Jurnal Gizi dan Dietetik Indonesia
(Indonesian Journal of Nutrition and Dietetics)
Vol 13, Issue 6, 2025: 407-420

Available online at: https://ejournal.almaata.ac.id/index.php/IJND
p-ISSN 2303-3045 e-ISSN 2503-183X
DOI: https://dx.doi.org/10.21927/ijnd.2025.13(6).407-420

Formulation of biscuits made from red rice bran, mocaf flour, and pumpkin seeds enriched with inulin as an alternative functional food for patients with type 2 diabetes mellitus

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ABSTRAK

Latar Belakang: *DM Tipe 2 merupakan gangguan metabolik yang ditandai hiperglikemia akibat penurunan sekresi insulin oleh sel β-pankreas. Upaya pengendaliannya dilakukan melalui pengaturan pola makan. Biskuit berbahan bekatul beras merah, tepung mocaf, dan biji labu kuning yang diperkaya inulin berpotensi sebagai camilan alternatif bagi penderita DM Tipe 2.*

Tujuan: *Penelitian ini bertujuan menganalisis karakteristik organoleptik serta kandungan gizi biskuit berbasis bekatul beras merah, mocaf, dan biji labu kuning dengan penambahan inulin, meliputi kadar air, abu, protein, lemak, karbohidrat, antioksidan, gula reduksi, serat pangan, pati total, amilosa, amilopektin, pati resisten, indeks glikemik, dan beban glikemik.*

Metode: *Penelitian menggunakan rancangan acak lengkap dengan tiga ulangan dan empat formulasi perbandingan bekatul beras merah dan mocaf, yaitu F1(30%:70%), F2(40%:60%), F3(50%:50%), dan F4(60%:40%). Uji organoleptik dianalisis menggunakan Kruskal-Wallis dan Mann-Whitney, sedangkan kandungan gizi menggunakan ANOVA dan Duncan.*

Hasil: *Hasil penelitian menunjukkan bahwa terdapat perbedaan nyata pada mutu hedonik (warna, tekstur, rasa pahit) dan kandungan gizi, kecuali gula reduksi. Kadar air 11,48–13,58%, abu 3,30–4,78%, lemak 18,69–21,19%, protein 12,49–15,27%, karbohidrat 47,14–53,92%, antioksidan 16,4–41,89%, gula reduksi 2,76–3,14%, serat pangan total 1,19–3,56%, pati total 35,03–40,42%, amilosa 8,76–9,76%, amilopektin 26,26–30,66%, pati resisten 0,81–2,60%, indeks glikemik 27,46–84,14. Formulasi terbaik adalah F3 (50% bekatul beras merah:50% mocaf) dengan beban glikemik 1,64–9,81 per takaran saji (10–60 g) dengan kategori rendah.*

Kesimpulan: *Biskuit berbahan bekatul beras merah, mocaf, dan biji labu dengan inulin memiliki indeks dan beban glikemik rendah, sehingga berpotensi diaplikasikan sebagai camilan sehat bagi penderita DMT2.*

KATA KUNCI*: bekatul beras merah; biji labu kuning; biskuit; diabetes mellitus tipe 2; tepung mocaf*

ABSTRACT

Background: *Type 2 DM is a metabolic disorder marked by hyperglycemia due to reduced insulin secretion from pancreatic β-cells. Dietary management is essential to control blood glucose levels. Biscuits made from red rice bran, mocaf, and pumpkin seeds enriched with inulin are proposed as an alternative snack for individuals with T2DM.*

Objectives: *This study aimed to analyze organoleptic and nutrient content of biscuits formulated with red rice bran, mocaf, and pumpkin seeds enriched with inulin, including moisture, ash, protein, fat, carbohydrates, antioxidants, reducing sugars, dietary fiber, resistant starch, glycemic index, and glycemic load.*

Methods: *Completely randomized design (CRD) with three replications was applied. Four formulations of red rice bran and mocaf were tested: F1(30%:70%), F2(40%:60%), F3(50%:50%), and F4(60%:40%). Organoleptic were assessed using Kruskal-Wallis and Mann-Whitney tests, while nutrient content was analyzed by ANOVA and Duncan's test.*

Results: *The study revealed significant differences in hedonic quality tests for color, texture, bitter taste, and nutritional content, except for reducing sugars. Nutrient values ranged as follows: moisture 11.48–13.58%, ash 3.30–4.78%, fat 18.69–21.19%, protein 12.49–15.27%, carbohydrates 47.14–53.92%, antioxidants 16.4–41.89%, reducing sugars 2.76–3.14%, total dietary fiber 1.19–3.56%, starch 35.03–40.42%, amylose 8.76–9.76%, amylopectin 26.26–30.66%, resistant starch 0.81–2.60%, and GI 27.46–84.14. The selected formulation F3(50%:50%), with GL values of 1.64–9.81 per 10–60 g serving, classified as low.*

Conclusions: *Biscuits formulated with red rice bran, mocaf flour, and pumpkin seeds enriched with inulin may be a potential snack for patient with T2DM.*

KEYWORDS*:* *biscuits; mocaf flour; pumpkin seeds; red rice bran; type 2 diabetes mellitus*

Article info:
Article submitted on December 29, 2024
Articles revised on March 05, 2025
Articles received on August 19, 2025
Articles available online on November 28, 2025

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder characterized by hyperglycemia. According to the International Diabetes Federation (IDF) (2022), an estimated 536.6 million adults (10.5%) were living with type 2 diabetes mellitus (T2DM) in 2021, and this number is projected to increase to 783.1 million (12.2%) by 2045. The prevalence of T2DM sufferers in Indonesia in 2013 was 2.1% and increased in 2018 to 24.11% (1). The prevalence of DM based on a doctor's diagnosis increased from 1.5% in 2018 to 1.7% in 2023 (2). The prevalence of DM in Central Java was 15.77% in 2015 and increased to 22.1% in 2016. DM is ranked 2nd for non-communicable diseases after hypertension (3). Based on data from the 2018 Riskesdas, the prevalence of diabetes mellitus (DM) sufferers in Surakarta City was 2.21%, which is the most significant incidence of DM in Central Java (1).

One of the causes of T2DM is a state of oxidative stress that can induce insulin resistance in peripheral tissues and damage insulin secretion in pancreatic beta cells. In addition, hyperglycemia is also involved in the process of free radical formation. Hyperglycemia causes glucose autooxidation, protein glycation, and activation of the polyol metabolic pathway which then accelerates the formation of reactive oxygen compounds. Excessive lipid oxidation can form radical compounds, so antioxidant compounds are needed to reduce them (4).

Regular physical activity accompanied by weight management and the implementation of a healthy lifestyle is a way to prevent and treat T2DM (5,6). In addition, low-glycemic index (GI) diets significantly improve glycemic control in patients with metabolic diseases, particularly type 2 diabetes mellitus. The meta-analysis demonstrated reductions in HbA1c and fasting blood glucose levels. These benefits are attributed to the consumption of carbohydrates with low GI and glycemic load, and foods rich in dietary fiber and antioxidants, making such dietary strategies effective for managing glycemic responses in T2DM patients (7). Dietary fiber in food can reduce the level of carbohydrate absorption which will affect the control of increased blood glucose (8,9). Dietary fiber will enter the large intestine to be fermented by bacteria in the large intestine to form SCFA (Short-chain fatty acid). The formation of SCFA will activate the secretion of GLP1 (Glucagon-like peptide 1), GIP (Glucose-dependent insulinotropic polypeptide), PPY (Peptida YY) hormones which will increase the sensitivity of insulin hormones, thus causing a decrease in blood glucose levels (10). Consumption of foods with a low glycemic index and glycemic load, high in fiber and antioxidants is highly recommended for diabetic patients, because it plays a role in lowering blood glucose levels to 9.97-15.3 mg/dL and has been proven to be able to reduce HbA1c levels by 0.26-0.55% (7).

Red rice bran is a rich source of protein, dietary fiber, fat, vitamin E, and bioactive compounds with antioxidant properties. Meza et al. (2024) reported that the protein content of rice bran ranges from 12% to 22%, depending on rice variety and extraction method, with red rice bran generally exhibiting higher protein levels than white rice bran. The dietary fiber content of rice bran ranges from 7% to 11%, comprising both soluble and insoluble fiber, which supports digestive health by promoting the growth of beneficial gut microbiota and enhancing the production of short-chain fatty acids (11). In addition, red rice bran contains anthocyanins, known for their antioxidant activity. The antioxidant capacity of red rice bran is indicated by an IC50 value of 21.98 mL extract/g DPPH, total phenolic content of 4.38 mg/100 g bran, and anthocyanin content of 109.33 mg/100 g bran. A lower IC50 value denotes higher antioxidant activity, classifying red rice bran as having very strong antioxidant potential (12).

In addition to its antioxidant potential, functional food ingredients are also evaluated based on their impact on blood glucose levels. One such ingredient is mocaf flour, which has a low glycemic index when compared to wheat flour. The glycemic index of mocaf flour is 46 and the fiber content is 1.9% - 3.4% (13), Despite its adequate carbohydrate and fiber content, mocaf is relatively low in protein, thus requiring the incorporation of protein-rich ingredients to enhance its nutritional value (14).

Pumpkin seeds (Cucurbita moschata) are a promising ingredient due to their high arginine and dietary fiber content. Every 100 grams of pumpkin seeds contain approximately 9.32 g of arginine and 18.4 g of dietary fiber. Arginine is beneficial for T2DM management as it plays a role in the regeneration of pancreatic β-cells, stimulation of insulin secretion, and improvement of pancreatic enzyme function. Pumpkin seeds are also rich in pectin, which helps regulate blood glucose levels and reduce insulin demand. Moreover, supplementation with pumpkin seed protein has been shown to improve glucose and insulin levels, reduce blood pressure, and decrease markers of oxidative stress. These effects were accompanied by favorable changes in plasma lipid profiles, leptin, and adiponectin levels, suggesting a potential role in managing insulin resistance and metabolic syndrome (15, 16).

Inulin is a well-studied prebiotic fiber that contributes to digestive health and glycemic control. It promotes the growth of beneficial gut microbiota and supports metabolic health without significantly altering the structural or sensory properties of food products (17, 18). The incorporation of inulin in biscuit formulations is considered beneficial for individuals with T2DM due to its ability to stabilize postprandial blood glucose levels.

Despite the promising properties of these individual ingredients, studies combining red rice bran, mocaf flour, pumpkin seeds, and inulin in a single food product are still limited, particularly in Indonesia. Zaddana et al. (2018) demonstrated that a formulation of biscuits using a 20% red rice bran and 40% purple sweet potato combination showed high antioxidant activity (IC50: 106.349 ppm), with 6.38% fiber, 6.92% protein, and 72.56% carbohydrates (12). Substituting 30% pumpkin seed flour in boba formulation increased protein and fiber contents by 4.95% and 7.7%, respectively (19). Furthermore, Putri et al. (2017) found that the optimal combination for biscuit formulation was 30% red rice bran flour and 4% red ginger extract, yielding biscuits with high antioxidant activity (85.94%) and favorable sensory characteristics (20). Other studies on red rice flour and mocaf-based cookies showed a low glycemic index (25.77–31.24), fat content of 26.13–26.58 g, protein content of 5.14–6.06 g, carbohydrates 62.35–64.76 g, and fiber 2.24–4.56 g (21).

The development of functional food products that incorporate low-glycemic index ingredients, rich in protein, fiber, antioxidants, and prebiotics, presents a promising nutritional strategy for diabetes management. Therefore, this study aimed to evaluate the organoleptic characteristics and nutritional composition including moisture, ash, protein, fat, carbohydrates, antioxidants, reducing sugars, dietary fiber, total starch, amylose, amylopectin, resistant starch, glycemic index, and glycemic load of biscuits formulated with red rice bran, mocaf flour, and pumpkin seeds, enriched with inulin.

MATERIALS AND METHODS

This study was conducted using a completely random design (CRD) with three replications. Four biscuit formulations were prepared using different ratios of red rice bran flour to mocaf flour, namely F1 (30%: 70%), F2 (40%:60%), F3 (50%: 50%) and F4 (60%:40%). This study was held between June to September 2024 in Nutrition Laboratory of the Faculty of Health Sciences, Universitas Kusuma Husada Surakarta for product development and Food and Nutrition Laboratory, UGM for nutrient analysis. This research was conducted fter obtaining valid ethical approval from the Kusuma Health Research Ethics Committee, Kusuma Husada University of Surakarta No. 2333/UKH.L.02/EC/IX/2024.

Table 1. Formulation of biscuits made from red rice bran, mocaf flour and yellow pumpkin seeds enriched with inulin

Ingredients	F1 (30% : 70%)	F2 (40% : 60%)	F3 (50% : 50%)	F4 (60% : 40%)
Red rice bran flour (g)	30	40	50	60
Mocaf flour (g)	70	60	50	40
Pumpkin seed (g)	25	25	25	25
Inulin (g)	10	10	10	10
Egg (pcs)	1	1	1	1
Corn flour (g)	10	10	10	10
Non nutritive sweetener (sachet)	2	2	2	2
Skimmed milk (g)	20	20	20	20
Margarine (g)	30	30	30	30
Vanilla paste (tsp)	1	1	1	1
Baking powder (tsp)	½	½	½	½

Note : pcs = pieces; tsp = teaspoon

The formulation of biscuits made from red rice bran, mocaf flour, and yellow pumpkin seeds enriched with inulin is presented in **Table 1**. The formulation was established through preliminary trials and guided by several previous studies, including research by Zaddana et al. (2018) on biscuits containing red rice bran and purple sweet potato; Rahmani et al. (2021) on the development of boba with pumpkin seed substitution; Putri et al. (2017) on the substitution of red rice bran flour and the level of red ginger extract; and Utami and Faida (2023) on the formulation of cookies using red rice flour and mocaf flour (12, 19, 20, 21).The biscuit making process began with weighing the ingredients according to the established formula in **Table 1**. The next stage was preparing the cream by mixing non-nutritive sweetener, margarine, and baking powder. Eggs were then added, and the mixture was beaten using a mixer at low speed for 1 minute until it became homogeneous. This step was intended to improve dough compactness, resulting in a crispier texture. This characteristic is attributed to egg white, which is known to contain up to 80% protein. After that, skimmed milk and vanilla paste were added and beaten at low speed. Other ingredients such as red rice bran flour, mocaf flour, pumpkin seeds, inulin extract and corn flour were then incorporated and mixed until homogeneous to ensure even appearance and taste. The cream mixture and the other ingredients were then combined to form the biscuit dough. The dough was shaped using a mold to achieve a uniform thickness of 5 mm and dimensions of 4 × 4 cm, using a biscuit molding tool. Once molded, the dough was placed on a baking sheet lined with baking paper and baked at a temperature of 130°C for 55 minutes. The organoleptic tests conducted included hedonic tests and hedonic quality tests. The attributes evaluated in the hedonic test included color, aroma, texture, taste, mouthfeel, aftertaste, and overall acceptability. The evaluation was carried out using a 5-point hedonic scale (1=strongly dislike; 2=dislike; 3= neutral/slightly like; 4=like; and 5= strongly like).

The hedonic quality test was conducted to assess specific perceptions related to product quality. The evaluated attributes included color, off-aroma, pleasant aroma, texture, sweet taste, bitter taste, mouthfeel, sweet aftertaste, and bitter aftertaste. For color, the scale ranged from 1= dark brown, 2=moderately dark brown, 3=light brown, 4=yellowish brown, to 5=golden yellow. Aroma attributes (off-aroma and pleasant aroma), taste attributes (sweet and bitter), and aftertaste attributes (sweet and bitter) were all rated using the same intensity scale: 1= very strong, 2= strong, 3=moderate/slightly strong, 4 = weak, and 5=very weak. Texture was evaluated from 1= very not crispy, 2= not crispy, 3=moderate/slightly crispy, 4 = crispy, to 5=very crispy. Mouthfeel was assessed using a scale where 1=very rough, 2= rough, 3=moderate/slightly rough, 4=not rough, and 5=very smooth. The panelists involved in the organoleptic test were 30 semi-trained panelists from undergraduate student from The Nutrition Department, Faculty of Health Sciences, Kusuma Husada University, Surakarta. The criteria for semi-trained panelists in this organoleptic test were individuals aged 20–30 years, having no allergies to the product ingredients, and possessing basic knowledge and experience in sensory evaluation tests. All formula of biscuit were analyzed for nutritional properties.

Moisture and ash content were analyzed using the gravimetric method (SNI 2973:2022), protein content using the Kjeldahl method (SNI 2973:2022), fat content using the Soxhlet method with Weibull modification SNI 2973:2022), and carbohydrate content using the by difference method (SNI 2973:2022), insoluble dietary fiber, soluble dietary fiber and total dietary fiber content using Enzymatic-Gravimetric method (AOAC:2016) (22, 23). Meanwhile, antioxidant activity was measured using DPPH method, reducing sugars content using Nelson-Somogyi method, total starch and amylose content using Direct Acid Hydrolysis method, amylopectin content using the by difference method, resistant starch content using Multienzyme method (24, 25, 26).

The glycemic index test was conducted using a one-shot case study design, which involved intentionally administering treatments to subjects using reference food (white bread) and biscuits, followed by measuring blood glucose after the treatment (27). A total of 54 grams of white bread containing 25 grams of available carbohydrates was measured based on the nutritional information listed on the packaging label, while the number of biscuits given was calculated based on proximate analysis results of each formula. The glycemic index, calculated using the trapezoid method to determine the area under the curve (AUC), involves measuring blood glucose levels at specific time intervals (e.g., 15, 30, 45, 60, 90, and 120 minutes) after consuming a test food. The glycemic load (GL) was calculated using the method of Vega-López et al. (2018) by multiplying the glycemic index (GI) by the available carbohydrate content of the biscuits made from rice bran, mocaf flour, and yellow pumpkin seeds enriched with inulin per serving, then dividing by 100 (28).

The chosen formula was identified using the Exponential Comparison Method (ECM), which is designed to establish the priority ranking of decision alternatives involving multiple criteria. In this process, the weighting is assigned based on the key components of the biscuit. The attributes used include the attributes from the organoleptic evaluation (hedonic test) (each at 5%), moisture, ash, fat, protein, carbohydrate, reducing sugar, dietary fiber, resistant starch content (each at 5%), antioxidant (10%), glycemic index (15%). These weights are then multiplied by the rank of each attribute for every biscuit formula. Finally, the resulting scores are summed to determine the overall ranking of each formula (29).

The data obtained was analyzed using SPSS version 25. Nutrient analysis and organoleptic test result were showed in mean and deviation standard. Organoleptic characteristics, except color, texture and bitter taste were analyzed using the Kruskal-Wallis test and if there is a real effect, it will be continued with Mann-Whitney's post-hoc test if there are significant differences between groups. Meanwhile, nutrient content, except reducing sugar was analyzed using ANOVA and and if there is a real effect, it will be continued with Duncan's post-hoc test if there are significant differences between groups. The level of statistical significantly different was at p<0.05.

RESULTS AND DISCUSSIONS

The hedonic test aims to determine the level of panelists' preference for a product without comparing it with other products. The attributes tested in the hedonic test consist of color, aroma, texture, taste, mouthfeel, aftertaste and overall. The assessment uses a scale of 1-5. The average value of the hedonic test results for biscuits is presented in the **Table 2**.

Table 2. Hedonic test results of biscuits made from red rice bran, mocaf flour and yellow pumpkin seeds enriched with inulin

Attribute	Treatment				
	F1 (30%:70%)	F2 (40%:60%)	F3 (50%:50%)	F4 (60%:40%)	p-value
Color	3.77±0.77 [a]	3.47±0.86[a]	3.63±0.81[a]	3.40±0.86[a]	0.205
Aroma	3.63±0.67 [a]	3.97±0.67[a]	3.60±0.86[a]	3.57±1.01[a]	0.233
Texture	3.23±0.63 [a]	3.60±0.62[a]	3.43±1.07[a]	3.40±0.72[a]	0.219
Taste	2.83±0.79 [a]	3.37±0.81[a]	3.30±1.18[a]	3.00±1.05[a]	0.091
Mouthfeel	3.00±0.64 [a]	3.00±0.64[a]	3.07±1.02[a]	3.10±0.89[a]	0.797
Aftertaste	2.90±0.77 [a]	3.10±1.03[a]	2.93±0.98[a]	3.03±0.99[a]	0.890
Overall	3.23±0.68 [a]	3.50±0.73[a]	3.27±1.05 [a]	3.00±0.87[a]	0.161

Note: The mean values followed by different letters in the same row indicate significant differences (p<0.05).
Scale : 1 = strongly dislike; 2 = dislike; 3 = neutral/slightly like; 4 = like; and 5 = strongly like

Based on **Table 3,** the preference scores for biscuit color ranged from 2.37 (moderately dark brown) in F3 to 3.90 (light brown) in F2. Although there was no significant difference in overall color preference among formulations (p>0.05), the hedonic quality scores for color showed a significant difference (p<0.05), particularly between F3 and other formulations. Pairwise comparisons revealed that F1 and F2 were not significantly different, whereas F3 and F4 were significantly different from the other formulations. The darker color in biscuits with higher red rice bran content is consistent with findings by Sofianti et al. (2021) and Damayanti et al. (2020), who reported that increased bran substitution leads to darker cereal-based products (30,31). This browning is attributed to the presence of phytochemicals such as anthocyanins and the Maillard reaction, which is a non-enzymatic reaction between reducing sugars and amino acids that produces melanoidin pigments during baking (31). At 121°C for 15 minutes, the red color of anthocyanin-rich red rice bran transitions to brown due to these reactions. In contrast, mocaf flour contributes to a lighter biscuit color. Its processing steps, including washing, soaking, and fermentation, help remove pigments and proteins that would otherwise promote browning, resulting in a whiter flour compared to conventional cassava flour (32).

The use of red rice bran and mocaf flour did not significantly affect panelists' preference for the aroma attribute (p>0.05). The aroma was assessed based on two sub-attributes: off-aroma and pleasant aroma. Based on **Table 3**, off-aroma scores ranged from 3.10 to 3.70 (moderate or slightly strong), with the highest in F2 and the lowest in F4. Pleasant aroma scores ranged from 2.67 to 2.80 (strong), with F3 having the highest score. Although the differences were not statistically significant, off-aroma in biscuits containing red rice bran may be linked to fat oxidation catalyzed by lipase enzymes naturally present in the bran. These enzymes accelerate lipid degradation, producing undesirable odors if not properly inactivated. Roasting has been reported to reduce this effect by deactivating lipase activity (33). The pleasant aroma was not notably influenced by the presence of mocaf flour, as its processing removes volatile and alkali compounds that typically contribute to strong odors (34).

Table 3. Hedonic quality test results of biscuits made from red rice bran, mocaf flour and yellow pumpkin seeds enriched with inulin

Attribute	Treatment				
	F1 (30%:70%)	F2 (40%:60%)	F3 (50%:50%)	F4 (60%:40%)	*p-value*
Color	3.87±0.94[a]	3.90±0.76[a]	2.37±0.96[b]	3.10±1.19[c]	0.000*
Off aroma	3.23±0.89[a]	3.70±0.65[a]	3.43±1.01[a]	3.10±0.99[a]	0.077
Pleasant aroma	2.73±0.74[a]	2.67±0.88[a]	2.80±0.85[a]	2.70±1.02[a]	0.887
Texture	2.87±0.86[a]	3.77±0.63[b]	4.13±0.82[c]	3.03±1.03[a]	0.000*
Sweet taste	3.43±0.73[a]	3.40±0.62[a]	3.33±1.09[a]	3.37±0.93[a]	0.979
Bitter taste	3.97±0.81[a]	3.43±0.94[b]	3.37±1.09[b]	2.67±1.24[c]	0.001*
Mouthfeel	3.10±0.96[a]	3.03±0.62[a]	2.77±1.16[a]	3.20±0.89[a]	0.305
Sweet aftertaste	3.57±0.77[a]	3.37±0.85[a]	3.50±0.86[a]	3.67±0.96[a]	0.647
Bitter aftertaste	3.87±0.94[a]	3.50±1.04[a]	3.20±1.06[a]	3.50±0.86[a]	0.126

Note: [a,b,c] The mean values followed by different letters in the same row indicate significant differences ($p<0.05$).
Scale:
Color: 1 = dark brown, 2 = moderately dark brown, 3 = light brown, 4 = yellowish brown, to 5 = golden yellow
Aroma (off-aroma and pleasant aroma), taste (sweet and bitter), and aftertaste (sweet and bitter): 1 = very strong, 2 = strong, 3 = moderate/slightly strong, 4 = weak, and 5 = very weak
Texture: 1 = very not crispy, 2 = not crispy, 3 = moderate/slightly crispy, 4 = crispy, to 5 = very crispy
Mouthfeel: 1 = very rough, 2 = rough, 3 = moderate/slightly rough, 4 = not rough, and 5 = very smooth

Based on **Table 3**, the use of red rice bran and mocaf flour did not significantly affect panelists' preference for biscuit texture ($p>0.05$), with scores ranging from 2.87 (not crispy, F1) to 4.13 (crispy, F3). However, significant differences were observed in the hedonic quality of texture ($p<0.05$). Further analysis revealed that F1, F2, and F3 were significantly different from one another, while F1 and F4 did not differ significantly. Textural variation among formulations may be influenced by the starch composition, particularly the amylose-to-amylopectin ratio. Amylose contributes to firmness and break resistance, whereas amylopectin yields a more fragile texture but acts as a binder that strengthens structural integrity (35). In addition, higher red rice bran substitution reduces gluten content, which is essential for forming the cohesive texture typically found in wheat-based products. Although red rice bran adds hardness due to its fiber content, the absence of gluten limits the structural matrix formation in the biscuits. Differences in protein and starch content, along with increased fiber levels from bran, also contribute to these textural changes (36).

Table 3 showed that the use of red rice bran and mocaf flour did not significantly affect panelists' preference for sweetness ($p>0.05$), with scores ranging from 3.33 to 3.43, categorized as moderate or slightly strong. However, significant differences were found in the hedonic quality of bitterness ($p<0.05$), with F1 having the highest score (3.93) and F4 the lowest (2.67). Pairwise comparisons indicated that F1 differed significantly from all other formulations, while F2 and F3 were not significantly different from each other but both differed from F4. The bitterness is likely associated with the saponin content in red rice bran. Additionally, the Maillard reaction during baking may contribute to bitterness through the formation of compounds resulting from the degradation of lipids, proteins, phosphatidylcholine, and amino acids (31).

Table 3 showed that there was no significant difference in panelists' preference for biscuit mouthfeel ($p>0.05$), with scores ranging from 2.77 (rough, F3) to 3.20 (moderate or slightly rough, F4). Although a slight variation was observed, the differences were not statistically significant across formulations. The hedonic quality of mouthfeel was also not significantly affected by the use of red rice bran ($p>0.05$). According to **Table 3** the use of red rice bran and mocaf flour did not significantly affect the sweet or bitter aftertaste of the biscuits ($p>0.05$). Sweet aftertaste scores ranged from 3.37 to 3.67, while bitter aftertaste scores ranged from 3.20 (F3) to 3.87 (F1), all categorized as moderate or slightly strong. The bitter aftertaste is likely related to the presence of saponins in red rice bran. Additionally, the Maillard reaction during baking may contribute to bitterness through the hydrolysis of amino acids and the oxidative degradation of lipids, phosphatidylcholine, and peptide (31). Overall is an assessment of the level of panelist preference for all attributes in biscuit products.

Table 4. Test results for the nutritional content of biscuits made from red rice bran, mocaf flour and yellow pumpkin seeds enriched with inulin

Nutrient Content (%)	Treatment				
	F1 (70%:30%)	F2 (60%:40%)	F3 (50%:50%)	F4 (40%:60%)	*p-value*
Moisture content	11.59±0.15[a]	12.57±0.08 [b]	13.58±0.04[c]	11.48±0.02[a]	0.000*
Ash content	3.30±0.02[a]	3.84±0.01[b]	4.17±0.05[c]	4.78±0.06[d]	0.000*
Fat	18.69±0.35[a]	18.98±0.33[a]	20.97±0.32[b]	21.19±0.27[b]	0.000*
Protein	12.49±0.44[a]	13.42±0.31[b]	14.16±0.08[c]	15.27±0.24[d]	0.000*
Carbohydrate	53.92±0.08[a]	51.2±0.70[b]	47.14±0.48[c]	47.29±0.47[c]	0.000*
Antioxidants	16.4±0.08[a]	24.09±0.08[b]	32.35±0.08[c]	41.89±0.08[d]	0.000*
Glycemic index	84.14±0.49[a]	47.40±1.03[b]	34.69±0.25[c]	27.56±0.49[d]	0.000*
Reducing sugars	2.92±0.0 [a]	3.14±0.04[a]	2.76±0.49[a]	2.99±0.01[a]	0.182
Insoluble dietary fiber	3.22±0.01[a]	2.88±0.01 [b]	1.74±0.01 [c]	1.16±0.01[d]	0.000*
Soluble dietary fiber	0.35±0.02[a]	0.29±0.01[b]	0.16±0.01[c]	0.04±0.00[d]	0.000*
Total dietary fiber	3.56±0.01[a]	3.16±0.00[b]	1.89±0.15[c]	1.19±0.01[d]	0.000*
Total starch	40.42±0.13[a]	37.21±0.06[b]	35.54±0.05[c]	35.03±0.45[d]	0.000*
Amylose	9.76±0.06[a]	9.48±0.05[b]	9.12±0.02[c]	8.33±0.01[d]	0.000*
Amylopectin	30.66±0.19[a]	27.73±0.11[b]	26.42±0.04[c]	26.70±0.44[c]	0.000*
Resistant starch	2.60±0.00[a]	2.22±0.01[b]	1.28±0.01[c]	0.81±0.01[d]	0.000*

Note: [a,b,c] The mean values followed by different letters in the same row indicate significant differences ($p<0.05$).

According to **Table 3**, panelists' overall preference for the biscuit formulations ranged from 3.00 (F4) to 3.50 (F2), categorized as neutral to slightly like on a 5-point hedonic scale. The use of red rice bran and mocaf flour did not significantly affect overall preference scores ($p>0.05$), indicating that all formulations were generally acceptable to the panelists. The analysis of the nutritional content of biscuits, as shown in Table 4, reveals significant differences in moisture content, ash, fat, protein, carbohydrates, antioxidants, glycemic index, insoluble dietary fiber, soluble dietary fiber, total dietary fiber, total starch, amylose, amylopectin and resistant starch across the formulations of biscuits made from red rice bran, mocaf flour, and pumpkin seed enriched with inulin.

According to **Table 4** and **Figure 1**, the nutritional composition of the biscuit formulations showed considerable variation. Moisture content ranged from 11.48% to 13.58%, ash content from 3.30% to 4.78%, fat from 18.69% to 21.19%, protein from 12.49% to 15.27%, and carbohydrates from 47.14% to 53.92%. **Figure 2** showed that antioxidant activity ranged from 16.4% to 41.89%, increasing proportionally with the amount of red rice bran flour. Formulations with higher red rice bran and lower mocaf flour content exhibited increased ash, fat, protein, and antioxidant levels, while showing decreased total starch, resistant starch, and dietary fiber (insoluble, soluble, and total).

When compared to the Indonesian National Standard (SNI 2973:2022), all formulations exceeded the maximum allowable moisture content (5%) and ash content (0.1%), but met the minimum protein requirement (≥5%) and maximum carbohydrate limit (<70%) for biscuits (22). The relatively high protein content is advantageous for individuals with type 2 diabetes mellitus, as proteins of high biological value enhance nitrogen absorption and utilization, thereby reducing metabolic waste and minimizing renal strain (37). Resistant starch content ranged from 0.81% to 2.60% across the formulations. According to the classification, resistant starch is categorized as very low (<1%), low (1–2.5%), and medium (2.5–5%) (38). Formulation F1 (2.60%)

falls into the medium category, whereas F2 (2.22%), F3 (1.28%), and F4 (0.81%) are classified as low and very low, respectively. The decreasing trend in resistant starch is associated with the reduced proportion of mocaf flour, which is relatively higher in starch content, and the increased use of red rice bran, which contributes more protein and fat but less digestible starch. The glycemic index (GI) was calculated using the trapezoid method to determine the area under the glucose response curve. Formulation F1 exhibited the highest GI (84.14), classified as high GI (≥70). In contrast, formulations F2 (47.40), F3 (34.69), and F4 (27.56) fell within the low GI category (≤55) (39).

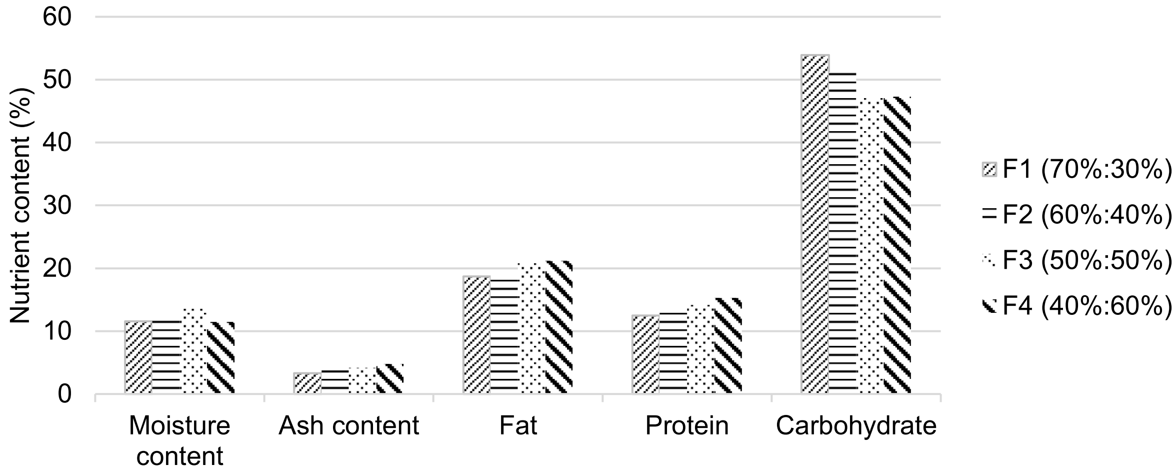

Figure 1. Comparison of the nutritional content of biscuits

The decline in GI values is attributed to increases in fat, protein, and fiber content, which are known to reduce postprandial glycemic responses. Fat and protein delay gastric emptying and carbohydrate digestion, while dietary fiber slows glucose absorption, potentially reducing carbohydrate uptake by up to 50% (9, 40). The type of carbohydrate also plays a critical role; simple carbohydrates tend to raise blood glucose levels more rapidly than complex carbohydrates (5). Additionally, the amylose-to-amylopectin ratio influences the glycemic response, with higher amylose content associated with reduced digestibility and thus a lower GI (39).

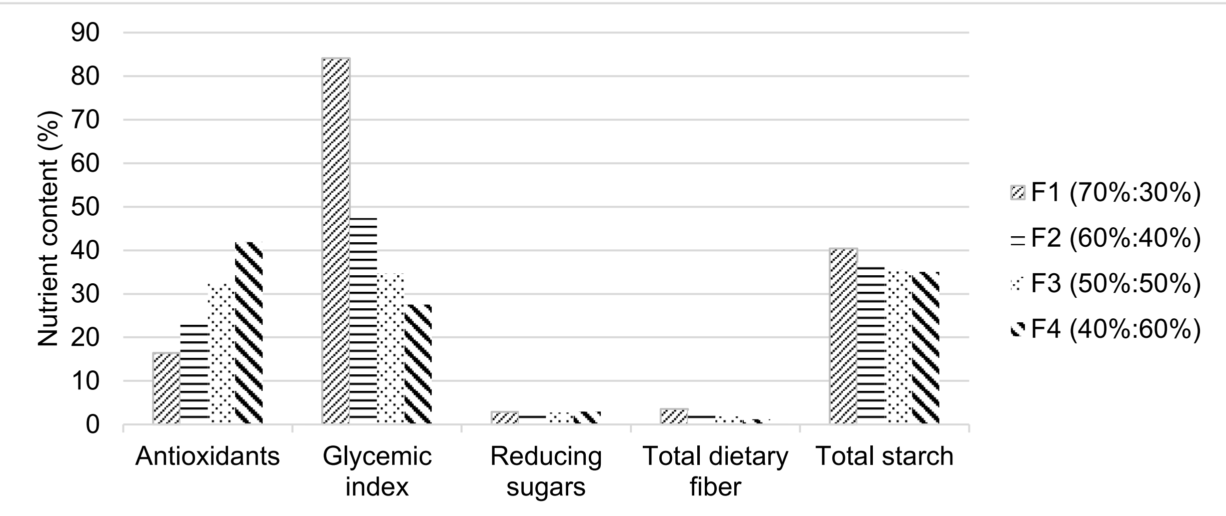

Figure 2. Comparison of the other nutritional content of biscuits

Table 5. Results of the selection of the preferred formulation using the Exponential Comparison Method (ECM)

Attribute	**Weight (%)**	**F1**		**F2**		**F3**		**F4**	
		Rank	**Score**	**Rank**	**Score**	**Rank**	**Score**	**Rank**	**Score**
Color	5	2	0.1	1	0.05	4	0.2	3	0.15
Aroma	5	3	0.5	1	0.05	2	0.1	4	0.2
Texture	5	2	0.1	4	0.2	1	0.05	3	0.15
Taste	5	4	0.2	1	0.05	2	0.1	3	0.15
Mouthfeel	5	4	0.2	1	0.05	3	0.15	2	0.1
Aftertaste	5	4	0.2	2	0.1	1	0.05	3	0.15
Overall	5	3	0.15	1	0.05	2	0.1	4	0.2
Moisture	5	2	0.1	3	0.15	4	0.2	1	0.05
Ash	5	1	0.05	2	0.1	3	0.15	4	0.2
Fat	5	1	0.05	2	0.1	3	0.15	4	0.2
Protein	5	4	0.2	3	0.15	2	0.1	1	0.05
Carbohydrates	5	4	0.2	3	0.15	1	0.05	2	0.1
Reducing sugar	5	2	0.1	4	0.2	1	0.05	3	0.15
Dietary fiber	5	1	0.05	2	0.1	3	0.15	4	0.2
Resistant starch	5	1	0.05	2	0.1	3	0.15	4	0.2
Antioxidant	10	4	0.4	3	0.3	2	0.2	1	0.1
Glycemic index	15	4	0.6	3	0.45	2	0.3	1	0.15
Total Score			2.90		2.35		2.25		2.50
Ranking			4		2		1		3

Note : The score for each attribute is calculated by multiplying the weight by the ranking of that attribute.

The selected formulation was determined using the Exponential Comparison Method (ECM), which involves assigning rankings and scores to each attribute (29). Different weights were assigned to each attribute based on the most critical quality attributes or the attributes prioritized for enhancement. Based on **Table 5**, it was determined that F3 is the selected formulation, achieving the highest total score of 2.25. The glycemic load (GL) calculation is carried out by determining the available carbohydrates in a single serving size of the selected formulation, specifically F3. According to Table 6. the available carbohydrates in the biscuits amount to 47.14 g per 100 g. For patients with diabetes mellitus, a serving of 10–40 grams of biscuits with a low glycemic load (<11) is recommended. A low glycemic load is defined as <11, a medium glycemic load as 11–20, and a high glycemic load as >20 (40).

Table 6. Glycemic load of biscuits made from red rice bran, mocaf flour and yellow pumpkin seeds enriched with inulin in the selected formulation

Serving Size (g)	**Available Carbohydrates per 100 g (g)**	**Available Carbohydrates per Serving (g)**	**Glycemic Load (GL)**	**Category**
10	47.14	4.71	1.64	Low
20	47.14	9.43	3.27	Low
30	47.14	14.14	4.91	Low
40	47.14	18.86	6.54	Low
50	47.14	23.57	8.18	Low
60	47.14	28.28	9.81	Low
70	47.14	33.00	11.45	Medium
80	47.14	37.71	13.08	Medium
90	47.14	42.43	14.72	Medium
100	47.14	47.14	16.35	Medium

Note : Range of glycemic load = low : <11; medium : 11–20; high : >20

The low glycemic index of biscuits made from red rice bran flour, mocaf (modified cassava flour), pumpkin seeds, and enriched with inulin affects the glycemic load of the biscuits. Additionally, the glycemic load of a food is also determined by the portion size consumed (41, 42). The recommended serving size for diabetes patients as a snack ranges from 10 to 60 grams, equivalent to one to six biscuits. In a single serving (10–60 grams), the glycemic load of the biscuits is found to be 1.64–9.81, which falls under the low glycemic load category. The glycemic load of a food or ingredient also depends on the quantity consumed. The glycemic load value increases as the amount of food consumed grows (42). Diabetes mellitus patients are advised to consume foods with a low glycemic index and glycemic load (39) (43). The findings of this study indicate that biscuits made from red rice bran flour, mocaf, pumpkin seeds, and enriched with inulin have a low glycemic index and glycemic load, making them a suitable alternative snack for individuals with Type 2 diabetes mellitus. The previous study suggested that the risk of Type 2 diabetes mellitus, cardiovascular diseases, and certain cancers can be reduced by consuming foods with a low glycemic load (44). Similarly, Vega-Lopez et al. (2018) and Zafar et al. (2019) reported that low glycemic index foods can improve glycemic control (44, 28).

CONCLUSION AND RECOMMENDATION

Biscuits formulated with red rice bran, mocaf, pumpkin seeds, and inulin showed significant differences in several nutritional and sensory quality attributes. Although overall preference was not significantly affected, formulations differed in color quality, texture, and bitterness. Given the low glycemic index and fiber content, these biscuits are potentially suitable as functional snacks for individuals with Type 2 diabetes. Further studies are recommended to assess long-term acceptability and physiological effects.

ACKNOWLEDGEMENT

This study was financially supported by the Ministry of Education, Culture, Research, and Technology of Republic Indonesia through Institute for Research and Community Service, Universitas Kusuma Husada Surakarta, for which we would like to express our gratitude and appreciation. We would like to thank Nutrition Laboratory of Kusuma Husada University of Surakarta for their assistance with this study. The authors have no conflict of interest.

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