International Journal of Agriculture and Biosciences 2026 15(2): 578-584.
https://doi.org/10.47278/journal.ijab/2025.211

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Utilization of Nano-calcium from Red Snapper (*Lutjanus malabaricus*) in Low-glycemic Snack Bar Products

Novia Anggraeni [1,*], Putut Har Riyadi [2], Raka Bachtiar Kuspradanarto [3] and Martina Widhi Hapsari [4]

[1]Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. H. Soedarto, S.H., Semarang 50275, Indonesia
[2]Department of Fish Product Technology, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. H. Soedarto, S.H., Semarang 50275, Indonesia
[3,4]Department of Food Technology, Faculty of Science and Technology, National Karangturi University, Jl. Raden Patah 182-185, Semarang 50227, Indonesia
*Corresponding author: novia.anggraeni@unkartur.ac.id

ABSTRACT

Adequate nutrition, including calcium, protein, and fat, is essential for balancing bodily requirements. These micronutrients and macronutrients are also crucial for individuals with type 2 diabetes mellitus. This study aimed to develop and evaluate a low-glycemic-index snack bar formula acceptable to consumers, determine the glycemic index and glycemic load of the best formula, and assess the effect of adding different concentrations of nano-calcium from red snapper on the antioxidant content of the snack bar. The experimental design was a completely randomized design with four treatments based on variations in the concentration of nano-calcium from red snapper bone: F0 (0%), F1 (5%), F2 (10%), and F3 (15%). The selected formula was determined by considering panelists' levels of preference and acceptance across all attributes. The chosen formula (F1) contains nutritional values including 18.04g (%w/w) protein, 13.05g (%w/w) fat, and 56.47g (%w/w) carbohydrates. One 50g serving of the snack bar contributes 15.1–37.5% of the Recommended Dietary Allowance (RDA) for individuals aged 17–29 years. This snack bar, made with robusta coffee extract and nano-calcium from red snapper, can be claimed as a source of protein, has a low glycemic index, and is rich in antioxidants.

Keywords: Type 2 diabetes mellitus, Low glycemic, Value added.

Article History
Article # 25-450
Received: 03-Aug-25
Revised: 17-Sep-25
Accepted: 05-Nov-25
Online First: 10-Dec-25

INTRODUCTION

Food security has become a primary global concern as the world population continues to rise and is projected to reach 9.3 billion by 2050 (Anggraeni, 2020). Red snapper (*L. campechanus*) production has increased annually. According to the Indonesian Ministry of Marine Affairs and Fisheries (KKP), fish production in the red snapper sector reached 20.54 million tons in 2022, representing a 5.49% increase from 2021, when it was 19.47 million tons. In red snapper processing activities, waste accounts for up to 15% and calcium concentration is 65–70% (Anggraeni et al., 2024). Red snapper is typically produced and marketed in fresh, filleted, or frozen forms. This processing generates several categories of waste, including scales, fins, skin, and bones, which have not been fully utilized. To increase the economic value of this waste, especially red snapper bones, they are well-suited for use as a calcium source, considering that calcium is essential for various metabolic processes in vital bodily functions, such as bone and tooth formation, a catalyst for biological reactions, and muscle contraction (Zhou et al., 2025).

Currently, have remained mainly underutilized. Therefore, they possess great potential for further development, given that fish bones contain the highest amount of calcium compared to other fish parts (Andreola et al., 2023). Red snapper bone flour also contains other essential nutrients, including fat, protein, and carbohydrates (Kaur et al., 2023). Nano-calcium can be added to food products, such as low-glycemic-index (GI)

Cite this Article as: Anggraeni N, Riyadi PH, Kuspradanarto RB and Hapsari MW, 2026. Utilization of nano-calcium from red snapper (*Lutjanus malabaricus*) in low-glycemic snack bar products. International Journal of Agriculture and Biosciences 15(2): 578-584. https://doi.org/10.47278/journal.ijab/2025.211

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snack bars. Low-glycemic-index foods are vital for people with diabetes, helping them meet their nutritional and fiber-intake needs. One factor contributing to the high prevalence of diabetes mellitus is dietary habits. Changes in diet and lifestyle also affect people's consumption patterns, leading to a lack of attention to the food they eat. According to Liu et al. (2024), foods with a high GI can increase blood sugar levels over time, which can lead to various diabetic complications. In addition, previous research (Thalib et al., 2021) on the addition of anchovy bone flour to snack bar products found that it elicited the highest panelist preference for taste, aroma, and texture.

Snack bars have emerged as a popular convenience food product appealing to diverse demographic groups, including both children and adults, due to their ease of consumption and variety of flavors. However, most snack bars currently available in the market are predominantly composed of carbohydrates, primarily derived from cereals, wheat flour, and sugar (Umami et al., 2021). The levels of protein, fiber, and micronutrients, such as calcium, are generally low in these products, rendering them suboptimal for meeting the population's nutritional requirements. To address this limitation, innovation in snack bar formulations is needed that not only provides energy from carbohydrates but also incorporates essential micronutrients such as calcium. One promising strategy is the fortification of snack bars with nano-calcium sourced from natural materials, such as red snapper fish bones. These fish bones, a by-product of the fishing industry, are rich in calcium, phosphorus, and collagen protein but remain underutilized (Rodrigues et al., 2021). The purpose of this study was to develop and evaluate a low-GI snack bar formula that is acceptable to consumers, determine the GI and GL content of the best snack bar formula, and assess the effect of adding different concentrations of nano-calcium from red snapper on the antioxidant content in the snack bar.

MATERIALS & METHODS

Ingredients included sago starch, black glutinous rice flour, nano-calcium red snapper, margarine, honey, peanuts, and robusta coffee extract. Chemicals used for chemical analysis include hexane solvent (for fat analysis) and reagents H_2SO_4, 40% NaOH, 4% H_3BO_3, 0.1 N HCl, selenium mix, methyl red indicator, and distilled water (for protein analysis). Equipment used included a digital scale, mixers, ovens, sieves, and standard laboratory apparatus.

Nano-calcium Production Process

The production of nano-calcium commenced with the extraction of calcium from red snapper bones using an alkaline method as developed by Anggraeni et al. (2024), with certain modifications. Initially, a coarse powder derived from red snapper bones was extracted with 1N NaOH at 100°C. The extract was subsequently cooled, filtered, and neutralized. Once neutralized, the extract was oven-dried at 50°C for 24h, yielding a fine white powder known as nano-calcium.

Snack Bar Production

The production of snack bars commences with milling raw materials into flour using a hammer mill (Lee et al., 2021), with subsequent modifications. The ingredients, including sago starch flour, black glutinous rice flour, margarine, and honey, are combined in a mixing bowl, with nano-calcium incorporated at 0, 5, 10, or 15% (w/w) of the total dry weight. Once the dough is homogeneous, it is molded and topped with rice crisps and peanuts. The prepared dough is then placed on a pre-greased baking tray and subjected to a two-stage baking process: initially at 150°C for 30 minutes, followed by an additional 20 minutes at the same temperature.

Organoleptic Analysis

The organoleptic test was conducted on snack bars made from red snapper fish bone flour, coffee extract, and sago flour using semi-trained panelists specializing in sensory evaluation. The panel consisted of at least 30 untrained individuals who had not previously participated in organoleptic tests. A hedonic test with a seven-point Likert scale (1 = like very much; 7 = dislike very much) was employed to assess panelists' preference levels for attributes including color, aroma, taste, and texture.

Proximate Analysis

The chemical content of the sample was determined using standard methods (AOAC 2005). The crude protein content was calculated by multiplying the total nitrogen factor by the crude protein content, which accounts for differences in carbohydrate content estimation.

Glycemic Index Analysis of Selected Snack Bars

Recruitment characteristics of prospective subjects were established using purposive random sampling, with verbal announcements requesting willingness to participate in the study. Prospective subjects included members of the general public who met the criteria and signed an informed consent form. Selected subjects met the inclusion criteria, including normal nutritional status (BMI 18.5–22.9 kg/m^2), fasting blood glucose between 70–100 mg/dL, and age 20–23 years. The food products evaluated for their GI were snack bars with coffee concentrations of 80, 100, and 120g, while the standard food product used as a comparator contained no added coffee extract. All test and reference foods provided 50g of available carbohydrates (by difference). According to Elfers et al. (2024), since the standard food is pure glucose, the amount given is 50g. The GI check was conducted with three repetitions: a white bread sample, a control sample, and finally the selected snack bar sample. Before checking their blood sugar, prospective subjects must fast for 8 hours and abstain from eating anything. Within 24 hours, research subjects consume samples in sequence and then have their blood sugar levels checked.

Determination of Glycemic Load

Glycemic Load (GL) is calculated by multiplying the GI by 100 and subtracting it from the carbohydrate content obtained from proximate analysis. The GL determination was carried out for the snack bar product using the selected formula: GL = (GI × carbohydrate content / 100).

Determination of Antioxidant Activity

The testing of antioxidant activity content was conducted at the Integrated Laboratory of Diponegoro University, Semarang, in August 2023. The determination of antioxidant activity was carried out using the quantitative DPPH (2,2-diphenyl-1-picrylhydrazyl) method based on the *Lambert-Beer* law.

Data Analysis

All collected data were subjected to statistical analysis using Microsoft Excel 2016 and SPSS version 26 (IBM Corp.). Before analysis, the data were tested for normality and homogeneity of variance. If the assumptions of normality and homoscedasticity were met, a one-way analysis of variance (ANOVA) was performed at the 95% confidence level ($\alpha = 0.05$). When significant differences among treatments were detected ($P<0.05$), Duncan's Multiple Range Test (DMRT) was conducted to determine specific differences between treatment means, including comparisons between experimental diets and the control diet.

RESULTS AND DISCUSSION

Formulation is predicated on providing adequate energy and essential nutrients, with a particular emphasis on protein content. The protein sources incorporated in the snack bar formulation include black glutinous rice flour and nano-calcium derived from red snapper fish bones. The energy and protein requirements in adults escalate concomitantly with body weight. Snack bars augmented with nano-calcium from red snapper bones are posited as a potential alternative protein source characterized by a low GI. Following multiple trials, a definitive formulation was established, comprising four distinct snack bar formulas. The experimental variable is the addition of nano-calcium from red snapper bones, with F0=0%, F1=5%, F2=10%, and F4=15%. This formulation is informed by prior studies (Canti and Martawidjaja 2024), which indicate that incorporating less than 50g of red snapper bone flour yields a dough texture most favored by children. Conversely, the addition of more than 50g of red snapper bone flour may result in a coarse texture and a propensity for a fishy odor, which is generally unappealing to children. This is attributed to the presence of large particles in the nano-calcium derived from red snapper bones (Yang et al. 2023).

In the F0 trial, the formulation served as a control without red snapper bone nanocalcium. The F1 formulation included a 5% addition of red snapper bone nano-calcium, F2 included a 10% addition, and F3 included a 15% addition. The concentrations of nano-calcium employed in this study were selected to assess the range of fortification that could confer functional benefits without compromising the sensory quality of the snack bar. The addition of 5% nano-calcium is anticipated to enhance calcium content with minimal impact on taste and texture. In contrast, the 10 and 15% additions were used to determine the threshold of consumer acceptance and the effects on the product's physicochemical properties, including hardness, color, and crispiness.

Furthermore, according to Verma et al. (2025), the incorporation of nano-calcium at levels of 10–15% in snack bars does not result in significant alterations in organoleptic properties. However, it can double the calcium content compared to conventional forms. Therefore, the variation in nano-calcium concentrations in this study aims to identify the optimal formulation that enhances the product's nutritional value without compromising sensory quality or product stability. The organoleptic evaluation was conducted using a single-phase standard hedonic test. This hedonic test was used to evaluate acceptance and preference for the red snapper bone-based snack bar. The scale ranged from 1 to 7, with 1 indicating "like very much" and 7 indicating "dislike very much." The hedonic test attributes included aroma, taste, texture, and color. Table 1 presents the results of the hedonic test on the snack bars. Aroma received the lowest mean score, whereas texture had the highest.

Table 1: Snack bar organoleptic test results

Formula	Organoleptic			
	Flavor	Taste	Texture	Color
F0	2.51±0.60	6.38±0.49	5.02±0.77	4.61±0.61
F1	3.35±0.76	6.81±0.49	6.16±0.76	4.19±0.87
F1	2.77±0.66	6.58±0.50	3.08±0.62	3.64±0.48
F3	3.40±0.49	6.53±0.50	4.11±0.86	4.20±0.88

Table 2: Proximate analysis of selected snack bar (F1)

Parameter	Value (F1)	USDA* Maximum
Proteins	18.01±0.08	9.38
Water content	11.10±0.40	11.26
Ash content	1.32±0.05	1.72
Fat content	13.05±0.20	10.93
Carbohydrate	56.47±0.12	66.72

In this study, the incorporation of snapper bone nanocalcium into snack bars yielded an average panelist preference score ranging from 2.51 to 3.4, indicating "dislike" to "somewhat dislike". The ANOVA test results indicated that variations in the ratio of red snapper bone nano-calcium significantly affected panelist preference for the aroma attribute. Subsequent DMRT tests revealed significant differences in aroma preference across the different formulations.

The organoleptic assessment of the odor parameter revealed that some panelists detected a fishy odor in snack bar samples fortified with nano-calcium derived from snapper bones. This phenomenon can be attributed to the intrinsic properties of the fish bone raw material, which retain characteristic volatile compounds typical of fish, such as trimethylamine (TMA), aldehydes, and free fatty acids, even after nanoencapsulation or particle-size reduction (Zhang et al., 2024). The fishy odor often intensifies with increasing nano-calcium (10–15%), because more volatile compounds are incorporated into the snack bar. Additionally, if the process of removing fat and protein from the bones is inefficient, leftover unsaturated fats can oxidize, creating a rancid or fishy smell (Aenglong et al. 2023). In contrast, the musty aroma sometimes detected in products made with black glutinous rice flour is likely due to oxidation of the rice's natural fats. This is particularly common if the flour has been stored for a long time or exposed to humidity. Black glutinous rice contains more fat than white rice and also has compounds that can react during processing or storage, leading to this musty aroma. Furthermore, the unsaturated fatty acids in black glutinous rice are prone to oxidation if the flour is not stored correctly in airtight containers at cool temperatures. The heat used during snack bar production can intensify this musty smell through reactions such as the Maillard reaction and the thermal degradation of fats, which some panelists found less appealing.

The findings from the hedonic assessment of the taste parameter indicate that incorporating 5% red snapper bone nano-calcium yielded a superior taste-preference score compared to the control group (lacking nano-calcium) and to treatments with 10 and 15% concentrations. This suggests that at the 5% concentration, the addition of nano-calcium enhances the product's taste profile without introducing an undesirable aftertaste. The observed increase in taste scores at this level is attributed to the presence of natural minerals and amino acids derived from the fish bones, which, in limited quantities, contribute to umami taste complexity, thereby enhancing the product's palatability (Darmanto et al., 2017). Furthermore, hydrolyzed collagen protein derived from fish bones may impart a subtle, mild flavor that remains unobtrusive when used in moderation (Kim et al., 2012). Conversely, at concentrations of 10 and 15%, panelists reported lower taste preference scores, likely due to a fishy or bitter aftertaste associated with higher concentrations of minerals and volatile compounds. Excessive calcium and nitrogen compounds from fish bones can produce flavors that are unfamiliar or less favored in carbohydrate-based snacks such as snack bars (Fetriyuna et al., 2023). As noted by Faria-Silva et al. (2020), the incorporation of fish- or bone-based protein ingredients in food products must be carefully regulated, as excessive use can result in a fishy taste and aroma that diminishes consumer acceptance. Therefore, a 5% concentration is deemed optimal for fortification, balancing enhanced calcium content with organoleptic acceptability, particularly regarding taste.

Table 3: Glycemic index of the control snack bar and formula

Treatment	Average weight (kg)	Average height (cm)	Period				
			0 min (mg/dL)	30 min (mg/dL)	60 min (mg/dL)	90 min (mg/dL)	120 min (mg/dL)
Control	62.5	162.125	76.2	139.3	110.8	90.3	84.1
F1	62.5	162.125	91.5	119.5	106.0	94.5	86.5

The incorporation of 5% red snapper bone nano-calcium yielded a hedonic texture score of 6.16, categorizing it as "like" on the 9-point scale. This score surpasses those of the control group and the 10 and 15% treatments, which tended to have a more complex, grittier texture due to excess minerals. At a 5% concentration, nano-calcium functions as a microfilling agent, enhancing the product's structure and density without compromising crispiness, thereby offering a more favorable texture (Anggraeni et al., 2020). Conversely, excessive addition can disrupt the structural balance, leading to an undesirable mouthfeel (Anggraeni and Hatmiyarni 2022). Consequently, the addition of 5% nano-calcium is an optimal formulation in terms of texture, as it enhances structure without adversely affecting mouthfeel. Overall, each formula has an acceptance rate exceeding 50%, indicating that most panelists endorse it. According to the hedonic analysis, formula F1 has the highest acceptance rate among F0, F3, and F4, except for color, where F1 shows a marginally higher acceptance than F0. Consequently, formula F1 is selected based on aroma, taste, and texture, with a red snapper bone nano-calcium concentration of 5%.

Table 2 presents a proximate analysis of a selected snack bar (F1). Moisture content can influence both the quality and shelf life of food products. Moisture content can be determined using several methods, including oven drying (Liang et al., 2023). The analysis showed that the selected snack bar formula had a moisture content of 11.10% (wet basis). This value meets the United States Department of Agriculture (USDA) standard, which allows a maximum of 11.26%. In a previous study, a catfish meal-based snack bar had a higher moisture content of 13.04% (wet basis). Ash content represents the inorganic components present in a food material. Food consists of both organic and inorganic components (Li et al. 2023). The combustion process removes organic matter, leaving only the inorganic residue. The ash content analysis of the selected snack bar formula yielded 1.32% (wet basis), which complies with the USDA standard that sets a maximum of 1.72%.

The fat content analysis revealed that the selected snack bar formula contained 13.05% fat (wet basis), which exceeds the USDA standard maximum of 10.93%. The fat content of a product is influenced by the ingredients used, particularly those that are sources of fat, such as margarine and peanuts. Adding margarine can significantly increase fat content, as it contains approximately 81% fat (Chen et al., 2025). Additionally, the highest fat contribution comes from the egg yolk used in the formulation. According to the Indonesian National Standardization Agency, egg yolk contains 31.9% fat (Maruddin et al., 2020).

The carbohydrate content of the selected snack bar formula was 56.47% (wet basis). This is lower than the USDA standard of 66.72%, but higher than the carbohydrate content of catfish bone meal snack bar (44.61% wet basis) and also higher than the values reported by Aminah et al. (2019), which ranged from 43.11 to 51.89%. The GI test of the selected snack bar was conducted to determine its effect on each panelist's blood glucose levels. Table 3 presents Glycemic index of the control snack bar and formula F1. The test was carried out in stages: a control snack bar (without coffee), followed by the selected F1 snack bar formula, with a 24-hour interval between tests. A total of 10 panelists participated, all of whom had signed ethical clearance forms and had fasted for 8 hours (with only ad libitum water consumption) before testing. According to ElSayed et al. (2024), an overnight fast is essential to prevent residual dietary sugars from influencing baseline blood glucose levels. The

Table 4: Determination of glycemic load for selected formula

Sample	Glycemic Index	Category IG	Carbohydrates/ Serving (g)	Glycemic Load	Category BG
Snack bar with added nano-calcium from snapper fish bones	14.22	Low	56.47	8.03	Low

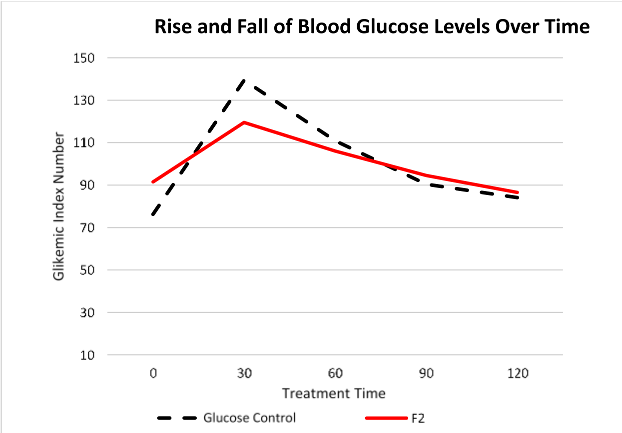

Fig. 1: Rise and fall of blood glucose levels over time.

Table 5: IC_{50} value of snack bar with added nano-calcium from snapper fish bones

No.	Treatment	Concentration (ppm)	IC_{50} (µg/mL)	Antioxidant Properties
1	Control	200	60.67	Low
2	F1	200	48.70	Very High

GI test was performed using the Easy Touch GCU 3-in-1 device (Taiwan). The panelists were aged between 17 and 29 years, with an average height of 162.12cm and an average body weight of 62.5kg. Each panelist consumed a 100 g serving of the F1 snack bar formula, which contained 56.47 g of carbohydrate (by difference). Blood glucose responses were recorded and are presented in Table 4.

Glucose levels were measured from blood taken from the panelists' fingertips at minute 0, meaning before they consumed the snack bar. Fig. 1 presents Rise and fall of blood glucose levels over time. The panelists then consumed it, and blood checks were repeated three times using the *Easy Touch* device. Blood glucose measurements were taken at 30, 60, 90, and 120min after serving. The average results for the control treatment at 0 minutes were 76.2mg/dL, at 30min were 139.3mg/dL, at 60min were 110.8mg/dL, at 90min were 90.3mg/dL, and at 120min were 84.1mg/dL. Meanwhile, for the F1 treatment, the average results were 91.5mg/dL at 0min, 119.5mg/dL at 30min, 106mg/dL at 60min, 94.5mg/dL at 90min, and 86.5mg/dL at 120min.

Based on the panelists' glucose levels, there are apparent differences between the two snack bar treatments. In the control snack bar treatment, glucose levels continued to rise until the 30th minute, then decreased but not sharply. In contrast, with the selected F1 snack bar formula, glucose levels rose from minute 0 to minute 30, but the increase was not steep; then there was a rather sharp decrease at minute 30, indicating that the glucose had been absorbed into the cells at that time. These findings are consistent with previous research by Lee et al. (2023) on rats, in which glucose continued to increase until minute 30 and then decreased thereafter.

The GI was calculated by multiplying the ratio of the area under the curve for the test food's glucose response to that of a reference glucose solution by 100: GI = [(Test Food AUC / Glucose AUC)] × 100. According to Table 4, the glycemic index of the snack bar made with red snapper nano-calcium and robusta coffee extract is 14.22%. This falls within the low GI range for food products, namely below 55. According to Chiavaroli et al. (2021), consuming foods with a low GI can improve insulin sensitivity, slow the rate of sugar absorption, and be beneficial as a blood sugar controller, thereby lowering the risk of complications in people with type 2 diabetes mellitus. The glycemic load of the F1 snack bar formula with 80g of robusta coffee extract is 8.03 (low GL).

The GL calculation was based on a serving size of the snack bar with 100g of robusta coffee extract. The glycemic load of the snack bar shows that consuming 100g, containing 56.47g of carbohydrates per serving, slowly increases blood glucose levels. This is influenced by the GI of the red snapper bone powder snack bar, which is enhanced by the addition of robusta coffee extract, considered to be in the low category. Quantitative testing of the antioxidant activity in this snack bar was conducted at the Integrated Laboratory of Diponegoro University using the DPPH method. The principle of measuring antioxidant activity is based on the change in the intensity of the purple color of DPPH, which is directly proportional to the concentration of the DPPH solution (Sukweenadhi et al., 2020). Based on the results of the antioxidant activity analysis of the snack bar made from red snapper nano-calcium, the Inhibitory Concentration ($IC_{50)}$ values are shown in Table 5.

Based on Table 5, the results show that the IC_{50} value in the control sample is 60.67µg/mL, whereas in the F1 sample, it is 48.7µg/mL. Thus, the F1 sample has a lower IC_{50} value than the control sample, indicating that the antioxidant activity in the selected F1 snack bar formula is very strong. This is supported by the statement from Pujimulyani et al. (2020) that the lower the IC_{50} value, the stronger the antioxidant activity; if the IC_{50} value is <50 µg/ml, then the antioxidant property is very strong. Therefore, this snack bar can be considered a source of antioxidants.

Conclusion

Four snack bar variants fortified with nano-calcium from red snapper fish were formulated. They were differentiated by the nano-calcium ratios: F0 (0%), F1 (5%), F3 (10%), and F4 (15%). The optimal formulation, designated F1, incorporates a 5% nano-calcium from red snapper. Sensory evaluation revealed that panelists favored the F1 snack bar, which includes nano-calcium from red snapper and robusta coffee extract, due to its superior taste, aroma, and texture. The selected formulation exhibits a moisture content of 11.10% (w/w), ash content of 1.32%, fat content of 13.05%, protein content of 18.04%, and an antioxidant level of 48.7 µg/ml. A single serving of the snack bar has a low GI (14.2%) and a low GL (8.0). Furthermore, the inclusion of coffee extract in the selected formulation results in a notably high antioxidant content, with an IC_{50} value below 50µg/mL, demonstrating the efficacy of nano-calcium from red snapper in mitigating oxidative effects that contribute to hyperglycemia. The use of fish bones in snack bar production could enhance the market value of red snapper bones in the fisheries industry.

DECLARATIONS

Funding: This research received financial support from Beasiswa Pendidikan Indonesia, BPI (The Indonesian Education Scholarship). Pusat Pelayanan Pembiayaan dan Asesmen Pendidkan Tinggi, PPAPT (Center for Higher Education Funding and Assessment), Ministry of Higher Education, Science, and Technology of Republic Indonesia. Lembaga Pengelola Dana Pendidikan, LPDP (Endowment Fund for Education Agency), Ministry of Finance of Republic Indonesia, as funders of this research activity.

Acknowledgement: Our gratitude goes to Beasiswa Pendidikan Indonesia, BPI (The Indonesian Education Scholarship). Pusat Pelayanan Pembiayaan dan Asesmen Pendidkan Tinggi, PPAPT (Center for Higher Education Funding and Assessment), Ministry of Higher Education, Science, and Technology of Republic Indonesia. Lembaga Pengelola Dana Pendidikan, LPDP (Endowment Fund for Education Agency), Ministry of Finance of Republic Indonesia, as funders of this research activity. Thanks to all academicians of the Faculty of Fisheries and Marine Science, Diponegoro University, and the Department of Food Technology, Faculty of Science and Technology, National Karangturi University, for their academic support.

Conflict of Interest: The author(s) declare no financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability: The additional data of this study can be obtained from the corresponding author upon a justified request.

Ethics Statement: The protocol described and executed in the present study was approved by the Bioethics Committee for Medical / Health Research of The Faculty of Medicine, Sultan Agung Islamic University (Protocol No. 193/V/2023/Komisi Bioetik).

Author's Contribution: Conceptualization: N.A; Methodology: P.A, and R.B.K; Investigation: M.W.; Formal analysis: N.A; Writing, reviewing, and editing: R.B.K.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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