value=0.036).
Conclusions: The combination of pumpkin seed flour, straw mushroom flour, and lesser yam as a binder had a significant effect on the proximate and amino acid profile of the meat analog.

INTRODUCTION

Cardiovascular diseases are a group of disorders that damage cardiovascular system, including the heart and blood vessels[1]. Globally, the number of individuals with cardiovascular diseases doubled between 1990 and 2019, increasing from 271 million cases in 1990 to 550 million cases in 2019[2]. Cardiovascular disease is responsible for 17.9 million deaths annually, accounting for 32% of the total global deaths[3]. Therefore, the prevention and treatment of cardiovascular disease is one of the targets in the *Sustainable Development Goals*, especially target 3.4 which aims to reduce premature mortality from non-communicable diseases by one-third in 2030[4].

Dyslipidemia refers to an abnormality in lipid levels, characterized by increased total cholesterol, *Low-Density Lipoprotein Cholesterol* (LDL-C), and triglyceride levels, with decreased *High-Density Lipoprotein Cholesterol* (HDL-C) levels[5]. Dyslipidemia is recognized as a major contributing factor to cardiovascular disease[1]. A study suggested that abnormal lipid levels (including total cholesterol, LDL-C, and triglycerides) are associated with the incidence of cardiovascular disease after 6 years follow-up[6]. Furthermore, a study conducted in Bali showed a significant correlation between total cholesterol and LDL-C levels and the incidence of hypertension[7].

Dyslipidemia has two types according to its etiology, primary and secondary dyslipidemia[5]. Primary

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dyslipidemia is caused by genetic abnormalities that affect lipid metabolism[8]. In contrast, secondary dyslipidemia is caused by other diseases, medication use, or unhealthy habits[9]. Secondary dyslipidemia occurs in 30–40% of individuals with dyslipidemia[9].

A high-fat diet is an unhealthy lifestyle factor that can contribute to secondary dyslipidemia[10]. The consumption of high-fat foods, particularly those containing saturated fats, can elevate LDL-C levels in the blood[11]. Consequently, foods high in saturated fats are often a concern for individuals with dyslipidemia.

Beef is one of the foods with a high content of saturated fat. The fat content in 100 g of beef (moderate and fatty cuts) ranges from 14.0 to 22.0 g[12]. Moreover, the saturated fat content in beef constitutes half of the total fat[13].

Meat analog has become a potential alternative to beef. Meat analog, or artificial meat, is made from plant-based ingredients and has fibrous characteristics similar to meat[14]. Consumers choose meat analog as a beef substitute to reduce the risk of metabolic diseases and because of its environmental benefits[15].

Pumpkin seeds are often considered as a byproduct of pumpkin (*Cucubirta moschata*) and not commonly consumed. Nevertheless, pumpkin seeds are a source of plant-based protein that can be utilized for meat analog production. The protein content of pumpkin seeds ranges from 30–40% of the total nutritional composition, with bioavailability ranging from 88–97%[16]. Pumpkin seeds contain more protein compared to other seeds or legumes[17]. Additionally, pumpkin seeds contain fiber and vitamin E, components that contribute to hypolipidemic effects[18,19].

Straw mushroom (*Volvariella volvacea*) is a type of mushroom commonly found in Indonesia, but its utilization remains limited. Straw mushroom contain high protein levels compared to other vegetables[20]. Additionally, straw mushroom has a low fat content, approximately 0.2 g per 100 g[12]. In relation to dyslipidemia, a study concluded that the intake of straw mushroom extract leads to a reduction in cholesterol levels[21]. This effect results from the high content of beta-glucan, a soluble fiber commonly found in the mushroom[22].

Lesser yam (*Dioscorea esculenta*) is one of the tubers commonly found in Indonesia and holds significant cultural value[23]. The carbohydrate content in lesser yam can be utilized as a binding agent for meat analogs. This is related to the interaction of carbohydrates with proteins in forming a fibrous texture that imitates beef[14]. In relation to dyslipidemia, consumption of lesser yam (in starch form) can reduce LDL-C and triglyceride levels, with an increase in HDL-C levels[24,25].

Therefore, this study aims to investigate the effect of the pumpkin seeds and straw mushroom combination with lesser yam as a binder on the proximate composition and amino acid profile of meat analog. This meat analog is designed to reduce the incidence of dyslipidemia. It is intended to mimic the characteristics of beef, particularly with its high protein content and complete amino acid profile, while maintaining a low fat content.

METHODS

Table 1. Meat Analog Formulation

Formulation	Ingredients (g)							
	Pumpkin Seed Flour	Straw Mushroom Flour	Lesser Yam Puree	Tapioca	Water	Salt	Shallots and Garlic	Pepper
F1	37.5	12.5	25	15	25	1	2	0.5
F2	25	25	25	15	25	1	2	0.5
F3	12.5	37.5	25	15	25	1	2	0.5

This study is an experimental study with completely randomized design. There are three formulations of pumpkin seed flour and straw mushroom flour, each with two replications. The ratios of pumpkin seed flour with straw mushroom flour in each formulation are F1 (75%:25%), F2 (50%:50%), dan F3 (25%:75%). Meanwhile, the amount of lesser yam puree added is consistent in each formulation.

Materials and Tools

The tools used are categorized into preparation tools, meat analog production tools, and chemical analysis tools. Preparation tools include knives, basins, steam blancher, grinders, drum dryers, cabinet dryers, sieves, and blenders. Meat analog production tools include basins, mixers, and steamers. Chemicals analysis tools include crucible, oven, desiccators, analytical balances, Kjeldahl flasks, destructive instruments, distillation apparatus, Erlenmeyer flasks, titration equipment, fat flasks, filter paper, Soxhlet extraction instrument, evaporating flasks, rotary evaporators, and High Performance Liquid Chromatography (HPLC).

The materials used are categorized into meat analog production materials and analysis materials. Meat analog production materials include pumpkin seeds, straw mushroom, lesser yam, tapioca, water, salt, shallots and garlic, and pepper powder (Figure 1). Analysis materials include $CuSO_4$, K_2SO_4, H_2SO_2, NaOH, Zn powder, aquades, HCl, 0.1% methyl red indicator, hexane solvent, potassium borate, buffer solution, and ortoftalaldehida (OPA) solution.

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(1) Water, (2) Lesser Yam Puree, (3) Tapioca, (4) Straw Mushroom Flour, (5) Pumpkin Seed Flour, (6) Garlic, (7) Shallots, (8) Pepper, dan (9) Salt

Figure 1. Meat Analog Materials

Preparation of Pumpkin Seed Flour

The sorted pumpkin seeds were soaked for 2 hours to remove phytic acid[26]. Then the pumpkin seeds were dried and crushed using a drum dryer (3rpm, T 140°C). Subsequently, the pumpkin seeds were ground using a grinder and sieved through a 60-mesh sieve.

Preparation of Straw Mushroom Flour

Straw mushrooms were sorted and washed to remove any residue. Then, the mushrooms are steam-blanched for 5 minutes to improve the color of the flour. Afterward, the straw mushrooms were ground using a grinder to facilitate the drying process. The straw mushrooms were dried using a drum dryer (3rpm, T 140°C) followed by cabinet dryer (1 hours, T 60°C). After drying, the straw mushrooms were ground again using a grinder and sieved through a 80-mesh sieve[27].

Preparation of Lesser Yam Puree

The lesser yam was sorted and peeled. Then, it was cut into 0,5-1 cm pieces and soaked in salt solution for 30 minutes. Afterward, the lesser yam was steamed for 30 minutes to make it easier to puree. The process was completed using a blender until it becomes a smooth puree.

Meat Analog Production

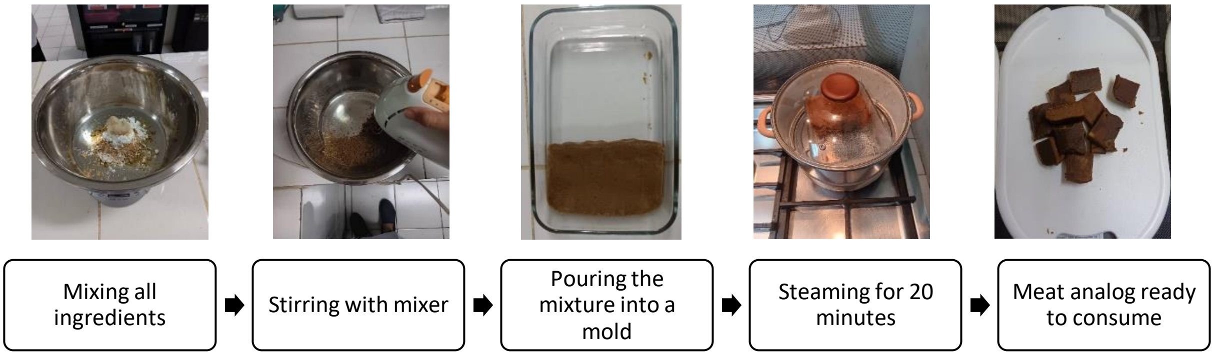

Figure 2. Meat Analog Production

Meat analog production is based on the study by Bintoro *et. al* (2023)[28]. All ingredients are blended according to the formulation (Table 1). The mixture is blended using a mixer until homogeneous. Then, the mixture is poured into a mold with dimensions 20x10x4 cm (lxwxh), with the height set at 1.5 cm. The mixture is steamed for 20 minutes and the meat analog is subsequently cut into pieces with dimensions of 5x4 cm (lxw).

Proximate Analysis: Moisture Content

Moisture content analysis was conducted using the gravimetric method. First, a crucible was dried in an oven for 30 minutes (T 105°C). Then, the crucible was cooled with desiccator for 30 minutes. Approximately 5 g of sample were weighed and crushed. Then, the sample was placed in the crucible and its weight was recorded. The crucible with sample was dried with oven for 6 hours. Afterward, the crucible was cooled with desiccator for 30 minutes. The moisture content was calculated using the following formula.

$$\%moisture = \frac{A - B}{C} x100\%$$

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Notations:
A = The Crucible and Sample Weight Before Drying
B = The Crucible and Sample Weight After Drying
C = The Sample Weight Before Drying

Proximate Analysis: Ash Content

Ash content analysis was conducted using the gravimetric method. First, the crucible was dried in a furnace for 30 minutes (T 600°C). Then, crucible was cooled in a desiccator for 30 minutes. The sample was crushed and weighed, approximately 5 g. The crucible containing the sample was dried with furnace for 6 hours (T 400-600°C). Afterward, the crucible containing the sample was cooled in a desiccator and weighed. The ash content was calculated using the following formula.

$$\%ash = \frac{A - B}{C} x100\%$$

Notations:
A = The Crucible and Sample Weight Before Drying
B = The Crucible and Sample Weight After Drying
C = The Sample Weight Before Drying

Proximate Analysis: Protein Content

Protein content analysis was conducted using Kjeldahl method, which involves 3 stages: destruction, distillation, and titration. In the first stage, the sample was crushed and weighed (±1 g). Then, the sample was placed into a Kjeldahl flask along with 3 g of $CuSO_4$, 7 g of K_2SO4, and 15-25 ml of H_2SO_4 and the mixture was homogenized. The Kjeldahl flask was heated using destructive instrument until the mixture becomes clear and smoke-free.

The next stage, distillation, the destruction solution was transferred into a distillation flask. Then, 25 ml of NaOH 50%, Zn powder, dan aquades were added until the flask is half-filled. In the other hands, 25 ml of HCL 0.1 and 5 drops of methyl red indicator were added to the receiving flask for distillation. The distillation process was conducted until the distillate volume reaches ±100 ml. Finally, titration was conducted on the distillate NaOH 0.1 N until the solution turns orange. Protein content was calculated using the following formula.

$$\%protein = \frac{(V_2 - V_1)NaOH\ x\ N\ NaoHx14{,}008xfk}{sample\ weight\ (mg)x\ 10} x100\%$$

Notations:
V_1 = Volume of NaOH Used for Blank Titration
V_2 = Volume of NaOH Used for Sample Titration
fk = Protein Conversion Factor (6.25)

Proximate Analysis: Fat Content

Fat content analysis was conducted using Soxhlet method. First, the fat flask was dried in an oven and then cooled in a desiccator. The flask was weighed and set up in the Soxhlet apparatus. The sample was prepared by grinding ±2 g of sample, then the sample was wrapped in tubular form with filter paper. The filter paper containing the sample was placed in Soxhlet apparatus, and hexane solvent was added until the sample is fully submerged. The Soxhlet apparatus was connected to a condensor and extraction was carried out for 6–8 hours. Afterward, the flask containing the extracted fat was placed in an oven for 1 hour to evaporate the remaining solvent. The fat flask was cooled and weighed to determine the fat content. The fat content was calculated using the following formula.

$$\%fat = \frac{W_2 - W_1}{sample\ weight} x100\%$$

Notations:
W_1 = Weight of the Empty Fat Flask
W_2 = Weight of the Fat Flask with the Extracted Fat

Proximate Analysis: Carbohydrate Content

Carbohydrate content analysis was conducted using by difference method. Then, carbohydrate content was calculated using the following formula.

%Carbohydrate
= 100% - (%Moisture + %Ash + %Protein + %Fat)

Amino Acid Profile Analysis

Amino acid profile analysis, which includes aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, and arginine, was conducted using HPLC with four stages, protein hydrolysate preparation, drying, derivatization and injection, and amino acid analysis. First, protein hydrolysate preparation stage, the sample was weighed (0.1 g) and crushed. Then, 10 ml of HCl 6N was added to the mixture. The mixture was heated in an oven for 24 hours (T 100°C). Second, drying stages was conducted using rotary evaporator for 15–30 minutes, with the addition of 5 ml of HCl 0.01 N. Then, the sample was filtered using Milipore filter paper. Third, derivatization and injection stage, the sample was mixed with potassium borate buffer solution at ratio 1:1. Then, the mixture was combined with OPA solution at ratio 1:5. Afterward, the result was filtered using Whatman filter paper and injected into HPCL. Amino acid content was calculated using the following formula.

$$\%AA = \frac{Sample\ areaxCxFpxMw}{Standard\ areaxSample\ weight} x100\%$$

Notations:
AA = Amino Acid
C = Amino Acid Standard Concentration
Fp = Dilution Factor
BM = Molecular Weight of Each Amino Acid

Data Analysis

The results of the proximate and amino acid profile analyses were analyzed using analysis of variance (ANOVA). If the ANOVA results indicated significat diffrences, the analysis was followed by Duncan's Multiple Range (DMRT). This study received ethical approval from Komite Kode Etik Penelitian Universitas Pembangunan Nasional "Veteran" Jakarta under approval number: 54/II/2024/KEP, dated February 22, 2024.

RESULTS AND DISCUSSIONS

Raw Material Characteristics

The primary raw materials consist of pumpkin seed flour, straw mushroom flour, and lesser yam puree were analyzed for proximate composition and amino acid profiles. Table 2 presents the results of proximate analysis. Based on the analysis, lesser yam puree has the highest moisture content because of the preparation method, which includes steaming. Pumpkin seed flour has the highest protein and fat content compared to the other two materials. Pumpkin seeds contain a high protein content, range from 30-40%[16]. Furthermore, the high fat content in pumpkin seeds is dominated by unsaturated fats, specifically oleic acid and linoleic acid[26]. Straw mushroom flour has the highest ash and carbohydrate content compared to the others. The high ash content indicaates a high mineral composition[29]. Additionally, the carbohydraye content in the mushroom is dominated by fiber[27].

Table 2. Raw Materials Proximate Analysis Results

Parameter	Pumpkin Seed Flour	Straw Mushroom Flour	Lesser Yam Puree
Moisture (%)	3.38	4.32	89.52
Ash (%)	5.33	8.24	0.02
Protein (%)	38.50	31.31	1.01
Fat (%)	10.88	2.51	<0.02
Carbohydrate (%)	41.91	53.62	9.45

The results of amino acid profile analysis of raw materials are presented in Table 3. Amino acids are the bulding blocks of protein, forming polypeptide chains through covalent bonds between individual amino acids[30]. Pumpkin seed flour has the highest total amino acid profile comapared to straw mushroom flour and lesser yam flour. This indicates that pumpkin seed flour contains the highest protein content (Table 2). A total of 15 amino acids were successfully identified in the three raw materials, consisting of essential, non-essential, and semi-essential amino acids. Glutamate was found to be the highest amino acid in pumpkin seed flour. Meanwhile, leucine was the highest in straw mushroom flour, and arginine was the highest in lesser yam puree.

Table 3. Raw Materials Amino Acids Profile Resuluts

Parameter	Pumpkin Seed Flour	Straw Mushroom Flour	Lesser Yam Puree
Aspartic Acid (%)	3.03	3.00	0.05
Threonine (%)	0.95	1.35	0.02
Serine (%)	1.65	1.37	0.03
Glutamic (%)	7.10	4.62	0.08
Glycine (%)	1.85	1.28	0.02
Alanine (%)	1.51	2.06	0.03
Valine (%)	1.59	1.41	0.03
Methionine (%)	0.58	0.23	<0.00
Isoleusine (%)	1.27	2.33	0.02
Leusine (%)	2.45	5.39	0.04
Tyrosine (%)	1.01	0.86	<0.00
Phenylalanine (%)	1.66	1.41	0.03
Histidine (%)	1.19	0.83	0.06
Lysine (%)	1.34	1.51	0.04
Arginine (%)	4.93	1.45	0.10
Total Amino Acid Profile (%)	**32.11**	**29.07**	**0.57**

Moisture Content

Moisture content is a crucial factor that can affect the shelf life and quality of food. High moisture content leads to reduced shelf life and quality of food[27]. The impact of moisture content on food includes changes in its physical and chemical properties, as well as an increased risk of microbiological spoilage[31]. The variance analysis results showed no significant differences in meat analog moisture content based on ratio of pumpkin seed flour and straw mushroom flour (Table 4).

The meat analog moisture content ranged from 42.19 to 42.29%. This result is higher than study by Lindriati et. al (2018), which reported a moisture content range for meat analog from 12.90 to 23.10%[32]. The high moisture content resulted from the additional ingredients in meat analog, lesser yam, which has a relatively high moisture content (Table 2). Additionally, the steaming process used for preparing meat analog increases the moisture content due to water absorption during processing[33].

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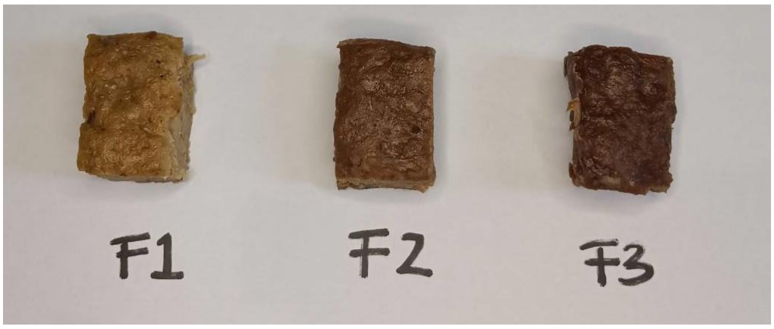

Figure 3. Formulation of Each Meat Analog

Ash Content

Ash content represents the mineral content of a product[29]. The higher the ash content, the the higher mineral content in the product. Table 4 shows the results of variance analysis, indicating a significant difference in the ash content of meat analog. Further Duncan analyis reveals that the ash content of F1 differs significantly from F2 and F3. However, no significant difference in ash content was found between F2 and F3. The raw materials used may contribute to the difference. This is supported by analysis of raw materials, which shows that the ash content of straw mushroom flour was 8.2%, while the ash content of pumpkin seed flour was 5.33% (Table 2). Consequently, a higher proportion of straw mushroom flour compared to pumpkin seed flour results in a higher ash content.

The meat analog ash content ranged from 2.81 to 4.34%. The meat analog has higher ash content compared to beef, which ranges between 0.5% and 1.4%[12,34]. This is due to the plant-based ingredients used in meat analog, which contain minerals derived from soil (trace elements). However, the high ash content in meat analog does not correlate with high bioavailability or bioaccessibility of the mineral content[35].

Protein Content

Protein is one of the most important components in side dishes. The protein content in animal-based material is generally high in both quality and quantity compared to plant-based amerta[36]. However, complementing various plant-based proteins can be a strategy to meet protein requirements. The variance analysis results showed no significant differences in meat analog protein content based on ratio of pumpkin seed flour and straw mushroom flour (Table 4). The raw materials used in the meat analog contain high protein content. However, pumpkin seed flour contains 38.50% protein, which is higher than mushroom flour at 31.31% (Table 2). The high protein content in pumpkin seed flour can be explained by the accumulation of proteins in seeds used during the germination process[37]. Meanwhile, the mushroom group is known to have higher protein content compared to vegetables, fruits, and grains[38]. Protein content in mushroom is often utilized as a meat alternative due to its relatively high protein quality, which contributes to health benefits and has a lower environmental impact[39].

Meat analog protein content in this study ranged from 15.50 to 16.90%. This is higher than study by Wibawa et. al (2023), which showed that the protein content in meat analog ranged from 9.05 to 11.90%[40]. The variation is a result of the different protein sources and the quantities used in meat analog product. The protein content of meat analog in this study is also comparable to beef, which ranges between 17.5% to 19.6%[12].

Fat Content

Fat content in food is considered by consumers because its association with an increased risk of various diseases, including cardiovascular disease[6]. This is primarily attributed to the cholesterol and saturated fatty acids present in the food. The variance analysis results showed significant differences in meat analog fat content based on ratio of pumpkin seed flour and straw mushroom flour (Table 4). Differences were observed in all three formulations. A higher proportion of pumpkin seed flour compared to straw mushroom flour resulted in increased fat content in the meat analog. This is because pumpkin seed flour has a relatively high fat content (10.88%), compared to straw mushroom flour fat content (2.51%) (Table 2). The high fat content in pumpkin seeds is predominantly composed of unsaturated fatty acids[26], which are known for their health benefits, including anti-inflammatory effects[20].

The fat content of meat analog in this study ranged from 3.86 to 14.08%. This is higher than the fat content reported by Wibawa et. al (2023), which ranged from 0.25 to 1.21%[40]. However, the fat content in this study is comparable to the de Angelis (2020) study, which had meat analog with fat content ranging from 4.26 to 8.93%[41]. Furthermore, the fat content in this study tends to be lower than beef, which has a fat content ranging from 10 to 22%[12]. Low fat content in meat analog is an advantage because it can be used to reduce the risk of dyslipidemia.

Table 4. Meat Analog Proximate Analysis Results

Parameter	Formulation			p-value
	F1	F2	F3	
Moisture (%)	42.19±2.022[a]	42.23±1.576[a]	42.29±1.152[a]	0.998
Ash (%)	2.81±0.417[a]	3.63±0.040[b]	4.34±0.113[b]	0.020*
Protein (%)	16.90±0.989[a]	16.03±0.332[a]	15.50±0.289[a]	0.227

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Parameter	Formulation			p-value
	F1	F2	F3	
Fat (%)	14.08±0.106[a]	9.78±0.360[b]	3.86±0.374[c]	0.000*
Carbohydrate (%)	24.01±0.509[a]	28.31±0.919[b]	33.98±1.124[c]	0.004*

The proportions of pumpkin seed flour and straw mushroom flour in meat analog are F1 (75%:25%), F2 (50%:50%), and F3 (25%:75%); similar superscript notations (a,b,c) within the same parameter indicate no significant difference based on Duncan's test (p-value≥0,05); symbol (*) denotes paramters with significant differences.

Carbohydrate Content

Predominantly, meat analog contains carbohydrate ranging form 10% to 30%[42]. This carbohydrate content contributes to forming a fibrous texture similar to beef[43]. The variance analysis results showed significant differences in carbohydrate content based on ratio of pumpkin seed flour to straw mushroom flour (Table 4). Furthermore, Duncan's test revealed significant differences among three formulations. Two raw materials, pumpkin seed flour and straw mushrom flour, have high carbohydrate contents, 41.91% and 53.62%, respectively (Table 2). However, the carbohydrate content in straw mushroom flour is higher than in pumkin seed flour, resulting in an increased carbohydrate content in the meat analog as the proportion of mushroom flour increases. It is known that carbohydrate content in mushrooms consist mainly of dietary fiber[27]. Additionally, supplementary ingredients, such as lesser yam puree and tapioca, contribute to the carbohydrate content in meat analog. These ingredients are carbohydrate sources that can serve as staple foods[23].

The carbohydrate content of meat anaog in this study is ranged from 24.01 to 33.98%. This is lower than the carbohydrate content in the study by Wibawa et. al (2023), which reported meat analog with carbohydrate content ranging from 45.84 to 53.37%[40]. However, the carbohydrate content in this study is higher compared to commercial meat analogs with carbohydrate content ranging from 9.63 to 20.31%[44]. The ingredients used are responsible for these differences.

Amino Acid Profile

Amino acids are the building blocks of proteins, linked by peptide bonds[30]. Animal-based foods, such as meat, are known to have a complete amino acid profile compared to plant-based foods[45]. However, a combination of plant-based foods can fulfill daily amino acid requirements.

The amino acid profile analysis in this study was conducted to compare the amino acid content of the meat analog with beef. This is because the production of meat analog aims to resemble the characteristics of beef, including having a complete amino acid profile. The variance analysis results showed no significant differences in the total amino acid profile based on ratio of pumpkin seed flour to straw mushroom flour (Table 5). However, this study observed a decrease in the total amino acids across the formulations. This decrease may be attributed to the reduction of pumpkin seed flour. This is supported by raw material analysis, which shows that the total amino acid content of pumpkin seed flour is higher than that of straw mushroom flour (Table 2). Furthermore, the total amino acid profile of meat analog in this study is lower than total amino acids in beef, which ranges from 22.15 to 24.85%[46].

This study successfully analyzed 15 amino acids, including essential, non-essential, and semi-essential amino acids. Table 5 shows that arginine was the highest amino acid in F1, while glutamate was the highest amino acid in F2 and F3. Additionally, methionine was the limiting amino acid in all formulations.These results were similiar to beef, which has glutamate as the highest amino acid and methionine as the lowest[46].

Table 5. Meat Analog Amino Acid Profile Results

Parameter	Formulation			p-value
	F1	F2	F3	
Aspartic Acid (%)	1.18±0.091[a]	1.18±0.021[a]	1.14±0.063[a]	0.796
Threonine (%)	0.46±0.021[a]	0.53±0.007[b]	0.57±0.021[b]	0.019*
Serine (%)	0.72±0.056[a]	0.69±0.007[a]	0.67±0.035[a]	0.572
Glutamic (%)	2.67±0.219[a]	2.37±0.091[a]	2.17±0.091[a]	0.092
Glycine (%)	0.66±0.056[a]	0.61±0.014[a]	0.55±0.028[a]	0.130
Alanine (%)	0.68±0.042[a]	0.72±0.028[a]	0.76±0.028[a]	0.204
Valine (%)	0.69±0.134[a]	0.71±0.098[a]	0.61±0.035[a]	0.632
Methionine (%)	0.18±0.014[a]	0.14±0.035[a]	0.10±0.014[a]	0.092
Isoleusine (%)	0.45±0.028[a]	0.64±0.289[a]	0.44±0.028[a]	0.483
Leusine (%)	0.81±0.070[a]	1.22±0.671[a]	0.70±0.035[a]	0.467
Tyrosine (%)	0.58±0.035[a]	0.46±0.134[a]	0.51±0.021[a]	0.437
Phenylalanine (%)	1.10±0.070[a]	0.95±0.282[a]	1.08±0.035[a]	0.662
Histidine (%)	0.96±0.077[a]	0.79±0.261[a]	0.83±0.021[a]	0.590
Lysine (%)	0.58±0.014[a]	0.62±0.007[a,b]	0.65±0.021[b]	0.036*
Arginine (%)	2.85±2.078[a]	1.48±0.247[a]	1.15±0.035[a]	0.437
Total Amino Acid Profile (%)	**14.60±3.019[a]**	**13.16±0.332[a]**	**11.97±0.374[a]**	**0.435**

The proportions of pumpkin seed flour and straw mushroom flour in meat analog are F1 (75%:25%), F2 (50%:50%), and F3 (25%:75%); similar superscript notations (a,b) within the same parameter indicate no significant difference based on Duncan's test (p-value≥0,05); symbol (*) denotes paramters with significant differences.

Variance analysis was also conducted on the 15 amino acids that were successfully identified. Table 5 shows that only 2 of 15 amino acids, lysine and threonine, have significant differences based on ratio of pumpkin seed flour and straw mushroom flour. Afterward, Duncan's test for lysine revealed a significant difference between F1 and F3, but no significant difference between F2 and the other formulations. Additionally, Duncan's test for threonine revealed a significant difference between F1 and the other formulations, but no significant difference between F2 and F3. The higher proportion of straw mushroom flour compared to pumpkin seed flour explained the differences obeserved in lysine and threonine. This is supported by the raw materials analysis, which showed higher lysine and threonine content in mushroom flour compared to pumpkin seed flour (Table 2).

Threonine is one of the essential amino acids that has influence in dyslipidemia. A study by Ma *et. al* (2020) observed that obese rats given a high fat diet with threonine supplementation (3% in drinking water) showed a trend of reduced body weight, body fat mass, triglyceride levels, total cholesterol, and LDL-C[47]. On the other hand, the essential amino acid lysine was found to have no impact on dyslipidemia, according to the same study.

The strenghts of this study, such as using the local food, providing an alternative to beef, and having the amino acid profile that closely resembles beef. However, the limitations of this study, such as not conducting the shelf life analysis, still using other binding (tapioca), and only addressing total fat content, which does not allow for identification of the fat types.

CONCLUSIONS

The results conclude that the proportion of pumpkin seed flour and straw mushroom flour has a significant differences in ash, fat, carbohydrate, as well as threonine and lysine content. The nutritional content of the meat analog in this study is not significantly different from other studies about meat analogs. However, the fat content is higher, although still lower compared to beef. Pumpkin seed flour, with its relatively high fat content, plays a role in increasing the fat content of meat analog. Meanwhile, the amino acids of meat analog in this study are quite similar to beef, with glutamate as the highest amino acid and methionine as the limiting amino acid. A suggestion for future research is to process the meat analog using alternative method, such as extrusion. This process may reduce the moisture content and extend the shelf life of meat analog.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest regarding this article. This research was funded by PT Indofood Sukses Makmur Tbk. under contract number SKE.037/X/CC/X/2023.

AUTHOR CONTRIBUTIONS

SM: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, visualization, dan writing – original draft; NN: methodology, resources, supervision, validation, dan writing – review & editing; AF: supervision, validation, dan writing – review & editing.

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