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## Original Research

### Development of food for special dietary uses of diabetes based on oyster mushroom and brown rice

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**Running title:** Development of Food for Special Dietary Uses of Diabetes

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**Key words:** brown rice; diabetes food; oyster mushroom; special dietary

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**Significance for public health:** The coadministration of oyster mushrooms and brown rice has shown significant efficacy in regulating blood glucose levels. This intervention has received positive acclaim from the general public and demonstrates great potential for individuals managing diabetes. The formulation of Food for Special Dietary Uses (FSDU) that integrates this combination could present a valuable option for improving the well-being of individuals with diabetes, yielding notable societal benefits.

## **Abstract**

Diabetes patients often struggle to meet their energy requirements, prompting the suggestion of utilizing Food for Special Dietary Uses (FSDU). Previous research has shown the efficacy of oyster mushrooms' B-glucan in controlling hyperglycemia and insulin resistance. Brown rice, high in magnesium and fiber with a low glycemic index, is known to lower blood glucose levels. Utilizing both ingredients in FSDU may provide products with recommended energy value, nutrient ingredients, and glycemic index. This study aimed to measure the energy, protein, fat, carbohydrate, fiber, glucose, and glycemic index of four mixed formulations of oyster mushrooms and brown rice, along with a control.

The study used a true experimental design with a completely randomized research design. The formulations included: P1=9% mixture of moringa and fish flour, P2=10% mixture of carrot and fish flour, F3=11% mixture of moringa and tempeh flour, and F4=12% tempeh flour. The total energy was measured using various methods: adiabatic oxygen bomb calorimeter for energy, Kjeldahl for protein, Soxhlet for fat, by-difference for carbohydrates, AOAC enzymatic-gravimetric for total dietary fiber, anthrone for total sugar, and blood glucose tests at 0, 15, 30, 45, 60, 90, and 120 minutes post-test-food ingestion for glycemic index measurements. Statistical analysis was performed using the One-Way ANOVA and Kruskal-Wallis tests.

The research results indicate significant differences in energy, protein, fat, carbohydrates, total sugar, and fiber values among groups ( $p < 0.01$ ). The lowest glycemic index was found in P4 (44.32, medium category). Further analysis indicated that P4 had higher total energy and fat, but lower carbohydrates, total sugar, and glycemic index. According to the Multiple Attribute Zeleny Method, the P4 formula was the best formula for all parameters.

The most effective formula, containing brown rice, oyster mushrooms, and tempeh flour (P4), could be a beneficial option for improving the health of individuals with diabetes in the community.

## Introduction

Diabetes mellitus (DM) is the most prevalent type of diabetes, accounting for more than 90% of all diabetes cases worldwide.<sup>1</sup> The World Health Organization (WHO) predicts an increase in DM cases in Indonesia from 8.4 million in 2000 to 21.3 million by 2030.<sup>2</sup> In line with that, data from the International Diabetes Federation (IDF) rank Indonesia as the fifth country worldwide in terms of DM prevalence.<sup>1</sup> The results of the Basic Health Research in 2018 showed an increase in the prevalence of DM from 6.9% in 2013 to 8.5% in 2018.<sup>3</sup> These data emphasize the imperative for continuous efforts in DM management and prevention.

The etiology of DM is multifactorial and can be classified into two categories: unmodified and modified. Unmodifiable risk factors for DM include race, ethnicity, family history, age, history of gestational DM, and history of low birth weight. Meanwhile, modifiable risk factors for Type 2 Diabetes Mellitus (T2DM) include overweight (BMI  $\geq$  23 kg/m<sup>2</sup>), lack of physical activity, hypertension ( $\geq$  140/90 mmHg), dyslipidemia (HDL  $<$  35 mg/dL and/or triglycerides  $>$  250 mg/dL), unhealthy diet characterized by high sugar and low fiber intake.<sup>4,5</sup> Nutritional therapy plays a pivotal role in managing DM patients.<sup>2</sup> Recommendations include limiting high glycemic index foods, increasing fiber-rich foods, and reducing saturated fat consumption. Furthermore, adherence to a consistent meal schedule, as well as careful attention to meal type and quantity, are crucial for DM patients. Despite these recommendations, according to Harmawati (2020), DM patients encounter obstacles in adhering to dietary guidelines, including the fear of sugar consumption, dissatisfaction with prescribed diets, and boredom with menu options. Currently, the development of Food for Special Dietary Uses (FSDU), catering specifically for DM patients, remains limited.<sup>6</sup> This is a challenge for DM patients in meeting their nutritional needs. FSDU for DM patients constitutes a specialized food formula designed to meet the dietary requirements of DM patients, providing an alternative or supplementary option that aligns with their daily nutritional needs and intake considerations.<sup>7</sup>

The preference for rice among Indonesians is very high, given its status as a staple food in daily meals.<sup>8</sup> However, people with diabetes mellitus (DM) must carefully consider their consumption of white

rice due to its high glycemic index, ranging from 50 to 87. As a result, many choose to substitute white rice with alternatives, such as brown rice.<sup>9</sup> Brown rice offers benefits for diabetes management due to its high fiber content, along with essential vitamins and minerals.<sup>10,11</sup> In line with that, the research conducted by Handayani et al. (2020) proved that brown rice consumption leads to reductions in blood glucose and HbA1C levels among respondents with DM.<sup>12</sup> Similarly, a study by Golzarand et al. (2021) showed that brown rice consumption significantly contributes to weight loss, improved lipid profiles, and lower fasting blood glucose levels compared to white rice.<sup>13</sup>

In the development of Food for Special Dietary Uses (FSDU) for T2DM patients, the inclusion of other functional foods, such as oyster mushrooms, is also needed. Oyster mushrooms, well-known for their high-fiber food, are a popular choice in the community. Moreover, they contain  $\beta$ -glucan, known as phytoinsulin, which is insulin derived from plants. This compound stimulates  $\beta$ -pancreatic cells in the body to produce more insulin, thus reducing blood glucose levels.<sup>14</sup> Additionally, white oyster mushrooms are also rich in fiber, which, when fermented within the body, increases the production of glucagon-like-peptide-1, helping in glycemic control in the body. Research indicates that the administration of oyster mushroom *infusa* extract exhibits a greater ability to reduce blood glucose levels compared to antidiabetic oral drugs.<sup>15</sup>

Considering this background, it is essential to research the development of food products utilizing brown rice and oyster mushrooms as the main ingredients in making FSDU for T2DM patients. This study aimed to develop FSDU products made from brown rice and oyster mushrooms with various flavors derived from fish flour, carrot flour, moringa flour, and tempeh. These products are expected to meet the energy content and nutrient requirements and maintain low GI, aligning with the specifications outlined for FSDU intended for individuals with DM and adhering to commercial FSDU standards.

## **Materials and Methods**

This research consisted of two main stages: product development and glycemic index testing. The first stage used a True Experiment design with a Complete Randomized Design. The sample consisted of

four modified formulations along with one control formula. The basic ingredients of FSDU remained the same across all four formulations, consisting of brown rice and oyster mushrooms. However, each formulation varied in the additional ingredients, which included fish flour, tempeh flour, carrot flour, and moringa flour. The additional ingredients were incorporated to provide additional protein to FSDU. According to Zetta (2018), for food research, each food sample underwent three repetitions for analysis, resulting in a total of 15 research sample units.<sup>16</sup> The second stage of the research involved quantitative research using a quasi-experimental method with a pre-posttest design. The objective was to determine the differences in glycemic index value between the four FSDU products developed compared to FSDU commercial products.

#### ***Energy, Macronutrients, Sugar, and Fiber Analysis Procedure***

Protein content was determined using the Kjeldahl method, with measurements conducted three times. The analysis process involved three stages: deconstruction, distillation, and titration. Fat was analyzed using the Soxhlet method. Carbohydrate analysis was carried out using by-different methods in proximate analysis, calculated using the formula:  $100\% - (\text{water content} + \text{ash content} + \text{fat content} + \text{protein content})$ . Energy content analysis was carried out using the Adiabatic Oxygen Bomb Calorimeter method. The sugar content, including reducing and non-reducing sugars, was measured using the Anthrone test with Spectrophotometry. The amount of dietary fiber contained in the product was determined using the enzymatic-gravimetric method.

#### ***Glycemic Index Measurement Procedure***

Ten subjects in this study were selected through purposive sampling. The inclusion criteria included willingness to participate by signing informed consent, normal nutritional status (Body Mass Index 18.5-25 kg/m<sup>2</sup>), age between 18-24 years, normal blood pressure, no history of diabetes mellitus, kidney disease, and liver disease, no family history of DM, no digestive disorders, no psychological stress and healthy, not taking medication, and normal fasting glucose levels <110 mg/dl. The exclusion criteria were failure to fast for 10 hours prior to data collection, illness, and the decision to withdraw from the study.

Materials used in the glycemic index test were four developed FSDU products, commercial FSDU products, and white bread as a reference/standard food; all products were prepared with an equivalent of 25 g of carbohydrate. The procedure in the glycemic index test involved fasting for 10 hours (22.00-08.00 WIB), followed by blood glucose levels measurement at 08.00 (minute 0). Test foods, including white bread, were consumed within 10 minutes, and blood glucose levels were measured at 15, 30, 45, 60, 90, and 120 minutes post-consumption. The same procedure was repeated for the glycemic index testing of the developed FSDU and commercial FSDU, conducted consecutively at a two-day interval. The results of blood glucose levels at each measurement time were plotted on the x-axis (time in minutes) and y-axis (blood glucose levels). Incremental Area Under Curve (IAUC) calculation used a Time Series Response Analyzer. Determination of the glycemic index value was determined by comparing the IAUC area among the developed FSDU products, commercial FSDU products, and white bread as reference food.<sup>18</sup>

#### ***Procedure for Determining the Best Treatment***

The best formulation was determined using the Multiple Attribute method (Zeleny, 1982), considering parameters such as energy content, protein, fat, carbohydrates, sugar, fiber, and glycemic index. The steps involved determining the ideal value of each parameter, calculating the degree of density (dk), and calculating the degree of density (Lp). The best treatment was selected based on the treatments with the minimum number of values.

#### ***Statistical Analysis***

Data were presented as Mean  $\pm$  SD for normally distributed data and Median  $\pm$  SE for non-normally distributed data. The data normality test was assessed using the Shapiro-Wilk test. For non-normally distributed data, various transformations such as squared variables, tripled variables, 1/variable, log<sub>10</sub>variable, and variable roots were used. Homogeneity of variance was examined using the Levene Test for normally distributed data. If the data were normally distributed and homogenous, the One-Way ANOVA test was applied, followed by the Bonferroni Post Hoc test to identify differences between treatments. Non-



normally distributed data were analyzed using the Kruskal-Wallis test. If  $p < 0.05$ , indicating a significant difference, the analysis proceeded with the Mann Whitney U Post Hoc test to determine the difference between treatments. A significance level of  $p < 0.05$  was utilized to determine statistical significance. All experimental procedures were approved by the Research Ethics Committee of Malang State Health Polytechnic (441/KEPK-POLKESMA/2022).

## **Results and Discussion**

The development of Food for Special Dietary Uses (FSDU) for Diabetes Mellitus patients based on brown rice and oyster mushrooms is an alternative food product utilizing local food ingredients. This food product was formulated into four distinct formulations, each incorporating protein-rich flour ingredients (Figure 1).

Table 1 shows the formulation of FSDU development based on brown rice and oyster mushroom. The results of energy and nutrient analysis of FSDU product development are presented in Table 2. Among the formulations, P4 exhibited the highest energy, protein, and fat values, while P3 contained the highest carbohydrate value, and P4 had the highest dietary fiber and sugar levels. The t-test results showed significant differences in at least one group regarding the values of energy, protein, fat, carbohydrates, water content, ash content, dietary fiber, and total sugar., attributed to variances in the additional ingredients. The addition of fish and moringa flour (P1), fish and carrot flour (P2), tempeh and moringa flour (P3), and tempeh flour (P4), aimed to provide a variety of aromas and flavors, impacted the energy and nutrient content of the products.

All developed formulations showed higher energy content compared to P0. These were attributable to the addition of fat sources such as soybean oil and coconut oil. Fat plays an essential role in increasing the calorie content of food products, due to its high energy contribution compared to other nutrients.<sup>19</sup> Importantly, soybean and coconut oil are rich in unsaturated fats that contain Polyunsaturated Fatty Acids

(PUFA) and Monounsaturated Fatty Acids (MUFA), making them safe and beneficial for patients with T2DM.<sup>2,20</sup> Moreover, all formulations exhibited high protein content and were not significantly different from P0. The consumption of high-protein products can increase satiety, as protein digestion triggers the release of the hormone cholecystokinin.<sup>22</sup>

The fat content in all developed formulations exceeded that of P0. The low-fat content in P0 can be attributed to single-nutrient ingredients such as maltodextrin, inulin, vegetable oil, and isomaltulose. P0 primarily derived its fat content from vegetable oil alone. Conversely, the higher fat content in the developed products stemmed not only from soybean oil and coconut oil but also from various whole food ingredients such as oyster mushroom flour, brown rice flour, tempeh flour, fish flour, carrot flour, and moringa flour. Soybean oil and coconut oil are unsaturated fats that contain PUFA and MUFA fatty acids; therefore, they can act as antioxidants and are safe for consumption by T2DM patients.<sup>2,20</sup>

P0 contained higher carbohydrates and sugar compared to the developed formulations. Designed as a substitute for the main meal, P0 contained optimal carbohydrates while remaining safe for consumption by patients with T2DM. In addition, P0 had a low glycemic index (GI) that could help in controlling blood glucose levels. Maltodextrin, a constituent of P0 and a product of starch hydrolysis, contains a complex structure that facilitates the slow release of blood glucose, thus preventing rapid spikes in blood glucose levels. Although P0 contained the highest sugar level and was significantly different from all formulations, its ingredient composition differed from the developed formulation. P0 utilized an artificial sweetener, sucralose, while the developed formulations used granulated sugar. Artificial sweeteners such as sucralose and stevia are commonly used to control obesity and diabetes mellitus due to their zero-calorie content.<sup>23,24</sup>

The dietary fiber of P0 was proven to be significantly different from all the developed formulations. Each developed formulation exhibited higher dietary fiber content than P0 due to the high dietary fiber content in the main ingredients of the formulations. Oyster mushrooms are rich in beta-glucan, a type of water-soluble fiber.<sup>15</sup> Beta-glucan is mainly contained in the cell wall of oyster mushrooms, especially white oyster mushrooms, with 9.1 grams of beta-glucan soluble fiber per 100 grams of white oyster mushrooms.<sup>25</sup>

Water-soluble dietary fiber offers numerous health benefits, especially for people with diabetes mellitus. Research by Fairudz and Nisa (2015) stated that beta-glucan consumption can help reduce blood glucose and cholesterol levels.<sup>26</sup> Moreover, water-soluble dietary fiber can promote prolonged satiety by forming a viscous gel in the digestive system, thereby slowing down the nutrient absorption process.<sup>25,26</sup> Additionally, water-soluble dietary fiber has been shown to enhance insulin sensitivity in DM patients.<sup>27</sup>

FSDU for T2DM patients, as outlined by the Indonesian Food and Drug Authority (BPOM) Regulation No. 1/2018, is expected to contain 1.5-5 grams of protein, 2.22-2.78 grams of fat, and 11.25-16.25 grams per 100 kcal (Table 3). Table 3 reveals that the protein content in all formulations adheres to the standards set by the Indonesian Food and Drug Authority (BPOM). However, the fat and carbohydrate levels in the formulations fail to meet the regulatory requirements. Specifically, the fat content remains inadequate, while the carbohydrate content exceeds the recommended range. Table 4 shows the calculation of best treatment based on zeleny formula.

Peak fluctuations in blood glucose levels upon consumption of FSDU product development were observed as follows: P1 at 137.33 mg/dl, P2 at 136.83 mg/dl, P3 at 121.5 mg/dl, P4 at 110 mg/dl, P0 (commercial product) at 114 mg/dl, and standard white bread at 114 mg/dl (minute 30) (Figure 1a). The glycemic index (GI) value was calculated using the Incremental Area Under Curve (IAUC) method. The GI calculation showed that P4 had the best GI value, which was within the medium category (55-70), while P1, P2, and P3 had high glycemic index values (>70) (figure 2b, 2c). Overall, the developed FSDU prototype contains  $\beta$ -glucan as a source of water-soluble dietary fiber derived from brown rice and oyster mushrooms.  $\beta$ -glucan, a polysaccharide with a linear  $\beta$ -(1,3) branch chain  $\beta$ -(1,6) bond structure, acts as an immunomodulator and a source of prebiotics that can stimulate probiotics in the colon.<sup>28</sup> Based on a systematic review and meta-analysis, oat  $\beta$ -glucan with a molecular weight of >300 kg/mmol significantly reduced IAUC and peak glucose by 23% and 28%, and insulin by 22% and 24%<sup>29</sup>. The mechanism of  $\beta$ -glucan in reducing blood glucose levels involves forming a lasting satiety effect by increasing luminal viscosity, thereby reducing food transit speed in the stomach and nutrient absorption in the intestine.<sup>11</sup>

The determination of the best treatment level involves seven parameters: carbohydrate, protein, fat, energy, fiber, sugar, and GI. Each parameter holds equal importance. The results identified P4 as the optimal FSDU (Table 4). The composition of P4, compared to other formulations, is the addition of tempeh flour. According to research by Mawarno and Putri (2022), adding tempeh flour increases the caloric value of food products due to increased protein value.<sup>30</sup> Soybean fermentation in tempeh production can increase protein content by up to 32%.<sup>31</sup> Overall, this brown rice and mushroom-based product formulation provides nutrients beneficial for DM patients due to the high content of fiber,  $\beta$ -glucan, Mg, and Manganese, which can help regulate blood glucose.<sup>9,12</sup>

## **Conclusions**

The study concluded that the optimal Food for Special Dietary Uses for Diabetes Mellitus (DM) patients was a formulation containing brown rice and oyster mushrooms supplemented with tempeh flour. This conclusion was drawn after evaluating various parameters including energy, protein, fat, carbohydrates, fiber, sugar, and glycemic index content. However, further studies are needed to explore the development of this formulation with diverse flavor variants, aiming to enhance acceptability while ensuring the provision of health benefits to DM patients.

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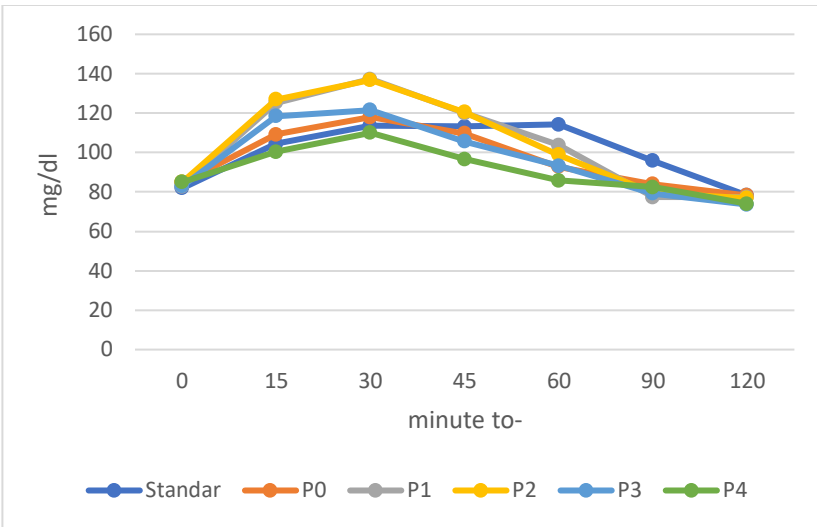
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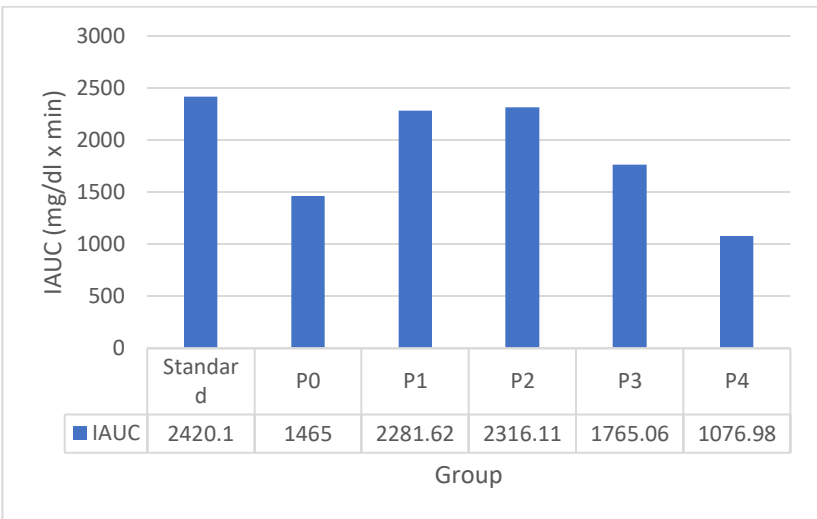


**Figure 1. Product development of FSDU OF DM**

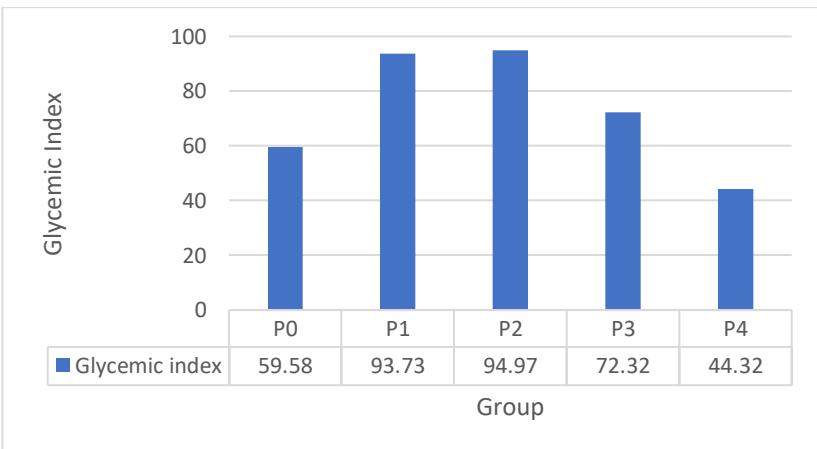




(a)



(b)



(c)

**Figure 2. Glycemic Index of Control Product and FSDU of DM Product Development**

**Description:**

(a) Average Blood Glucose Levels of Respondents at 0, 15, 30, 45, 60, 90, and 120 minutes

(b) Average Area Under Curve Respondents

(c) Average Glycemic Index

**Standard** White bread

**P0** FSDU of DM Commercial Product

**P1** FSDU of DM with added fish-moringa flour

**P2** FSDU of DM with added fish-carrot flour

**P3** FSDU of DM with added tempeh-moringa flour

**P4** FSDU of DM with additional tempeh flour

**Table 1. Formulation of FSDU development based on brown rice and oyster mushroom**

Ingredients	Composition of Ingredients for Each Formulation			
	Formula 1 (P1)	Formula 2 (P2)	Formula 3 (P3)	Formula 4 (P4)
	grams	grams	grams	Grams
Mushroom flour	60	60	60	60
Brown Rice	375	375	375	375
Soybean Oil	5	5	5	5
Coconut Oil	30	3	3	3
Sugar	12	12	12	12
Moringa Fishmeal	50	-	-	-
Carrot Fish Flour	-	52	-	-
Moringa Tempeh flour	-	-	60	-
Tempeh Flour	-	-	-	62
Prediction of nutritional content of brown rice and oyster mushroom-based FSDU *				
Carbohydrate (gram)	360.71	362.59	363.09	362.20
Protein (gram)	64.98	63.68	63.37	64.18
Fat (gram)	46.29	47.96	48.52	46.72
Energy (kcal)	2109.34	2126.71	2134.08	2119.42

Notes: \*) Calculated based on TKPI (2017)<sup>17</sup>

**Table 2. Energy and nutrient value per 100 grams**

Measurements	P0	P1	P2	P3	P4	P value
Energy, Median $\pm$ SE, kcal	377 $\pm$ 0.00 <sup>a</sup>	395 $\pm$ 0.00 <sup>a,b,c</sup>	391 $\pm$ 0.33 <sup>a,b</sup>	397 $\pm$ 0.00 <sup>b,c</sup>	404 $\pm$ 0.33 <sup>c</sup>	0.01*
Protein, Median $\pm$ SE, g	13.84 $\pm$ 0.03 <sup>a,b</sup>	13.80 $\pm$ 0.07 <sup>a</sup>	14.52 $\pm$ 0.01 <sup>b</sup>	13.47 $\pm$ 0.05 <sup>a</sup>	13.88 $\pm$ 0.06 <sup>a,b</sup>	0.02*
Fat, Median $\pm$ SE, g	0.44 $\pm$ 0.015 <sup>a</sup>	6.13 $\pm$ 0.06 <sup>b,c</sup>	5.45 $\pm$ 0.09 <sup>a,b</sup>	6.16 $\pm$ 0.02 <sup>b,c</sup>	7.75 $\pm$ 0.02 <sup>c</sup>	0.01*
Carbohydrate, Median $\pm$ SE, g	79.43 $\pm$ 0.02 <sup>a</sup>	71.23 $\pm$ 0.20 <sup>a,b</sup>	71.02 $\pm$ 0.10 <sup>a,b</sup>	71.81 $\pm$ 0.10 <sup>a,b</sup>	69.77 $\pm$ 0.09 <sup>b</sup>	0.01*
Water Content, Median $\pm$ SE, %	4.06 $\pm$ 0.04 <sup>a</sup>	6.13 $\pm$ 0.06 <sup>b,c</sup>	6.31 $\pm$ 0.02 <sup>c</sup>	5.95 $\pm$ 0.04 <sup>a,b</sup>	6.19 $\pm$ 0.03 <sup>b,c</sup>	0.01*
Ash Content, Mean $\pm$ SD, %	2.22 $\pm$ 0.03 <sup>a</sup>	2.74 $\pm$ 0.07 <sup>b</sup>	2.68 $\pm$ 0.02 <sup>b</sup>	2.50 $\pm$ 0.04 <sup>c</sup>	2.46 $\pm$ 0.08 <sup>c</sup>	0.00*
Dietary Fiber, Mean $\pm$ SE, %	4.12 $\pm$ 0.017 <sup>a</sup>	7.65 $\pm$ 0.046 <sup>b</sup>	4.2 $\pm$ 0.016 <sup>b</sup>	7 $\pm$ 0.04 <sup>b</sup>	4.26 $\pm$ 0.02 <sup>b</sup>	0.01*
Sugar, Mean $\pm$ SD, %	22.07 $\pm$ 0.59 <sup>a</sup>	14.22 $\pm$ 0.23 <sup>b</sup>	13.59 $\pm$ 0.07 <sup>b</sup>	13.75 $\pm$ 0.24 <sup>b</sup>	10.88 $\pm$ 0.16 <sup>c</sup>	0.00*

Description:

P0 = FSDU of DM Commercial Product

P1 = FSDU of DM with added fish-moringa flour

P2 = FSDU of DM with added fish-carrot flour

P3 = FSDU of DM with added tempeh-moringa flour

P4 = FSDU of DM with additional tempeh flour

\*Kruskal Wallis

\*\* One-way ANOVA

<sup>a,b,c</sup> Notification of further test results

**Table 3. Comparison of FSDU formulation with BPOM FSDU standard per 100 kcal**

Nutrients	Formulation Groups				BPOM FSDU Standard
	P1	P2	P3	P4	
	Median ± SE	Median ± SE	Median ± SE	Median ± SE	
Protein (g)	3.49 ± 0.07	3.71 ± 0.01	3.39 ± 0.05	3.43 ± 0.06	2.5-5
Fat (g)	1.55 ± 0.06	1.39 ± 0.09	1.55 ± 0.02	1.91 ± 0.02	2.22 – 2.78
Carbohydrate (g)	18.03 ± 0.20	18.16 ± 0.10	18.08 ± 0.10	17.26 ± 0.09	11.25-16.25

Description:

P0 = FSDU of DM Commercial Product

P1 = FSDU of DM with added fish-moringa flour

P2 = FSDU of DM with added fish-carrot flour

P3 = FSDU of DM with added tempeh-moringa flour

P4 = FSDU of DM with additional tempeh flour

**Table 4. Calculation of Best Treatment Based on Zeleny Formula**

<b>Formulation</b>	<b>L1</b>	<b>L2</b>	<b>L infinite</b>	<b>Total</b>	<b>Best Ranking</b>
P1	0.1344	0.0070	0.0747	0.2161	4
P2	0.1588	0.0090	0.0653	0.2331	3
P3	0.1280	0.0043	0.0542	0.1865	2
P4	0.1143	0.0056	0.0620	0.1819	1