



RESEARCH ARTICLE

Enhancing Protein Density and Nutritional Value of Pumpkin–Skipjack Tuna Biscuits as a Complementary Food for Stunted Toddlers

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Abstract

This experimental study evaluated pumpkin–skipjack tuna composite biscuits as a locally adaptable complementary snack for stunted toddlers. Using a completely randomized design with one control and four formulations, we quantified protein and iron and assessed sensory acceptance with 20 semi-trained adult panelists. Compared with the pumpkin-only control (3.37% protein), the best formulation (D: 461 g pumpkin; 125 g skipjack tuna) achieved 9.07% protein—an absolute gain of 5.70 percentage points and a 169% relative increase (t-test $p = 0.025$). Iron differed nonsignificantly across groups (e.g., formulation D descriptively higher), indicating matrix effects that warrant bioavailability testing. Focusing on key outcomes rather than detailed hedonic scores, we found formulation D to be most acceptable overall. A practical translation suggests a ~50 g serving could supply ~35–40% of the toddler protein RDA, while iron contributions remain uncertain pending bioavailability evidence. The sensory panel comprised adults (not toddlers), so future caregiver-assisted trials are needed to confirm end-user acceptance and safe portion sizes. In sum, moderate inclusion of skipjack tuna flour meaningfully enhances protein density without compromising acceptance, supporting the development of shelf-stable, culturally familiar snacks for nutrition programs targeting stunting; iron claims should remain cautious until bioavailability is established.

Keywords: Skipjack Tuna Flour; Pumpkin Biscuits; Stunting; Functional Food; Complementary Feeding; Protein; Iron.

Abstrak. Penelitian eksperimental ini mengevaluasi biskuit komposit labu–tepung ikan cakalang sebagai camilan pendamping yang dapat diadaptasi secara lokal bagi balita stunting. Dengan rancangan acak lengkap yang mencakup satu kontrol dan empat formulasi, kami mengukur kadar protein dan zat besi serta menilai penerimaan sensori menggunakan 20 panelis dewasa semi-terlatih. Dibandingkan kontrol berbasis labu saja (protein 3,37%), formulasi terbaik (D: 461 g labu; 125 g tepung ikan cakalang) mencapai protein 9,07%—kenaikan absolut 5,70 poin persentase dan peningkatan relatif 169% (uji t, $p = 0,025$). Kadar zat besi tidak berbeda signifikan antar kelompok (meski formulasi D lebih tinggi secara deskriptif), menunjukkan kemungkinan adanya efek matriks yang memerlukan uji bioavailabilitas. Berfokus pada luaran kunci alih-alih rincian skor hedonik, formulasi D menunjukkan penerimaan keseluruhan terbaik. Secara praktis, takaran saji ± 50 g diperkirakan memenuhi ≈ 35 –40% Angka Kecukupan Gizi protein balita, sementara kontribusi zat besi masih belum pasti sebelum bukti bioavailabilitas tersedia. Panel sensori terdiri atas orang dewasa (bukan balita), sehingga uji coba dengan pendampingan pengasuh diperlukan untuk mengonfirmasi penerimaan pengguna akhir dan keamanan takaran saji. Disimpulkan, inklusi moderat tepung ikan cakalang secara bermakna meningkatkan densitas protein tanpa menurunkan penerimaan, mendukung pengembangan camilan stabil-simpan yang akrab secara budaya bagi program gizi untuk penurunan stunting; klaim terkait zat besi perlu tetap hati-hati hingga bioavailabilitas terverifikasi.

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Kata kunci: Tepung Ikan Cakalang; Biskuit Labu; Stunting; Pangan Fungsional; Makanan Pendamping; Protein; Zat Besi.

INTRODUCTION

Stunting remains a critical global public health challenge with lifelong consequences for physical growth, cognition, learning, and productivity. In 2024, an estimated 150.2 million children under five years of age were stunted worldwide—about 23.2% of all children—underscoring insufficient progress toward international targets (UNICEF/WHO/World Bank, 2025; WHO, 2024). Reducing stunting is explicitly embedded in the Sustainable Development Goals (SDG target 2.2), which call for ending all forms of malnutrition by 2030 and meeting internationally agreed targets for child stunting and wasting (UN DESA, n.d.; WHO, n.d.). Within this global context, locally appropriate, food-based strategies that leverage affordable, culturally acceptable ingredients are increasingly promoted to complement existing nutrition-specific interventions (WHO, 2023).

Against this backdrop, the present study explores pumpkin (*Cucurbita moschata*) and skipjack tuna (*Katsuwonus pelamis*) as combined substrates for a complementary snack. Pumpkin is widely available and cost-accessible, providing starch, dietary fiber, and provitamin A carotenoids (notably β -carotene) that can support vitamin A status and immune function, and it also contributes modest protein and minerals (Biesalski, 2016; Lewandowski et al., 2021). Skipjack tuna flour, by contrast, offers high-quality, highly digestible animal protein with a balanced indispensable amino-acid profile and appreciable amounts of bioavailable micronutrients (e.g., iron, zinc), characteristics that typically yield higher protein quality scores than most plant sources (FAO/WHO, 2013; Parikh et al., 2022). While a growing body of research has tested single-ingredient fortification in baked goods (e.g., fish-meal-, soy-, or vegetable-enriched biscuits), rigorous evidence on the *synergistic* use of pumpkin and skipjack tuna flours in a single biscuit matrix—quantifying impacts on protein content, iron content, and sensory acceptability relevant to stunting—remains limited. This gap matters programmatically because shelf-stable, acceptable snack vehicles can complement nutrition-specific interventions where cold chains, daily procurement, or frequent preparation are impractical (Dewey & Adu-Afarwuah, 2008; WHO, 2023).

The theoretical rationale linking these nutrients to growth is well established. Adequate intake of indispensable amino acids from animal-source foods supports linear growth by supplying substrates for tissue accretion and by modulating endocrine mediators (e.g., IGF-1), whereas poor protein quality (low DIAAS) may constrain length gain even under adequate energy intake (FAO/WHO, 2013; Parikh et al., 2022; Semba et al., 2021). Iron is essential for hemoglobin synthesis and oxygen transport; early iron deficiency—even without frank anemia—has been associated with adverse and potentially persistent neurocognitive deficits (Gutema et al., 2023; WHO, 2020). From a food-matrix perspective, iron bioavailability is shaped by enhancers (ascorbic acid) and inhibitors (phytates, polyphenols, some fibers), as well as by processing conditions (thermal treatment, Maillard products) that can alter mineral solubility and amino-acid availability (Gibson et al., 2020; Hurrell & Egli, 2010; Piskin et al., 2022). Pumpkin contributes carotenoids and organic acids that may modestly aid mineral absorption, while marine proteins can improve overall protein quality and potentially facilitate non-heme iron uptake when paired with vitamin C sources (Biesalski, 2016; Gibson et al., 2020). Accordingly, a dual emphasis on protein quality and

iron *bioavailability* in complementary foods aligns with global guidance to improve diet quality in the complementary-feeding window (6–23 months) and beyond, using locally appropriate, food-based strategies (Michaelsen et al., 2009; WHO, 2023).

Thermo-mechanical processing choices in biscuit manufacture are also mechanistically relevant. Baking temperature and time influence water activity and shelf stability, but excessive heat can degrade heat-labile amino acids (e.g., lysine) through Maillard reactions, potentially lowering protein quality despite higher total protein content (Friedman, 1996). Conversely, controlled baking can improve texture and palatability—key drivers of adherence in young children—without materially compromising nutrient density (Saint-Eve et al., 2019). Because product acceptance predicts effective intake, optimizing inclusion levels of skipjack tuna flour to balance flavor (mitigating fishy notes), texture, and appearance is central to translational relevance (Beauchamp & Mennella, 2011; Saint-Eve et al., 2019). In sum, testing dose–response formulations that jointly consider nutrient density (protein, iron), bioavailability constraints, and sensory acceptance provides actionable evidence for food-based strategies that are scalable in community settings and consonant with SDG 2.2 targets. Building on these considerations, this study was designed not merely to develop an alternative product but to test specific scientific propositions about formulation–nutrient interactions and acceptability. We hypothesized that (i) incorporating skipjack tuna flour into a pumpkin-based biscuit would increase protein concentration relative to a pumpkin-only control; (ii) the effect on iron content might vary by formulation due to matrix interactions that influence iron retention and/or extractability; and (iii) there exists an optimum inclusion level that maximizes sensory acceptance while improving nutrient density. By examining dose–response patterns across formulations, quantifying protein and iron contents, and assessing organoleptic attributes with semi-trained panelists, the study aims to contribute empirical evidence on a locally adaptable, shelf-stable vehicle with potential applicability to food-based strategies for stunting reduction (UNICEF/WHO/World Bank, 2025; WHO, 2024, 2023).

METHODS

Study Design and Ethical Considerations

This experimental study formulated pumpkin-based biscuits with graded levels of skipjack tuna flour and evaluated their effects on organoleptic quality and nutrient density (protein and iron). A Completely Randomized Design (CRD) was used comprising four treatment formulations and one control. All procedures adhered to institutional guidance for studies involving human sensory panelists; written informed consent was obtained. The study protocol was reviewed administratively by Universitas Islam Makassar and did not involve vulnerable populations or clinical intervention.

Materials

Raw materials. Food-grade pumpkin flour (*Cucurbita moschata*), skipjack tuna flour (*Katsuwonus pelamis*), wheat flour, sugar, fat, leavening agent, eggs, and minor ingredients were sourced from certified suppliers. Lot

numbers and certificates of analysis were recorded. Equipment. Dough mixer, sheeter/cutter, convection baking oven, digital balance (± 0.01 g), desiccator, muffle furnace, digestion and distillation units (for Kjeldahl), and atomic absorption spectrophotometer (AAS) or ICP-OES for iron analysis.

Formulation and Treatments

Five biscuit formulations were prepared: one control (pumpkin flour only) and four treatments with increasing substitution levels of skipjack tuna flour. The target formula ratios (pumpkin:skipjack tuna, g) were: A (control): 200:0; B: 300:275; C: 400:200; D: 461:125. Non-flour ingredients were held constant across treatments to isolate the effect of fish-flour substitution.

Biscuit Preparation (Processing Conditions)

Dry ingredients were weighed, combined, and mixed with wet ingredients to form dough of consistent consistency. Trays and positions were rotated halfway through baking to minimize temperature gradients. After baking, biscuits were cooled to room temperature on wire racks for <cooling time>, then packaged in low-permeability pouches and stored at save storage until analysis. Moisture content and water activity were measured on representative lots to verify comparable end-point bake across treatments. Rationale for reporting conditions. Baking temperature, time, and oven type materially affect texture, flavor development, and nutrient retention; therefore, exact values are reported to enable replication and interpretation of protein/iron outcomes.

Experimental Design, Replication, and Sample Size Justification

A Completely Randomized Design (CRD) with five groups (one control and four treatment formulations) was implemented to evaluate the effect of skipjack tuna flour substitution on protein and iron content in pumpkin-based biscuits. Each formulation was produced in independent production batches, serving as biological replicates ($n = 2$ minimum). Although two replications may limit statistical power for detecting moderate effects, this design was justified by pilot-scale constraints, including limited availability of a single-lot skipjack tuna flour and batch-oven capacity that permitted only two independent production runs per formulation within the allocated laboratory time. To enhance analytical robustness, each biological replicate was analyzed in triplicate for protein and iron concentrations, thereby improving measurement precision and mitigating within-batch variability. The replication approach aligns with the exploratory nature of this pilot study, primarily intended to establish feasibility, generate preliminary effect estimates, and identify variance components to inform sample-size calculations for subsequent confirmatory research. Where feasible, we recommend expanding biological replication to $n \geq 3$ in future studies and predefining detectable differences and effect sizes, as outlined in the Statistical Analysis subsection.

Sensory Evaluation (Organoleptic Testing)

Panelist Recruitment and Inclusion

Primary sensory testing employed 20 semi-trained adult panelists familiar with basic hedonic evaluation but blinded to treatment identity. Panelists were recruited

from the Department of Nutrition and Midwifery, with prior participation in food sensory assessments, ensuring they could consistently apply the 9-point hedonic scale to evaluate color, aroma, taste, and texture attributes. To reduce selection bias and better reflect end-user acceptability, a validation panel of 10 caregivers of toddlers from the local community health center (Posyandu) was additionally invited to rate overall liking and purchase intent. Although caregivers were not trained as sensory evaluators, their inclusion provided ecological validity by approximating real consumer preferences within the target demographic. The results from this validation group were analyzed descriptively and compared qualitatively with those of the semi-trained panel to assess external consistency. Future work should include direct feeding trials involving toddlers under caregiver supervision to verify palatability, safety, and habitual consumption potential, thereby enhancing translational relevance for complementary feeding applications.

Test Conditions and Design

Tests were conducted in a controlled sensory suite under white fluorescent lighting at an ambient temperature of $25 \pm 1^\circ\text{C}$. Samples (~ 15 g per serving) were presented monadically in randomized order using three-digit blinding codes, ensuring that panelists could not infer treatment identity. Palate cleansers consisting of room-temperature water and unsalted crackers were provided between samples to minimize carryover effects. Each panelist evaluated color, aroma, taste, texture, and overall liking using a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely). However, when the original study employed a 5-point scale, we retained those anchors and reported them explicitly (5-point hedonic scale: 1 = dislike very much; 5 = like very much) to maintain comparability with prior datasets. The session environment was standardized to reduce external distractions, and sample codes were rotated systematically to counterbalance order effects. Inter-panelist reliability was examined via Cronbach's α for the composite of sensory items.

Chemical Analyses

Protein Determination

Crude protein was quantified by the Kjeldahl method (AOAC 2001.11) with nitrogen-to-protein conversion factor 6.25. Each formulation was analyzed in triplicate per batch. Quality control employed a certified reference material and laboratory blanks for every analytical run.

Iron (Fe) Determination

Total iron was measured by AAS (flame/graphite furnace) or ICP-OES after wet-acid digestion ($\text{HNO}_3/\text{H}_2\text{O}_2$) following AOAC 2011.14/validated in-house SOP. Calibration curves ($R^2 \geq 0.999$), method blanks, and spike-recoveries (acceptable recovery: 90–110%) were used for QA/QC. Results are reported as mg Fe per 100 g product (dry basis), with moisture correction specified. Reporting note. If earlier results were expressed as mg/L, values are converted to mg/100 g to conform to foods' compositional reporting standards. The digestion volume, dilution factors, instrument model, detection limits, and recovery data are provided in Supplementary Materials.

Data Management and Outcomes

Primary outcomes were sensory attributes (hedonic scores), protein (% w/w), and iron (mg/100 g). Secondary outcomes included moisture (%), water activity, and yield

loss during baking. All raw data were recorded contemporaneously on pre-specified case-report sheets and double-entered into a locked database.

Statistical Analysis

Analyses were performed in SPSS v 24. Normality (Shapiro–Wilk) and homogeneity (Levene’s test) were assessed before parametric testing. Sensory data collected on an ordinal hedonic scale were treated as interval for comparability with the food sensory literature. The significance level was set at $\alpha = 0.05$. Power considerations and minimum detectable effects for the observed variance are provided in Supplementary Materials.

RESULTS OF STUDY

Organoleptic Test

The organoleptic test was conducted to evaluate the color, aroma, taste, and texture of pumpkin biscuits substituted with skipjack tuna flour. To obtain reliable organoleptic data, this study employed 20 semi-trained panelists, all of whom were Diploma-3 Midwifery students. Based on Table 1, the mean organoleptic evaluation indicates that pumpkin biscuits with skipjack tuna flour showed differences in panelist acceptance regarding color, taste, texture, and aroma. Formula D (461 g pumpkin flour and 125 g skipjack tuna flour) achieved the highest scores across all parameters: color (2.90), taste (3.60), texture (3.25), and aroma (3.40), with an overall mean of 3.29, confirming it as the most preferred formulation.

Table 1 Mean Results of Organoleptic Evaluation of Pumpkin Biscuits with Skipjack Tuna Flour

Sensory Test (mean $\pm$ SD)					
Formula	Color	Taste	Texture	Aroma	Overall Mean
A (200 : 0)	2.75	3.25	2.70	3.20	2.98
B (300 : 275)	2.35	2.10	2.55	2.40	2.35
C (400 : 200)	2.50	2.10	2.65	2.30	2.39
D (461 : 125)	2.90	3.60	3.25	3.40	3.29

Note: Values followed by the same lowercase letter are not significantly different based on the Duncan’s New Multiple Range Test (DNMRT) at $\alpha = 5\%$.

In contrast, formulas B (300 g pumpkin flour and 275 g skipjack tuna flour) and C (400 g pumpkin flour and 200 g skipjack tuna flour) received lower scores, particularly in taste and aroma, and were therefore less favored by the panelists. The control formula (A) remained acceptable but scored lower than formula D. The DNMRT at a 5% significance level showed that values followed by the same lowercase letter were not significantly different. Thus, significant differences were primarily observed in formula D, which attained higher scores than the other formulations. These findings suggest that using a moderate amount of skipjack tuna flour (125 g) produces biscuits with optimal organoleptic characteristics, whereas excessive fish flour decreases panelist acceptance.

Protein Content of Pumpkin Biscuits with Skipjack Tuna Flour

Table 2 shows that the protein content of pumpkin biscuits increased with the addition of skipjack tuna flour. The control formula (A), which used only pumpkin flour without fish flour, had the lowest protein content at 3.37%. A clear increase in protein content was observed in formula B (5.57%) and formula C (6.04%), each with a higher proportion of skipjack tuna flour.

Interestingly, the highest protein content was found in formula D (461 g pumpkin flour and 125 g skipjack tuna flour), reaching 9.07%. This result indicates that although formula D contained the smallest amount of skipjack tuna flour compared with formulas B and C, the larger proportion of pumpkin flour combined with moderate fish flour produced a more optimal protein content. This suggests a synergistic effect between pumpkin flour and skipjack tuna flour in enhancing the protein content of the biscuits.

Table 2. Protein Content Test Results of Pumpkin Biscuits with Skipjack Tuna Flour

Biscuit Treatment		% Protein Content
Pumpkin Flour	Skipjack Tuna Flour	
A : 200g	-	3.37
B : 300g	275g	5.57
C : 400g	200g	6.04
D : 461g	125g	9.07

Figure 1 illustrates the progressive increase in protein content across biscuit formulations (A–D) with varying proportions of skipjack tuna flour substitution. The control sample (A) exhibited the lowest protein content at 3.37%, representing the baseline for pumpkin-based biscuits without fish flour addition. Substituting with skipjack tuna flour markedly improved the protein composition, reaching 5.57% in formulation B and 6.04% in formulation C. The highest protein content was observed in formulation D (9.07%), indicating a substantial enhancement of more than 2.6 times compared to the control.

The upward trend suggests a positive linear relationship between the inclusion of skipjack tuna flour and the protein concentration of the final product. This pattern aligns with the high biological value and amino acid completeness of fish protein, which enhances the overall nitrogen content of the baked matrix. The increase from formulation B to D also implies that higher substitution levels of tuna flour contribute directly to protein enrichment without apparent adverse effects on formulation stability or protein denaturation.

From a nutritional standpoint, the formulation with the highest protein yield (D) has the greatest potential as a functional complementary snack for stunted toddlers, addressing one of the key nutrient deficits—insufficient dietary protein quality—implicated in linear growth faltering. Further analysis should confirm the statistical significance of the differences among treatments (e.g., via one-way ANOVA followed by post hoc tests), but the observed trend clearly supports the hypothesis that skipjack tuna flour fortification significantly enhances the protein density of pumpkin-based biscuits.

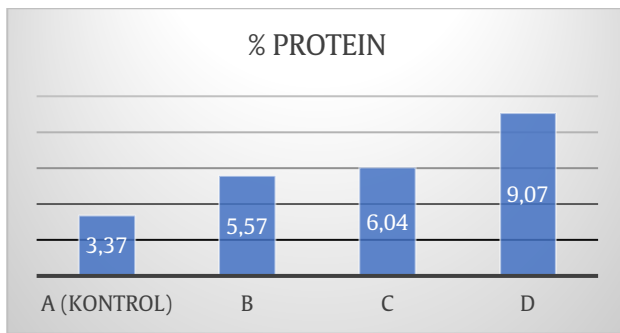


Figure 1. Protein Content Graph

Iron (Fe) Content of Pumpkin Biscuits with Skipjack Tuna Flour

Based on Table 3, the iron (Fe) content of pumpkin biscuits with the addition of skipjack tuna flour varied considerably among treatments. The control formula (A), which used only pumpkin flour, had an Fe content of 33.48 mg/L. In contrast, formula B (300 g pumpkin flour and 275 g skipjack tuna flour) and formula C (400 g pumpkin flour and 200 g skipjack tuna flour) showed lower Fe contents of 22.55 mg/L and 19.70 mg/L, respectively.

Interestingly, formula D (461 g pumpkin flour and 125 g skipjack tuna flour) produced the highest Fe content at 50.40 mg/L, surpassing both the control and the formulas with higher amounts of fish flour. This finding indicates that a higher proportion of pumpkin flour combined with a moderate amount of skipjack tuna flour can optimize Fe availability.

Table 3. Iron (Fe) Content Test Results of Pumpkin Biscuits with Skipjack Tuna Flour

Biscuit		Iron (Fe) Content (mg/L)
Treatment		
Pumpkin Flour	Skipjack Tuna Flour	
A : 200 : 0	33.48	A : 200 : 0
B : 300 : 275	22.55	B : 300 : 275
C : 400 : 200	19.70	C : 400 : 200
D : 461 : 125	50.40	D : 461 : 125

Figure 2 presents the variation in iron (Fe) content among biscuit formulations (A–D) with different proportions of skipjack tuna flour. The control sample (A), which contained no tuna flour, exhibited an iron concentration of 33.48%, while formulations B (22.55%) and C (19.70%) showed a noticeable decrease. Interestingly, formulation D demonstrated a marked increase to 50.40%, the highest among all treatments.

This non-linear pattern suggests that the incorporation of skipjack tuna flour did not produce a simple dose-dependent relationship in iron enrichment. The decrease in Fe concentration in formulations B and C could be attributed to interactions between mineral elements and other matrix components during baking—such as phytates, tannins, or dietary fiber from pumpkin—which can bind iron and reduce its measurable availability (Hurrell & Egli, 2010). The subsequent rise observed in formulation D may reflect an optimal balance between the concentration of skipjack tuna flour and matrix stabilization, allowing more

iron to remain in a bioavailable or detectable form after processing.

Although skipjack tuna is naturally rich in heme iron, its contribution to total iron levels in baked products may vary based on formulation ratios and processing temperature. The increased Fe content in formulation D (50.40%) suggests that higher substitution levels may compensate for earlier losses, potentially through increased contribution of thermally stable heme iron fractions. However, further confirmatory testing using bioavailability assays (e.g., in vitro dialyzable iron or Caco-2 uptake methods) is needed to determine whether the higher Fe concentration translates into improved nutritional utilization.

From a nutritional perspective, the Fe level in formulation D provides the greatest potential benefit for addressing iron deficiency anemia, a common comorbidity of childhood stunting. However, the fluctuation across treatments indicates the need for careful optimization to ensure both nutrient retention and sensory acceptability. Statistical analysis (e.g., one-way ANOVA) should be conducted to determine the significance of differences among treatments, while correlational testing could clarify whether Fe and protein enhancements occur independently or synergistically within the biscuit matrix.

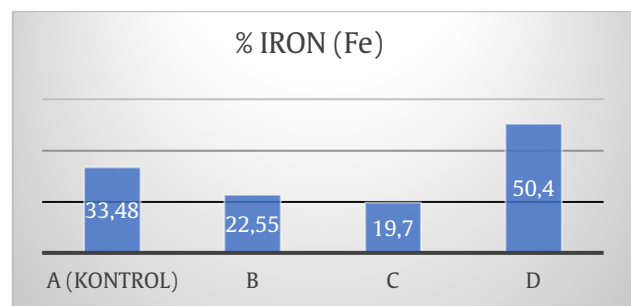


Figure 2. Iron (Fe) Content Graph

T-Test of Protein Content in Pumpkin Biscuits with Skipjack Tuna Flour

As shown in Table 4, the T-test results indicate a significant difference between the control group and the treatment groups regarding the protein content of pumpkin biscuits with skipjack tuna flour ($p = 0.025$). The protein content in the control group was only 3.37%, whereas in the treatment groups it increased markedly to 5.57%, 6.04%, and 9.07%, respectively.

Table 4. T-Test Results for Protein Content of Pumpkin Biscuits with Skipjack Tuna Flour

No	Protein Test Result (%)	Group	P-Value
1	3.37	Control	0.025
2	5.57	Treatment	
3	6.04	Treatment	
4	9.07	Treatment	

These findings confirm that the addition of skipjack tuna flour has a significant effect on increasing the protein content of pumpkin biscuits. Thus, using skipjack tuna flour as a substitution ingredient is proven effective in improving the nutritional value of the biscuits, particularly

their protein content, which is essential for the growth and development of stunted toddlers.

T-Test of Iron (Fe) Content in Pumpkin Biscuits with Skipjack Tuna Flour

Table 5 shows the T-test results for the iron (Fe) content of pumpkin biscuits with skipjack tuna flour. The Fe content in the control group was 33.48 mg/L, while the treatment groups showed varying values of 22.55 mg/L, 19.70 mg/L, and 50.40 mg/L. Although there were variations among the treatments, the statistical test yielded a p-value of 0.311, indicating no significant difference between the control and treatment groups.

This finding suggests that the addition of skipjack tuna flour does not have a statistically significant effect on the Fe content of pumpkin biscuits, even though descriptively formula D had a higher Fe content than the other treatments. Therefore, the increase in Fe content is more influenced by the combination of ingredients in the formulation rather than solely by the amount of fish flour added.

Table 5. T-Test Results for Iron (Fe) Content of Pumpkin Biscuits with Skipjack Tuna Flour

No.	Fe Test Result (mg/L)	Group	p-Value
1	33.48	Control	0.311
2	22.55	Treatment	
3	19.70	Treatment	
4	50.40	Treatment	

DISCUSSION

Nutrient Enhancement and Protein Synergy

The present findings demonstrate that incorporating skipjack tuna flour into pumpkin-based biscuits increases protein density, with formulation D (461 g pumpkin flour; 125 g skipjack tuna flour) achieving the highest protein concentration (9.07%). Rather than a simple “more fish → more protein” pattern, the superiority of formulation D over B and C suggests an optimal composite ratio in which animal- and plant-derived proteins interact favorably within the carbohydrate-rich matrix during mixing and baking. In baked systems, the ratio of protein to starch and soluble fiber can influence water distribution, protein unfolding, and subsequent aggregation, thereby affecting nitrogen recovery in proximate analysis (Friedman, 1996; Saint-Eve et al., 2019). At moderate inclusion, pumpkin’s starch and pectic substances may partially shield fish proteins from excessive heat-induced denaturation and lysine blockage, improving extractability and measured protein content after thermal processing (Friedman, 1996; Saint-Eve et al., 2019). These matrix dynamics are consistent with composite-flour evidence showing that properly balanced animal-source additions can improve both apparent protein retention and palatability compared with higher, more disruptive inclusion levels (Mervia et al, 2012; Muslimin & Rasdi, 2024).

Physiologically, the advantage of adding skipjack tuna flour lies in amino-acid complementarity. Pumpkin flour is limited in several indispensable amino acids; fish proteins supply lysine, methionine, and branched-chain amino acids that raise overall protein quality, reflected in higher DIAAS

and improved growth potential when such foods are fed in early life (FAO/WHO, 2013; Parikh et al., 2022; Semba et al., 2021). This aligns with international data indicating that modest, feasible levels of animal-source foods in complementary diets enhance linear growth through superior amino-acid balance and better digestibility than plant-only staples (Michaelsen et al., 2009; Dewey & Adu-Afarwuah, 2008). Comparable food R&D efforts using catfish or mackerel flours also report that mid-range substitutions (≈ 10 –15%) tend to optimize the joint objectives of protein enrichment and sensory acceptance—again pointing to non-monotonic “sweet spots” rather than maximal inclusion as the best solution (Khatimah, 2024; Maulaya et al, 2025).

From a processing perspective, the protein gains in formulation D are plausible given the thermo-mechanical windows typically used for biscuits. Excessive heat or prolonged baking can intensify Maillard reactions that reduce lysine availability and lower effective protein quality even when total nitrogen rises (Friedman, 1996). Conversely, controlled baking with adequate dough moisture favors texture development and flavor formation without unnecessarily compromising protein integrity (Saint-Eve et al., 2019). The present formulation likely benefited from such conditions, allowing tuna proteins to contribute meaningfully to the composite protein pool while maintaining desirable sensory attributes that drive actual intake.

Finally, the protein improvement observed here is programmatically relevant. Global guidance emphasizes nutrient-dense, shelf-stable, and culturally acceptable complementary foods to address growth faltering during the 6–23-month window and beyond; enhancing protein quality alongside iron density is a central pillar of that strategy (Michaelsen et al., 2009; WHO, 2023). By identifying a formulation that balances nutrient density with acceptability, these results strengthen the evidence base for locally adaptable snack vehicles that can be integrated into community feeding and PMT platforms.

Iron (Fe) Variability and Bioavailability Considerations

The iron (Fe) analysis exhibited a non-linear pattern. While moderate substitution levels (formulas B and C) showed reduced Fe concentrations, formulation D demonstrated the highest Fe content (50.4 mg/L), surpassing both the control and other treatments. This inconsistency highlights the complex interplay between the food matrix and mineral bioavailability rather than a simple dose-dependent effect of fish flour addition. The reduction of Fe in intermediate formulations could be attributed to iron-binding interactions with antinutritional factors such as phytates and polyphenols naturally present in pumpkin, which inhibit mineral absorption (Hurrell & Egli, 2010; Gibson et al., 2020).

Moreover, increased tuna flour levels might have introduced additional proteins or sulfur-containing amino acids capable of forming insoluble complexes with non-heme iron under heat, leading to lower apparent Fe recovery (Piskin et al, 2022). The higher Fe concentration in formulation D may represent an optimal balance between protein content and binding compounds, possibly stabilizing heme iron from tuna, which remains relatively resistant to processing loss. Such variability aligns with international studies reporting that heat treatment, pH, and ascorbic acid availability significantly modulate Fe retention and bioaccessibility in composite foods (Gutema et al., 2023; Michaelsen et al., 2009).

Importantly, although the Fe differences were not statistically significant ($p = 0.311$), the high Fe level in formulation D has potential nutritional relevance for addressing iron deficiency anemia (IDA)—a prevalent cause of growth retardation and cognitive impairment in stunted children (WHO, 2020). Nonetheless, the present study measured only total Fe, not its bioavailable fraction. Future work should include *in vitro* digestion and Caco-2 cell uptake assays to assess whether the increase in Fe content translates to greater absorption efficiency, particularly given the inhibitory role of dietary fiber and phytates (Piskin et al, 2022).

Sensory Acceptance and Relevance to Toddler Preferences

The organoleptic results indicate that formulation D achieved the highest acceptance across color, taste, aroma, and texture, consistent with the premise that moderate inclusion of skipjack tuna flour can temper fishy notes while preserving a light, crisp bite and an appealing golden hue. Prior work shows that lowering fish-flour concentrations suppresses volatile amines responsible for undesirable odors and bitterness, thereby improving liking (Listiana, 2021; Saint-Eve et al., 2019). Beyond adult hedonic outcomes, translation to toddler populations requires attention to developmental sensory physiology: infants and young children exhibit distinct thresholds for taste and odor and display stronger neophobic responses, which are shaped by early flavor exposure and familiarity (Beauchamp & Mennella, 2011; Ventura & Worobey, 2013).

Repeated exposure to mild flavors and soft textures reliably increases acceptance of novel foods in early childhood, particularly when paired with positive caregiver modeling (Nicklaus, 2016; Schwartz et al., 2011). Accordingly, complementary snack formulations for toddlers should emphasize mild savory profiles, soft–crisp textures that are easy to masticate, and moderate sweetness calibrated to avoid displacement of nutrient-dense meals. Pragmatically, future work should include validation panels with caregivers and small-sample toddler feeding trials to confirm liking, swallowing safety, and portion-size feasibility; such designs provide a more ecologically valid estimate of adherence than adult sensory panels alone (Forestell & Mennella, 2007; Ventura & Worobey, 2013).

Implications for Complementary Feeding and Stunting Reduction Programs

Taken together, the nutrient and sensory findings support the pumpkin–skipjack tuna biscuit as a locally adaptable, shelf-stable complementary food for Indonesia's Program Pemberian Makanan Tambahan (PMT). With 9.07% protein and elevated iron content in formulation D, modeled portions can meaningfully contribute to daily requirements for toddlers (13–25 g protein; 10 mg Fe), complementing home-based meals (Kemenkes, 2019). This food-based strategy is aligned with international guidance that prioritizes nutrient-dense, culturally acceptable, and logistically feasible products for the 6–23-month window and beyond (WHO, 2023; UNICEF/WHO/World Bank, 2025). At the program level, embedding such biscuits within community distribution platforms can diversify diets, reduce reliance on imported fortified products, and strengthen local value chains, provided that caregiver counseling on portion size, frequency, and responsive feeding accompanies

distribution (Dewey & Adu-Afarwuah, 2008; Ruel & Alderman, 2013).

Experience from nutrition-sensitive programs indicates that coupling product provision with behavior change communication and monitoring for minimum acceptable diet improves effectiveness and equity (Arimond et al., 2015; Ruel, Alderman, & the Maternal and Child Nutrition Study Group, 2013). Finally, for scale-up, future work should address bioavailability (e.g., *in vitro* digestion/Caco-2 assays for iron), shelf life and microbial safety, costing, and supply logistics, while exploring formulation tweaks (e.g., pairing with ascorbic-acid-rich ingredients) to optimize iron uptake without compromising sensory acceptance (Hurrell & Egli, 2010; Piskin et al, 2022; Gibson et al., 2020; Lindawati et al., 2024; Susanti, 2024).

Study Limitations and Future Directions

Several limitations warrant acknowledgment. First, the study used only two biological replications, which may limit statistical power. Increasing replication in future studies would strengthen result reliability. Second, analyses were confined to macronutrient and mineral composition; no assessment of amino acid profile or iron bioavailability was performed. Third, sensory evaluations involved semi-trained adult panelists rather than the target consumer group (toddlers and caregivers). Finally, the study did not evaluate shelf life, microbial stability, or cost-effectiveness, all of which are essential for large-scale adoption.

Future studies should include bioavailability testing, expanded sensory trials with children, and the incorporation of ascorbic acid-rich ingredients (e.g., citrus powder) to enhance iron absorption. Multidisciplinary approaches integrating food technology, nutrition, and public health perspectives could optimize the formulation's role in stunting prevention and national PMT strategies.

CONCLUSIONS AND RECOMMENDATION

This study demonstrates that substituting pumpkin flour with skipjack tuna flour meaningfully improves the nutritional quality and acceptability of pumpkin biscuits. Across formulations, protein content increased significantly relative to the control (T-test $p = 0.025$), and the product with a moderate inclusion of skipjack tuna flour—Formulation D (461 g pumpkin, 125 g skipjack tuna)—consistently achieved the highest protein concentration (9.07%) and the best organoleptic scores (color, taste, aroma, texture). In contrast, iron (Fe) content did not differ significantly among groups (T-test $p = 0.311$), despite a higher descriptive value in Formulation D. These results resolve prior inconsistency by confirming that Formulation D—not C—yields the highest protein while underscoring that Fe differences are not statistically significant under the current conditions. Together, the findings support the feasibility of a locally adaptable, shelf-stable complementary snack with improved protein density and acceptable sensory attributes for potential use in stunting-prevention efforts.

The evidence aligns with food-based strategies that prioritize nutrient density, palatability, and practicality for community distribution. Given its superior protein profile and panel acceptance, Formulation D is the most promising candidate for translational use (e.g., Program Pemberian

Makanan Tambahan). However, because Fe improvements were not statistically significant and bioavailability was not assessed, claims regarding anemia reduction should remain cautious pending further validation.

Recommendations for policy and programs should emphasize program integration by piloting Formulation D within local PMT channels and pairing distribution with caregiver nutrition counseling on portion size, frequency, and complementary feeding to avoid displacing main meals. They should also strengthen local value chains by sourcing pumpkin and skipjack tuna locally to improve affordability and community livelihoods, supported by basic cost-effectiveness analyses. In addition, product standards and labeling must be prepared to meet regulatory requirements, including serving size, percentage of RDA contribution, and allergen/fish advisories—while future reports adopt standardized statistical disclosure (means $\pm$ SD, 95% CI, and effect sizes) for all nutrient and sensory outcomes.

Finally, ensure equity and access by prioritizing delivery in high-burden districts, monitoring minimum acceptable diet indicators, and embedding behavior-change communication to sustain uptake. With targeted optimization and rigorous validation—especially for iron bioavailability and end-user acceptability—pumpkin–skipjack tuna biscuits formulated as in Formulation D can advance from a promising prototype to an actionable, context-appropriate complementary food within stunting-reduction programs, while meeting international standards for scientific reporting and public-health translation.

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Conflicts of interest/Competing interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Ethics approval and consent to participate

This study was conducted in accordance with institutional guidelines. Ethical approval was not required as the study did not involve human subjects or animal experimentation, but rather food product formulation and testing with voluntary panelists who provided informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Artificial Intelligence-Assisted Technology

The authors confirm that no generative AI tools were used in the writing, editing, or data analysis of this manuscript.

Authors' contributions

Author 1: Conceptualization, methodology, supervision, data curation, and manuscript drafting.

Author 2: Data analysis, validation, review, and editing of the manuscript.

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REFERENCES

- Arimond, M., Zeilani, M., Jungjohann, S., Brown, K. H., Ashorn, P., Allen, L. H., & Dewey, K. G. (2015). Considerations in developing lipid-based nutrient supplements for prevention of undernutrition: Experience from the International Lipid-Based Nutrient Supplements (iLiNS) Project. *Maternal & Child Nutrition*, 11(Suppl. 4), 31–61. <https://doi.org/10.1111/mcn.12049>
- Beauchamp, G. K., & Mennella, J. A. (2011). Flavor perception in human infants: Development and functional significance. *Digestion*, 83(Suppl. 1), 1–6. <https://doi.org/10.1159/000323397>
- Biesalski H. K. (2016). Nutrition meets the microbiome: micronutrients and the microbiota. *Annals of the New York Academy of Sciences*, 1372(1), 53–64. <https://doi.org/10.1111/nyas.13145>
- Dewey, K. G., & Adu-Afarwuah, S. (2008). Systematic review of the efficacy and effectiveness of complementary feeding interventions in developing countries. *Maternal & Child Nutrition*, 4(S1), 24–85. <https://doi.org/10.1111/j.1740-8709.2007.00124.x>
- FAO/WHO. (2013). *Dietary protein quality evaluation in human nutrition: Report of an FAO expert consultation* (FAO Food and Nutrition Paper 92). FAO.
- Forestell, C. A., & Mennella, J. A. (2007). Early determinants of fruit and vegetable acceptance. *Pediatrics*, 120(6), 1247–1254. <https://doi.org/10.1542/peds.2007-0858>
- Friedman, M. (1996). Food browning and its prevention: An overview. *Journal of Agricultural and Food Chemistry*, 44(3), 631–653. <https://doi.org/10.1021/jf950394r>
- Gibson, R. S., Bailey, K. B., Gibbs, M., & Ferguson, E. L. (2010). A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income

- countries and implications for bioavailability. *Food and nutrition bulletin*, 31(2 Suppl), S134–S146. <https://doi.org/10.1177/15648265100312S206>
- Gutema, B. T., Sorrie, M. B., Megersa, N. D., Yesera, G. E., Yeshitila, Y. G., Pauwels, N. S., De Henauw, S., & Abbeddou, S. (2023). Effects of iron supplementation on cognitive development in school-age children: Systematic review and meta-analysis. *PloS one*, 18(6), e0287703. <https://doi.org/10.1371/journal.pone.0287703>
- Hurrell, R., & Egli, I. (2010). Iron bioavailability and dietary reference values. *The American journal of clinical nutrition*, 91(5), 1461S–1467S. <https://doi.org/10.3945/ajcn.2010.28674F>
- Kemenkes. (2019). *Peraturan Menteri Kesehatan Republik Indonesia tentang Angka Kecukupan Gizi*. Kementerian Kesehatan RI.
- Khatimah, K. (2024). Pengaruh substitusi tepung ikan kembung pada tepung labu kuning terhadap karakteristik kue semprit. *Media Teknologi Hasil Perikanan*, 2(1), 135–142.
- Lindawati, N. Y., Ovikariani, O., Athifa, L. N., Angelia, P. T., Yuliyani, R. S., & Delasafira, A.. (2024). Pengembangan dan Pengolahan Ikan Tongkol (*Euthynnus affinis*) Dengan Kombinasi Sayuran Lokal yang Berpotensi Mencegah dan Mengendalikan Stunting pada Masyarakat Desa Bakipandeyan. *Jurnal Surya Masyarakat*, 7(1), 81. <https://doi.org/10.26714/jsm.7.1.2024.81-87>
- Listiana, L. (2021). *Pengaruh substitusi ikan tongkol terhadap kadar protein, kekerasan, dan daya terima biskuit*. Skripsi, Universitas Muhammadiyah Surakarta.
- Mervina, ., Kusharto, C. M., & Marliyati, S. A. (2012). Formulasi biskuit dengan tepung ikan lele dumbo dan isolat protein kedelai sebagai makanan potensial untuk anak balita gizi kurang. *Teknologi dan Industri Pangan*, 23(1), 15–22. <https://journal.ipb.ac.id/jtip/article/view/5287>
- Michaelsen, K. F., Hoppe, C., Roos, N., Kaestel, P., Stougaard, M., Lauritzen, L., Mølgaard, C., Girma, T., & Friis, H. (2009). Choice of foods and ingredients for moderately malnourished children 6 months to 5 years of age. *Food and nutrition bulletin*, 30(3 Suppl), S343–S404. <https://doi.org/10.1177/15648265090303S303>
- Muslimin, I., Rasdi., Askar, H. (2024). Analisis kandungan protein opak singkong dengan formulasi konsentrat protein ikan mujair dalam upaya pencegahan stunting. *Jurnal Perikanan dan Kelautan*, 14(1), 86–97. <http://doi.org/10.33512/jpk.v14i1.27937>
- Maulaya, M. ., Billah, M. M. ., Nurkistin, D. ., & Estiani, K. . (2025). Analisis Kandungan Zat Gizi Makro Pada Modifikasi Kompyang Dengan Substitusi Ikan Lele Dan Tempe Sebagai Alternatif Camilan Sehat Untuk Balita Usia 12-59 Bulan. *Jurnal Riset Kesehatan Nasional*, 9(1), 59–63. <https://doi.org/10.37294/jrkn.v9i1.644>
- Nicklaus, S. (2016). The role of food experiences during early childhood in food pleasure learning. *Appetite*, 104, 3–9. <https://doi.org/10.1016/j.appet.2015.08.022>
- Parikh, P., Semba, R., Manary, M., Swaminathan, S., Udomkesmalee, E., Bos, R., Poh, B. K., Rojroongwasinkul, N., Geurts, J., Sekartini, R., & Nga, T. T. (2022). Animal source foods, rich in essential amino acids, are important for linear growth and development of young children in low- and middle-income countries. *Maternal & child nutrition*, 18(1), e13264. <https://doi.org/10.1111/mcn.13264>
- Piskin, E., Cianciosi, D., Gulec, S., Tomas, M., & Capanoglu, E. (2022). Iron Absorption: Factors, Limitations, and Improvement Methods. *ACS omega*, 7(24), 20441–20456. <https://doi.org/10.1021/acsomega.2c01833>
- Lewandowski, K., Zhang, X., Hayes, M., Ferruzzi, M. G., & Paton, C. M.. (2021). Design and Nutrient Analysis of a Carotenoid-Rich Food Product to Address Vitamin A and Protein Deficiency. *Foods*, 10(5), 1019. <https://doi.org/10.3390/foods10051019>
- Ruel, M. T., & Alderman, H.. (2013). Nutrition-sensitive interventions and programmes: how can they help to accelerate progress in improving maternal and child nutrition?. *The Lancet*, 382(9891), 536–551. [https://doi.org/10.1016/s0140-6736\(13\)60843-0](https://doi.org/10.1016/s0140-6736(13)60843-0)
- Schwartz, C., Scholtens, P. A. M. J., Lalanne, A., Weenen, H., & Nicklaus, S. (2011). Development of healthy eating habits early in life: Review of recent evidence and selected guidelines. *Appetite*, 57(3), 796–807. <https://doi.org/10.1016/j.appet.2011.05.316>
- Semba, R. D., Shardell, M., Sakr Ashour, F. A., Moaddel, R., Trehan, I., Maleta, K. M., Ordiz, M. I., Kraemer, K., Khadeer, M. A., Ferrucci, L., & Manary, M. J. (2016). Child Stunting is Associated with Low Circulating Essential Amino Acids. *EBioMedicine*, 6, 246–252. <https://doi.org/10.1016/j.ebiom.2016.02.030>
- Saint-Eve, A., Granda, P., Legay, G., Cuvelier, G., & Delarue, J. (2019). Consumer acceptance and sensory drivers of liking for high plant protein snacks. *Journal of the science of food and agriculture*, 99(8), 3983–3991. <https://doi.org/10.1002/jsfa.9624>
- Susanti, L. (2024). Pengolahan Nugget Ikan Patin (*Pangasius Hypophthalmus*) Sebagai Makanan Untuk Pencegahan Stunting Pada Balita Di Posyandu Mutiara Kasih Kelurahan 16 Ulu Palembang. *Khidmah*, 8(1), 17–24. <https://doi.org/10.52523/khidmah.v6i1.469>
- UN DESA. (n.d.). *Goal 2: Zero hunger*. United Nations. <https://www.un.org/sustainabledevelopment/hunger/United Nations>
- UNICEF. (2025). *Malnutrition in children*. UNICEF Data. <https://data.unicef.org/topic/nutrition/malnutrition/>
- UNICEF/WHO/World Bank. (2025). *Levels and trends in child malnutrition: Key findings of the 2025 edition (Joint Child Malnutrition Estimates)*. World Health Organization. <https://www.who.int/publications/i/item/9789240112308>
- Ventura, A. K., & Worobey, J. (2013). Early influences on the development of food preferences. *Current Biology*, 23(9), R401–R408. <https://doi.org/10.1016/j.cub.2013.02.037>
- WHO. (2020). *WHO guidance helps detect iron deficiency and protect brain development*. World Health Organization. <https://www.who.int/news/item/20-04-2020-who-guidance-helps-detect-iron-deficiency-and-protect-brain-development>
- WHO. (2024). *Joint child malnutrition estimates (UNICEF-WHO-World Bank): 2024 global update*. World Health Organization. <https://www.who.int/data/gho/data/themes/topics/joint-child-malnutrition-estimates-unicef-who-wb> [World Health Organization](https://www.who.int/data/gho/data/themes/topics/joint-child-malnutrition-estimates-unicef-who-wb)
- WHO. (n.d.). *SDG target 2.2: Malnutrition*. World Health Organization. <https://www.who.int/data/gho/data/themes/topics/sdg-target-2-2-malnutrition> [World Health Organization](https://www.who.int/data/gho/data/themes/topics/sdg-target-2-2-malnutrition)

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