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Effect of fish protein hydrolysate-enriched mocaf biscuits on fecal short-chain fatty acid levels in obese adults

Pengaruh biskuit mocaf yang diperkaya hidrolisat protein ikan terhadap kadar asam lemak rantai pendek feses pada orang dewasa obesitas

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Abstract

Obesity is linked to dietary patterns that influence gut microbiota balance and short-chain fatty acid (SCFA) production, which regulate energy metabolism and appetite. However, evidence regarding the effects of fish protein hydrolysates (FPH) on SCFA levels in humans remains limited. This study evaluated the effects of FPH-based mocaf biscuits on fecal SCFA levels in obese adult participants. A two-stage design was applied: (1) formulation and evaluation of four biscuit variants (F0–F3) and (2) a randomized controlled post-only trial involving 30 obese participants aged 20–50 years from Semarang, Indonesia. Participants consumed 60 g of biscuits daily for four weeks, along with a 1500 kcal/day diet. Anthropometric, dietary, and fecal SCFA data were collected, and statistical analyses were performed using Shapiro–Wilk, Mann–Whitney, and paired t-tests (α = 0.05). The F2 biscuits had the highest protein content (4.66%) and sensory acceptance ($p<0.05$). Baseline SCFA levels were comparable between the groups, and post-intervention differences were not significant ($p=0.071$), although the treatment group showed a slight decrease in visceral fat ($p=0.089$) and waist circumference ($p=0.094$). In conclusion, FPH-enriched mocaf biscuits improved protein quality and acceptability but did not significantly alter fecal SCFA levels in obese adults.

Keywords: Fish Protein Hydrolysate, Mocaf Biscuit, SCFA, Obesity, Functional Food.

Abstrak

Obesitas berkaitan dengan pola makan yang memengaruhi keseimbangan mikrobiota usus dan pembentukan asam lemak rantai pendek (SCFA), yang berperan dalam mengatur metabolisme energi dan nafsu makan. Namun, bukti mengenai pengaruh hidrolisat protein ikan (FPH) terhadap kadar SCFA pada manusia masih terbatas. Penelitian ini menilai pengaruh biskuit mocaf berbasis FPH terhadap kadar SCFA feses pada orang dewasa obesitas. Penelitian dilakukan dalam dua tahap: (1) formulasi dan evaluasi empat varian biskuit (F0–F3), serta (2) uji acak terkontrol dengan 30 peserta obesitas berusia 20–50 tahun di Semarang, Indonesia. Peserta mengonsumsi 60 gram biskuit per hari selama empat minggu dengan pola makan 1500 kkal per hari. Data antropometri, asupan gizi, dan kadar SCFA feses dianalisis menggunakan uji Shapiro–Wilk, Mann–Whitney, dan t-berpasangan (α = 0,05). Varian F2 memiliki kandungan protein tertinggi (4,66%) dan penerimaan sensorik terbaik ($p<0,05$). Meskipun kadar SCFA tidak berbeda signifikan antar kelompok ($p=0,071$), kelompok perlakuan menunjukkan penurunan kecil pada lemak visceral ($p=0,089$) dan lingkar pinggang ($p=0,094$). Kesimpulannya, biskuit mocaf yang diperkaya FPH meningkatkan mutu protein dan daya terima, tetapi belum memberikan perubahan signifikan pada kadar SCFA feses pada orang dewasa obesitas.

Kata Kunci: Hidrolisat Protein Ikan, Biskuit Mocaf, SCFA, Obesitas, Pangan Fungsional

Introduction

Obesity is a global health problem that affects more than 2 billion people. According to the World Health Organization (WHO, 2021), obesity causes more than four million deaths annually, making it a serious epidemic with challenging prospects for its control. In Indonesia, the prevalence of obesity among adults (>18 years) increased from 21.8% in 2018 to 23.4% in 2023 (Kemenkes RI, 2023). Lifestyle, genetic, and environmental factors are major contributors to obesity (Albuquerque et al., 2017; Nicolaidis, 2019; Sheikh et al., 2017).

Overweight and obesity are characterized by abnormal or excessive fat accumulation and are major risk factors for non-communicable diseases, including diabetes (Al Rahmad et al., 2020; Xu et al., 2021), cardiovascular diseases (Schroder et al., 2021), and various types of cancer including endometria and, breast cancer (Neuhouser et al., 2015). Epidemiological studies indicate that the quality of dietary protein and fat sources influences the risk of obesity, emphasizing the importance of dietary modification in prevention strategies.

Controlling food intake is challenging because calorie restriction often increases the feeling of hunger. Adjusting macronutrient composition, especially by increasing dietary protein and reducing carbohydrates, may help regulate satiety. Protein is the most satiating macronutrient because of its higher thermogenic effect than carbohydrates and fats (Nandar et al., 2019). High-protein diets (>25% of total energy) can promote satiety and energy expenditure (Zhu et al., 2023), although the effects may differ according to the protein source and amino acid composition (Hemler et al., 2022). High-protein intake influences appetite and energy balance and may modulate gut microbiota composition. Bioactive peptides from fish protein hydrolysate (FPH) can alter microbial metabolism and short-chain fatty acid (SCFA) production, both of which are closely linked to obesity prevention.

Dietary composition strongly affects the gut microbiota, which regulates digestion, metabolism, and immunity (Nova et al., 2022) (Hakozaki et al., 2020). The gut microbiota interacts with dietary components to influence host metabolic pathways, including bile acid and branched-chain amino acid metabolism, which contribute to obesity pathogenesis (Palmas et al., 2021; Pesta & Samuel, 2014). However, there is limited evidence regarding the effect of fish-derived bioactive proteins, particularly FPH, on SCFA levels in obese adults through functional food interventions.

Mocaf (modified cassava flour) biscuits were selected as a delivery vehicle because they are widely consumed, low-cost, and culturally familiar. Mocaf is a gluten-free local product rich in resistant starch and dietary fiber, both of which can support SCFA production and local food diversification. Thus, incorporating FPH into mocaf biscuits represents an innovative approach to combine local food resources with functional and nutritional benefits.

This study aimed to formulate and assess the nutritional and sensory quality of mocaf biscuits enriched with fish protein hydrolysate and evaluate their effect on fecal SCFA levels in obese adults. These findings are expected to provide evidence for the development of locally sourced, protein-based functional foods that support obesity management through microbiota modulation.

Methods

Design and Subjects

The study consisted of two main phases: the first phase involved the formulation of mocaf flour biscuit products using an experimental design, while the second phase comprised the intervention stage with a randomized control group design. The research was conducted from October 2024 to February 2025 and received ethical approval from the Health Research Ethics Committee (Komite Etik Penelitian Kesehatan/KEPK), Faculty of Medicine, Diponegoro University/Dr. Kariadi General Hospital under approval number 488/EC/KEPK/FK-UNDIP/IX/2024.

Sample size determination was based on the standard deviation and the expected clinical differences from previous studies. The calculation was performed using G*Power software, and the hypothesis test formula was applied to estimate the mean difference between the two independent populations (Kang & Huh, 2021).

The inclusion criteria encompassed active employees aged 20–50 years at the Semarang City Health Office who agreed to participate and signed informed consent forms. The female participants were neither pregnant nor menopausal. Subjects were required to meet the criteria for obesity (BMI ≥ 25 kg/m^2 and waist circumference ≥ 80 cm), have a sedentary lifestyle, not consume specific supplements or medications, not participate in any dietary program within the past month, and maintain a stable body weight over the previous three months. Respondents with allergies to the food ingredients used in the study were also excluded.

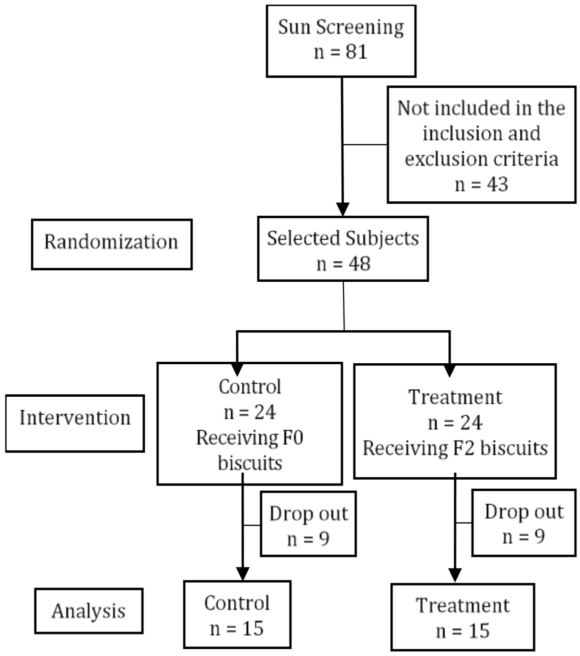

Figure 1. Study screening flowchart

The exclusion criteria included a history of chronic diseases (cardiovascular disease, diabetes, dyslipidemia, or hypertension), smoking habits or alcohol consumption, unintentional weight loss, vigorous physical activity prior to blood sampling, refusal to provide informed consent, withdrawal during the study period, and failure to complete all intervention stages. A total of 48 subjects were recruited, with 24 in the control group and 24 in the treatment group. Randomization was performed using a computer-generated algorithm (random.org). As nine participants dropped out during the study (37.5%), a per-protocol analysis was applied to include only participants who completed the entire intervention period (n = 15 per group). Attrition analysis confirmed no baseline differences between those who completed the study and those who dropped out. The research flowis presented in Figure 1.

Tools and Materials

The tools used in the preparation of mocaf biscuits with added fish protein hydrolysate included a digital food scale, electric oven, planetary mixer with balloon whisk and paddle attachments, mixing bowls, spatulas, baking trays, parchment paper, and metallized packaging materials.

The materials used in this study consisted of mocaf flour (Mocafine), shortening (Palmia®), maltodextrin (DE 10-12, Lihua), baking powder (Koepoe-Koepoe®), salt (DOLPIN®), food-grade ammonium bicarbonate (Chang®), soy lecithin (Solec N-TNA), EM 90% stevia extract (SXY stevia®), eggs, grated cheese (Emina®), dried parsley leaves (Yutakachi®), vanilla flavoring (ethyl vanillin, Eternal Pearl®), truffle flavoring (PT. Triefta Aroma Nusantara) and fish protein hydrolysate (PT Berikan Teknologi Indonesia). The fish protein hydrolysate was characterized by a moisture content of 7.90–7.96%, ash content of 2.65–2.70%, protein content of 12.38–12.65%, total fat content of <0.02%, and carbohydrate content of 76.80–76.96%.

Biscuit Preparation

The biscuit production was formulated into four variants: F0 as the control without the addition of fish protein hydrolysate (FPH), and F1, F2, and F3 with the addition of 30 g, 40 g, and 50 g of FPH, respectively, as presented in Table 1. The formulation was adapted from the study conducted by Araste et al. (2015) in accordance with the objectives of this research. It was further refined with several modifications to suit our experimental design.

Table 1. The intervention formula

Composition	F0		F1		F2		F3	
	(g)	%	(g)	%	(g)	%	(g)	%
Modified cassava flour (MOCAF)	100	53.96	100	53.96	100	53.96	100	53.96
Maltodextrin	50	26.98	20	10.79	10	5.40	0	0.00
Fish Protein Hydrolysate	0	0.00	30	16.19	40	21.58	50	26.98
Eggs	14	7.55	14	7.55	14	7.55	14	7.55
Shortening	13	7.01	13	7.01	13	7.01	13	7.01

Grated Cheese	4	2.16	4	2.16	4	2.16	4	2.16
Baking Powder	1.6	0.86	1.6	0.86	1.6	0.86	1.6	0.86
Ammonium Bicarbonate	1.6	0.86	1.6	0.86	1.6	0.86	1.6	0.86
Truffle Flavoring	0.53	0.29	0.53	0.29	0.53	0.29	0.53	0.29
Vanilla Flavoring	0.2	0.11	0.2	0.11	0.2	0.11	0.2	0.11
Dried Parsley Leaves	0.2	0.11	0.2	0.11	0.2	0.11	0.2	0.11
Salt	0.1	0.05	0.1	0.05	0.1	0.05	0.1	0.05
Soy Lecithin	0.05	0.03	0.05	0.03	0.05	0.03	0.05	0.03
Natural Sweetener (Stevia)	0.04	0.02	0.04	0.02	0.04	0.02	0.04	0.02

The assessment process commenced by establishing the priority order of each attribute and converting the attribute values into dimensionless scales. Subsequently, each attribute was weighted according to its rank and multiplied by the corresponding values on a dimensionless scale. The resulting products were summed, and the formulation with the highest total score was considered the most preferable.

Biscuit production was carried out at the Sustainable Diet and Biodiversity Laboratory, Integrated Laboratory Unit (UPT), Diponegoro University. The process involved mixing, kneading, molding, and baking. In the first stage, food flavorings (vanilla and truffle), stevia extract, baking powder, salt, grated cheese, shortening, lecithin, and eggs were placed in the mixer bowl and mixed for 3 min using a balloon whisk attachment at medium speed. Ammonium bicarbonate, previously dissolved in water, was then added, and the dough adhering to the bowl was scraped down with a spatula before mixing continued for an additional 5 min. Maltodextrin, fish protein hydrolysate, mocaf flour, dried parsley leaves, and water were then added and mixed using a paddle attachment for 5 min at low speed. The dough was then evenly rolled using a dough roller on a dough-sheet base. The sheeted dough was molded and weighed to obtain a weight of 4.0–4.4 g per piece of dough. The molded dough pieces were placed on baking trays lined with parchment paper and baked at 170 °C for approximately 27 min, resulting in biscuits weighing 3.0–3.3 g.

Organoleptic Test

A total of 66 untrained panelists (aged 20–50 years) from Semarang City participated in the organoleptic evaluation. The sensory evaluation was conducted using the hedonic method on the attributes of appearance, aroma, taste, texture, and overall acceptability, employing a 9-point scale, where a score of 1 indicated 'dislike extremely' and a score of 9 indicated 'like extremely.' To minimize carry-over effects, the panelists were provided with drinking water to rinse their mouths between evaluations.

Nutrient Content of Biscuits

The nutritional composition of the biscuit products was analyzed using gravimetric and drying methods to determine the moisture and ash contents. Lipid and protein contents were analyzed using the Soxhlet and Kjeldahl methods, respectively, while the carbohydrate content was determined using the by-difference method. According to the Association of Official Analytical Chemists (AOAC, 2005), these analytical procedures were applied to ensure accurate determination of the nutritional composition of the biscuits, with gravimetric methods for moisture and ash, Soxhlet and Kjeldahl methods for lipid and protein, and the by-difference method for carbohydrates.

Determination of the Best Formula

Multi-attribute decision-making using a Compensatory Model and Additive Weighting technique (MADCAW) was employed to identify the optimal formulation. The assessment process began with establishment a priority order for each attribute, followed by the conversion of the attribute values into a dimensionless scale. Subsequently, each attribute was assigned a weight according to its rank, and the dimensionless values were multiplied by their corresponding weights. The resulting products were aggregated, and the formulation with the highest total score was considered the best.

Intervention

Anthropometric data (body weight, height, BMI, waist circumference, and visceral fat) were collected before and after the intervention using an Omron® device (Karada HBF 214), Microtoise, and Metline. Fecal samples were collected only after the intervention to measure SCFA levels because of financial and logistic limitations in sample handling and GC analysis. Therefore, comparisons were made between groups at the post-intervention stage rather than within-group pre–post changes. A 24-hour food recall interview was conducted to assess dietary intake. All activities were conducted at the Semarang City Health Office and Pandanaran Primary Health Center.

The intervention was conducted over four weeks, during which participants received 60 g of biscuits per day, divided into 30 g in the morning and 30 g in the afternoon. Both groups received standardized dietary education on a 1500 kcal/day balanced diet plan to minimize interindividual dietary variation. This uniform instruction served as a control variable to ensure that any observed differences were attributable to the FPH biscuit intervention rather than to caloric restriction. Regular visits were scheduled to conduct interviews to collect data on physical activity using the IPAQ-SF questionnaire, as well as dietary intake through the 24-hour food recall.

Statistical Analysis

Data were analyzed using SPSS version 25, Normality was tested using the Shapiro–Wilk test for samples with fewer than 50 participants.

The Kruskal–Wallis test was employed to evaluate the effects of fish protein hydrolysate on nutrient content and hedonic scores, followed by the Mann–Whitney test for post-hoc analysis of significant results. The significance level was set at $P < 0.05$. Independent t-tests were applied to the participant characteristics, anthropometric measurements, SCFA levels, dietary intake, and physical activity.

Paired t-tests were used to analyze changes in anthropometric parameters and SCFA levels after the intervention. The choice of non-parametric and parametric tests was based on the data distribution results. To control for potential confounders, such as baseline dietary intake and physical activity, variables were compared between groups and confirmed to be not significantly different prior to the intervention. Therefore, no statistical adjustments were required in the final analysis.

Result and Discussion

Organoleptic Test

In the organoleptic evaluation, formula F2 obtained the highest score for appearance (6.27 ± 1.21), followed by F3 (6.24 ± 1.77) and F0 (6.17 ± 1.58), whereas F1 received the lowest score (6.02 ± 1.73). For texture, F3 achieved the highest score (6.29 ± 1.06), followed by F0 (6.20 ± 1.52), F2 (6.09 ± 1.06), and F1 (5.86 ± 1.27). Statistical analysis showed no significant differences ($p > 0.05$), indicating that the addition of 30, 40, and 50 g of fish protein hydrolysate did not affect the panelists' assessment of appearance or texture. The results of the organoleptic tests are presented in Table 2.

Table 2. Results of Organoleptic Testing of Biscuits

Sample	Quality Attributes				
	Landform	Aroma	Flavor	Texture	Overall
F0	6.17±1.58^{a}	6.17±1.55^{a}	6.17±1.56^{a}	6.20±1.52^{a}	6.39±1.39^{a}
F1	6.02±1.73^{a}	5.27±1.57^{b}	5.32±1.34^{b}	5.86±1,27^{a}	5.58±1.12^{ab}
F2	6.27±1.21^{a}	5.79±1.23^{ab}	5.89±1.06^{ac}	6.09±1.06^{a}	6.08±1.02^{bc}
F3	6.24±1.77^{a}	4.82±1.40^{c}	5.26±1.05^{bc}	6.29±1.06^{a}	5.44±0.94^{c}
p-value	0.899^{a}	0.000^{b}	0.000^{b}	0.238^{a}	0.000^{b}

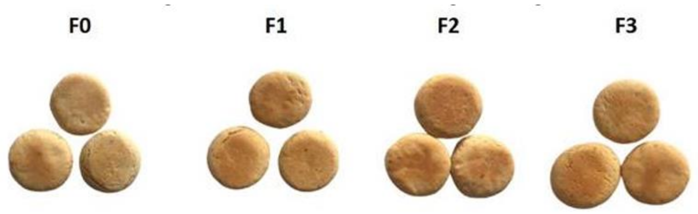

Figure 2. Biscuits with fish protein hydrolysate (HPI) F0 (maltodextrin 100%: 0% HPI), F1 (maltodextrin 40%: 60% HPI), F2 (maltodextrin 20%: 80% HPI), F3 (maltodextrin 0%: 100% HPI).

Data are presented as mean values followed by different superscript letters (a–c), indicating significant differences ($p < 0.05$) based on the Mann–Whitney U post hoc test. The scoring scale was as follows: 1 = extremely dislike; 2 = very much dislike 3 = dislike 4 = slightly dislike 5 = neutral 6 = slightly like 7 = like 8 = very much like and 9 = extremely like.

The overall appearance of the biscuits can be seen in Figure 2. The organoleptic test results indicated no significant differences in appearance and texture among the formulas ($p > 0.05$), with mean scores ranging from 6.0–6.3 for appearance and 5.86–6.29 for texture. This finding suggests that variations in formulation did not affect visual perception or physical properties. The Maillard reaction in biscuits contributed to browning, aroma formation, and flavor enhancement. This reaction occurs when proteins from flour or eggs interact with reducing sugars (glucose/fructose) at high baking temperatures (Handayani et al., 2017). This results in a golden-brown color, pleasant aroma, and more complex flavor profile. Consequently, in terms of appearance, all the formulas were considered visually comparable by the panelists.

However, aroma, taste, and overall acceptability showed significant differences among the formulas ($p < 0.05$), with lower scores observed for F3, likely due to changes in ingredient composition or processing that affected aroma and flavor compounds. Biscuit flavor and aroma are influenced by volatile compounds such as aldehydes, pyrazines, and furfural, which impart caramel, roasted, and nutty notes, and non-volatile compounds such as maltol, which enhances sweetness and flavor complexity (Su et al., 2022). The combination of raw materials and baking processes determines the biscuit characteristics, with factors such as temperature, pH, lipid oxidation, and interactions among volatile compounds shaping the aroma profile. Treatments such as excessive heating (±110 °C), oxidation, or the use of strong-flavored ingredients may disrupt the balance of volatile compounds, thereby reducing the panelists' preference (Putalan & Ariany, 2025). The lower aroma score of F3 can be attributed to the degradation of desirable volatile compounds or the formation of less-desirable compounds. Excessive use of fish protein hydrolysate and truffle flavoring may produce intense aroma and bitter taste.

The consistency of the texture scores across the formulas suggests that the ingredients and processing conditions were able to maintain the product structure. This is likely because differences in formulation did not affect key factors, such as moisture content, fat distribution, or protein structure (Kouhsari et al., 2022). The absence of significant differences also indicates that the panelists did not perceive meaningful variations in chewiness or density, confirming that texture was not a distinguishing factor among the samples.

The quality attributes that most strongly influenced overall acceptability were aroma and taste, as these play a central role in consumer satisfaction with food products. These findings reinforce the notion that aroma and taste are the primary determinants of product acceptance (Calín-Sánchez & Carbonell-Barrachina, 2021), while appearance and texture remain relatively consistent despite variations in the amount of fish protein hydrolysate used.

Nutrient Content of Biscuits

The nutrient contents (moisture, fat, carbohydrate, and energy) showed no significant differences among the formulas ($p > 0.05$). However, the protein content differed significantly ($p < 0.05$), indicating that variations in the biscuit formulation affected the protein levels, while other nutrient components remained unchanged. The nutrient contents of the four formulations are presented in Table 3.

Table 3. Nutrient content of biscuit formulations

Variable	Biscuit Formula				p-value
	F0	F1	F2	F3	
Water content (%)	3.08	2.75	4.27	4.12	0.364[b]
Protein (%)	2.52	3.51	4.66	4.54	0.005[a]
Fat (%)	9.20	9.53	8.85	8.55	0.146[b]
Carbohydrate (%)	78.47	74.12	74.09	74.39	0.262[b]
Energy (kal/100g)	395.76	384.99	396.47	383.41	0.622[b]

Food letter total (%)	5.69	8.79	9.26	6.53	0.128[b]

[a]: One Way Anova, [b]: Kruskal Wallis
Data presented as mean and median from two repetitions and duplicates (n = 4)

Nutrient analysis of different biscuit formulations showed that the moisture content ranged from 2.75% to 4.27%. These values comply with the Indonesian National Standard (SNI 2973:2011), which specifies a maximum moisture content of 5% (Susanto et al., 2018). No significant differences were observed among the formulas (p = 0.364), indicating that variations in the raw material composition did not substantially affect the moisture levels. This finding is important because low moisture content supports the shelf life of biscuits by inhibiting microbial growth (Belokurova et al., 2021).

However, the protein content showed significant differences (p = 0.005). The control formula (F0) had the lowest protein level (2.52%), while F2 (4.66%) and F3 (4.54%) exhibited higher values, suggesting that ingredient substitution enriched the protein content of the biscuits. This provides additional nutritional value, enhancing their role as a snacks. The incorporation of both plant- and animal-based protein sources in biscuit formulations contributed to improving nutritional quality and promoting satiety, thereby supporting the potential of biscuits as functional food (Hamzah et al., 2021).

Fat (8.55–9.53%), carbohydrate (74.09–78.47%), and energy (383.41–396.47 kcal/100 g) contents showed no significant differences among formulas (p > 0.05), indicating that variations in ingredients did not affect these components, despite slight variations in their values. The stability of the fat content across the formulas reflects the consistency in fat usage, which is important given the role of fat in determining the texture and overall quality of biscuits (Kouhsari et al., 2022).

The dietary fiber content was higher in the treatment formulas (8.79–9.26%) than in the control (5.69%), although the difference was not statistically significant (p = 0.128). Mocaf flour is a source of dietary fiber and a wheat substitute in gluten-free products. In addition to imparting a distinctive texture, mocaf also increases the mineral content (zinc, calcium, and magnesium) and provides a pH that supports the gelatinization process (Kristanti & Setiaboma, 2022). Thus, mocaf offers a favorable nutritional profile and potential as a healthy functional food ingredient (Khasanah et al., 2024; Tzani et al., 2025).

Overall, the results demonstrated that variations in the formulation affected the ash and protein content, whereas other components remained unchanged. Formula 2 improved the protein and fiber contents without compromising the sensory quality.

Determination of the Best Formula

Formula F2 was selected as the best formulation because it balanced both sensory quality and nutritional content. F2 obtained the highest scores for protein and dietary fiber, which are important for functional biscuits and digestive health, respectively. The taste and texture of F2 were also preferred by the panelists. F1 received the lowest scores, the control formula (F0) showed notable improvements, and F3 performed fairly well but remained below F2. Therefore, F2 was considered the most suitable option for developing healthy biscuits. The results for the best formulation are presented in Table 4.

Table 4. Best formula

Attributes	Weight (a)	F0 DV (b)	Score F0 (a x b)	F1 DV (c)	Score F1 (a x c)	F2 DV (d)	Score F2 (a x d)	F3 DV (e)	Score F3 (a x e)
Flavour	0.12	1.0	0.12	0.33	0.04	0.67	0.08	0.0	0.0
Protein	0.11	0.0	0.0	0.33	0.04	1.0	0.11	0.67	0.07
Energy total	0.1	0.0	0.0	0.67	0.07	0.33	0.03	1.0	0.1
Food letter	0.1	0.0	0.0	0.33	0.03	0.67	0.07	1.0	0.1
Carbohydrate	0.09	0.67	0.06	0.67	0.06	1.0	0.09	0.33	0.03
Texture	0.08	0.67	0.05	0.0	0.0	0.33	0.03	0.33	0.03
Aroma	0.08	1.0	0.08	0.33	0.03	0.67	0.05	0.0	0.0

Overall	0.07	1.0	0.07	0.33	0.02	0.67	0.05	0.0	0.0
Landform	0.07	0.33	0.02	0.0	0.0	1.0	0.07	0.67	0.05
Fat	0.06	0.33	0.02	0.0	0.0	0.67	0.04	1.0	0.07
Ash content	0.06	1.0	0.06	0.67	0.04	0.33	0.02	0.0	0.0
Water content	0.06	0.67	0.04	1.0	0.06	0.0	0.0	0.33	0.02

DV = Dimensionless Value

Subject Characteristics

The distribution of sex was balanced between the two groups (p ≥ 0.05), with each group consisting of four men and 11 women. The independent t-test results indicated no significant differences in the subject characteristics (p > 0.05), confirming that both groups were homogeneous. The participants had a BMI of 30–31 kg/m^2 and waist circumference of 90–95 cm, indicating class I obesity with high abdominal fat content. The average energy intake was 1700–1800 kcal, with adequate protein and fat intake but insufficient carbohydrate and fiber consumption. Physical activity levels were moderate in both groups.

Table 5. Subject Characteristics Before Intervention

Subject Characteristics	Control (n=15) Mean ± SD	Intervention (n=15) Mean ± SD	p-value
Gender*			1.000^c
Male	4 (36.37%)	4 (36.37%)	
Female	11 (63.63%)	11 (63.63%)	
Age (year)	37.4 ± 8.911	33.93 ± 6.273	0.228^a
Body weight (kg)	78.02 ± 8.60	76.93 ± 13.54	0.794^a
IMT (kg/m^2)	31.27 ± 2.25	30.21 ± 3.83	0.364^a
Waist circumference (cm)	95.47 ± 7.52	95.91 ± 9.97	0.892^a
Visceral Fat	14.87 ± 3.56	14.53 ± 6.09	0.856^a
Energy (kcal)	1822.19 ± 324.50	1773.24 ± 294.41	0.669^a
Protein (g)	62.50 (32.2 – 100.8)	56.77 ± 17.80	0.178^a
Total fat (g)	74.98 ± 26.18	62.07 ± 26.90	0.194^a
Carbohydrate (g)	181.13 ± 63.90	159.11 ± 67.80	0.106^b
Food letter (g)	11.50 ± 7.45	8.61 ± 5.74	0.243^a
Physical activity (MET-Min/Week)	1836.20 ± 1211.3	2243.53 ± 1578.00	0.853^a

^{a}: Independent t-test, ^{b}: Paired t-test, ^{c}: Chi-square

Changes in Body Composition During the Intervention

The analysis revealed no significant differences in body weight, BMI, waist circumference, or visceral fat between the control and treatment groups (p > 0.05). The control group showed slight increases in body weight and visceral fat, while the treatment group experienced small reductions in both parameters, accompanied by decreases in waist circumference in both groups and a stable BMI. The treatment group demonstrated the greatest reductions in visceral fat (5.02%) and waist circumference (1.24%), indicating that the intervention was more effective in reducing abdominal fat.

Table 6. Body Composition of Subjects During the Intervention

Variable	Group	Control (n=15) Mean ± SD	Intervention (n=15) Mean ± SD	p-value
Body weight (kg)	Before	78.02 ± 8.60	76.93 ± 13.54	0.794^a
	After	78.20±9.03	76.70 ± 13.30	0.721^a
	Δ	0.18 ± 0.43	− 0.23 ± 0.24	
	p-value	0.903^b	0.457^b	
BMI (kg/m^2)	Before	31.268 ± 2.25	30.210 ± 3.83	0.364^a
	After	31.331 ± 2.36	30.115 ± 3.60	0.284^a

	Δ	0.063 ± 0.11	– 0.095 ± 0.23	
	p-value	0.521[b]	0.466[b]	
Waist Circumference (cm)	Before	95.47 ± 7.52	95.91 ± 9.97	0.892[a]
	After	92.97 ± 7.97	94.72 ± 10.77	0.617[a]
	Δ	- 2.5 ± 0.45	– 1.19 ± 0.8	
	p-value	0.211[b]	0.445[b]	
Visceral Fat	Before	14.87 ± 3.56	14.53 ± 6.09	0.856[a]
	After	15.07 ± 3.83	13.80 ± 4.88	0.436[a]
	Δ	0.33 ± 3.89	– 0.73 ± 1.24	
	p-value	0.334[b]	0.195[b]	
Fecal SCFA Levels (After-Intervention Only)		67.94 ± 6.64	63.68 ± 5.74	0.071[a]

[a]: Independent t-test, [b]: Mann–Whitney U test

The findings of this study are consistent with those of previous studies demonstrating that fish protein hydrolysate supplementation can significantly impact appetite regulation, particularly by reducing cravings for sweet foods (Dale et al., 2019). Protein consumption plays an essential role in appetite regulation by enhancing satiety and suppressing hunger. High-protein diets have been positively associated with increased secretion of satiety-inducing hormones, such as GLP-1, which can lead to a decrease in food intake and assist in weight management (Choi, 2022). Additionally, interventions aimed at appetite suppression and improved metabolic health are particularly relevant for obesity management (Daskalaki et al., 2023). Specifically, fish protein hydrolysates have shown efficacy in reducing appetite and improving insulin sensitivity, contributing to favorable metabolic outcomes in obesity (Daskalaki et al., 2023; Labatjo et al., 2023). Neuroimaging studies have also indicated that appetite regulation is closely linked to brain activity, providing insights into the physiological mechanisms that govern appetite and satiety (Althubeati et al., 2022). Understanding these relationships highlights the potential of high-protein foods as effective tools for managing obesity and promoting healthier eating behaviors (Venegas et al., 2022; Putra et al., 2025).

Fecal SCFA Levels (Post-Intervention Only)

The SCFA level in the control group was 67.94 ± 6.64 µmol/L, whereas that in the treatment group was 63.68 ± 5.74 µmol/L. A small standard deviation indicates data homogeneity. Although the mean values differed, the intersubject variation remained within a reasonable range. Statistical analysis yielded p = 0.071, indicating no significant difference between groups. Rather than suggesting potential significance, this finding should be interpreted as showing a tendency toward increased variability, which warrants further investigation with larger sample sizes and longer intervention durations.

The absence of a significant difference may be attributed to various factors. First, SCFA production is primarily driven by the microbial fermentation of dietary fiber; in this study, both groups reported fiber intake below 10 g/day, which likely limited fermentation capacity. Second, the 4-week intervention duration may have been insufficient to allow measurable microbiota shifts, as previous studies have shown that meaningful SCFA modulation typically occurs after ≥8 weeks of consistent dietary change (Feng et al., 2022; Zhao et al., 2022).

The measurement of short-chain fatty acids (SCFAs) in fecal samples provides critical insights into the gut fermentation processes. Unlike traditional gas chromatography (GC) or liquid chromatography (LC) methods, SCFA extraction can be achieved more efficiently by homogenizing fecal samples in ultrapure water before centrifugation, which helps reduce interference from solid particles (Qi et al., 2024). SCFA concentrations are typically categorized, with values below 40 µmol/L suggesting low fermentation activity and levels above 60 µmol/L indicating high fiber bioavailability (Feng et al., 2022). The mean SCFA concentrations observed in both groups (>60 µmol/L) indicate relatively active fermentation, although the differences were not significant. This suggests that the dietary intervention did not disrupt fermentation processes but did not enhance them beyond habitual levels. Stable SCFA levels may also reflect the balance between increased protein intake and limited fiber availability, as protein fermentation alone contributes less to SCFA synthesis but may influence the microbial composition (Liu et al., 2022).

A limitation of this study is that SCFA levels were measured only in the post-intervention phase without baseline data, making it impossible to determine absolute changes from pre- to post-intervention phase. Therefore, future studies should include baseline SCFA measurements, longer intervention periods, and controlled dietary fiber intake to better capture the dynamic relationship between fish protein hydrolysates, gut microbiota, and SCFA production.

Analysis of Confounding Variables

Based on Table 7, the analysis of dietary intake indicates that the intervention had a significant effect on reducing energy and carbohydrate intakes and increasing protein intake, whereas fat intake and physical activity did not show meaningful changes. The similarity in dietary patterns between the groups suggests that the differences in SCFA levels are more likely attributable to the biscuit intervention rather than variations in the daily diet. However, the low fiber intake (<10 g/day) in both groups may have limited the potential increase in SCFA production in this study. In addition, the higher carbohydrate intake observed in the control group than in the intervention group may have contributed to the higher SCFA levels through the fermentation of complex carbohydrates by the gut microbiota.

Table 7. Results of Analysis of Dietary Intake and Lifestyle Factors

Variable	Group	Control	Intervention	p-value
Energy (kcal)	Before	1822.19 ± 324.5	1773.24 ± 294.41	0.669[a]
	After	1575.08 ± 318.93	1467.15 ± 192.17	0.271[a]
	Δ	-247.82 ± 5.57	-306,09 ± 102,24	
	p-value	0.047[c]	0.007[c]	
Protein (g)	Before	62.5 (32.2 – 100.8)	57.3 (30.8 – 88.8)	0.178[b]
	After	62.2 (48.2 – 133.4)	70 (46.9 – 122.6)	0.078[b]
	Δ	-0.3 (16 – 32.6)	12.7 (16.1 – 33.8)	
	p-value	0.776[d]	0.014[c]	
Fat (g)	Before	74.98 ± 26.18	62.07 ± 26.9	0.194[a]
	After	73.32 ± 22.4	65.35 ± 17.74	0.290[a]
	Δ	- 1.66 ± 0.22	3.28 ± 9.16	
	p-value	0.824[c]	0.651[c]	
Carbohydrate (g)	Before	181.13 ± 63.9	159.1 ± 67.8	0.106[b]
	After	172.2 ± 32.4	121.8 ± 59.15	0.002[a]
	Δ	- 8.93 ± 31.4	– 37.3 ± 8.65	
	p-value	0.550[c]	0.115[c]	
Physical activity (MET-Min/Week)	Before	1836.20 ± 1211.3	2243.53 ± 1578	0.853[a]
	After	1819.33 ± 1306.11	1925.8 ± 1330.5	0.945[a]
	Δ	- 16.87 ± 94.81	– 317.73 ± 247.5	
	p-value	0.920[c]	0.232[c]	

[a]: Independent t-test, [b]: Mann–Whitney U test, [c]: Paired t-test, [d]: Wilcoxon Test

Although no statistically significant changes were observed in body weight, BMI, waist circumference, or visceral fat ($p > 0.05$), a downward trend in visceral fat (−0.73 ± 1.24) and waist circumference (−1.19 ± 0.8 cm) was noted in the intervention group. These modest reductions, although not significant, align with the metabolic benefits previously associated with fish protein hydrolysate intake, such as improved satiety and lipid metabolism (Daskalaki et al., 2023). However, as the primary outcome of this study was SCFA levels, these secondary anthropometric trends should be interpreted as supportive observations rather than direct evidence of efficacy of the intervention.

Overall, sensory and nutritional analyses demonstrated that mocaf–FPH biscuits were well accepted and nutritionally enriched with higher protein and dietary fiber. Although the intervention did not significantly modify SCFA levels, the observed stability suggests that the product was metabolically well-tolerated and did not negatively affect gut fermentation. Considering the strong relationship between dietary composition and microbiota-mediated metabolism, further research incorporating higher-fiber formulations or longer supplementation durations is warranted to elucidate the synergistic effects of protein and fiber on SCFA modulation and obesity management.

Conclusion

Overall, incorporating fish protein hydrolysate (FPH) into biscuit formulations improved the protein content and maintained acceptable sensory qualities, particularly in terms of taste and texture. However, the primary outcome, fecal SCFA levels, did not show a statistically significant difference between the control and intervention groups, indicating that the short duration of the intervention and low dietary fiber intake (<10 g/day) likely limited the extent of SCFA production.

Despite this, the modest reductions in visceral fat and waist circumference observed in the intervention group suggest possible supportive metabolic effects of FPH consumption, although these should be interpreted with caution, given the small sample size and non-significant differences.

Future studies should include baseline SCFA measurements, a larger and more diverse participant sample, and longer intervention durations to better elucidate the relationship between FPH, dietary fiber, and SCFA production, as well as their combined effects on metabolic health. FPH-based biscuits represent a promising prototype of functional food with improved nutritional quality; however, further optimization, particularly through fiber enrichment and extended clinical evaluation, is necessary before translation into dietary practice.

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