

Catfish bone powder for mineral-enriched functional fish meatballs

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Abstract. Processing of catfish (*Clarias* spp.) produces substantial quantities of bone waste rich in calcium and phosphorus, which is typically discarded or relegated to low-value feed. The present study evaluated catfish bone powder (CBP) as a mineral fortificant in fish meatballs ("bakso ikan") by assessing nutritional, sensory, physicochemical, microbiological, and economic qualities. Five formulations (0, 5, 10, 15, and 20% CBP, substituting catfish meat) were prepared using a completely randomised design (CRD) with five replicates and analysed for proximate composition, calcium and phosphorus content, pH, water-holding capacity (WHC), lipid oxidation, texture, and 9-point hedonic acceptability over 24 hours of ambient storage (28±1°C). Microbiological safety of the CBP and the fortified product was confirmed through total plate count (TPC), total coliform, *Escherichia coli*, and *Salmonella* spp. analyses. CBP inclusion increased ash, calcium, and phosphorus content in a dose-dependent manner ($p < 0.01$), and all formulations complied with the Indonesian National Standard (SNI 7266:2017) for ready-to-eat fish meatballs. The 10% CBP formulation achieved the highest overall sensory acceptability ($p < 0.05$) and the best firmness and moisture retention, whereas pH declined progressively during storage across all treatments, most slowly in the control and most rapidly at 20% CBP, and lipid oxidation, WHC, and texture all changed in a dose- and time-dependent manner. Economic analysis indicated a reduction in raw-material cost and an increase in projected profit margin for the 10% CBP formulation relative to the control under a modelled market scenario. A 10% CBP inclusion level is recommended as a nutritionally enriched, microbiologically safe, sensory-acceptable, and cost-effective strategy that valorises aquaculture by-products in accordance with circular-economy principles.

Keywords: bone powder, calcium fortification, circular economy, food safety, functional food.

Introduction. Aquaculture is one of the fastest-growing food production sectors globally. In Indonesia, catfish (*Clarias* spp.) has become a leading commodity due to its high productivity, strong consumer acceptance, and relatively low production costs. However, both commercial and domestic fish processing generate significant quantities of by-products, such as heads, bones, and skin, which are often discarded or relegated to low-value animal feed. Notably, these by-products, especially bones, contain high concentrations of calcium and phosphorus, minerals that are essential for human health (Maksimenco et al., 2024).

Calcium deficiency is still a significant public health issue in many developing countries, including Indonesia, leading to stunted growth, skeletal abnormalities, and

impaired metabolic function. Dietary fortification with natural, accessible, and cost-effective sources such as fish bone represents a practical approach to remedying micronutrient deficiencies. Utilizing catfish bone waste as a fortificant supports both nutritional improvement and eco-friendly waste management, in line with circular economy principles (Anandya et al., 2025).

Fish meatballs, or “bakso ikan,” are a widely consumed, protein-rich food throughout Southeast Asia, appreciated because of their affordability, flavor, and versatility. Despite their popularity, traditional formulations usually lack essential micronutrients, particularly calcium and phosphorus, that are essential to skeletal health in children, pregnant women, and the elderly. According to the FAO (2023), calcium deficiency affects approximately 3.5 billion people globally, playing a role in conditions such as osteoporosis and impaired growth. The 2022 Indonesian Nutritional Status Survey (SSGI) found that 24.4% of children under five experience stunting, a condition strongly associated with chronic micronutrient deficiency (Badan Kebijakan Pembangunan Kesehatan, 2022).

Simultaneously, the aquaculture industry produces substantial quantities of underutilized waste, particularly fish bones. The Ministry of Marine Affairs and Fisheries (Pusat Data, Statistik, dan Informasi, 2021) reports that catfish (*Clarias spp.*) accounts for over 1.2 million tonnes of national aquaculture output annually, with approximately 15% comprising non-edible waste, mainly bone. These by-products are rich in hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), which provides a natural and bioavailable source of calcium and phosphorus. Repurposing this waste as food ingredients is consistent with the circular economy model and advances Indonesia’s pledge to Sustainable Development Goal 12 on efficient consumption and production (Young et al., 2026).

Previous research has incorporated catfish bone powder (CBP) from tuna, sardine, and milkfish to improve the nutritional quality of products such as biscuits, nuggets, and snacks (Patria et al., 2024; Amertaningtyas et al., 2026). However, those studies focused mainly on dry, low-moisture matrices and evaluated nutritional enrichment alone, without assessing physicochemical stability, sensory performance, microbiological safety, or economic feasibility during storage. An integrated assessment that combines physicochemical quality (pH, texture, WHC), sensory acceptance, microbiological safety, shelf-life behavior, and cost-effectiveness of bone-powder fortification in a high-moisture, ready-to-eat fish product, such as meatballs, remains unavailable. This gap is important because meatballs are stored and consumed under conditions that favour microbial growth and lipid oxidation, unlike the dry snack matrices previously studied, and the effects of CBP on physicochemical properties (pH, texture, WHC), sensory acceptance, microbiological safety, shelf-life, and cost benefit during short-term storage are not well documented. Given that the fortificant originates from a waste stream, microbiological verification is essential prior to recommending such products for human consumption. This study produced and evaluated fortified catfish meatballs containing 0, 5, 10, 15, and 20% CBP to determine the inclusion level that ideally balances nutritional enhancement, product quality, microbiological safety, consumer acceptability, and economic sustainability. It was hypothesized that moderate CBP inclusion would enhance the calcium and phosphorus content without damaging product integrity, safety, or short-term shelf life.

Material and Method

Chemicals and ingredients. All ingredients used were food-grade and locally sourced. Sodium tripolyphosphate (STPP), tapioca starch, salt, garlic, shallot, monosodium glutamate (MSG), black pepper and baking powder were obtained from certified suppliers in Kediri, East Java, Indonesia. Reagents for calcium, phosphorus, lipid oxidation and microbiological analyses were of analytical grade and obtained from Merck (Darmstadt, Germany); culture media (plate count agar, lauryl tryptose broth, EC broth, eosin methylene blue agar and selenite cystine broth) were obtained from Oxoid (Basingstoke, UK).

Sample collection and identification. Fresh catfish (*Clarias* spp.) and bone waste were obtained from a local fish processor in Kediri, East Java, Indonesia, in March 2025 (7°48'55"S, 112°01'02"E) (Figure 1). Species identification was carried out by S. Windarto (Department of Aquaculture, Universitas Diponegoro, Semarang) and verified morphologically. No voucher specimen was deposited because all samples were processed fresh.

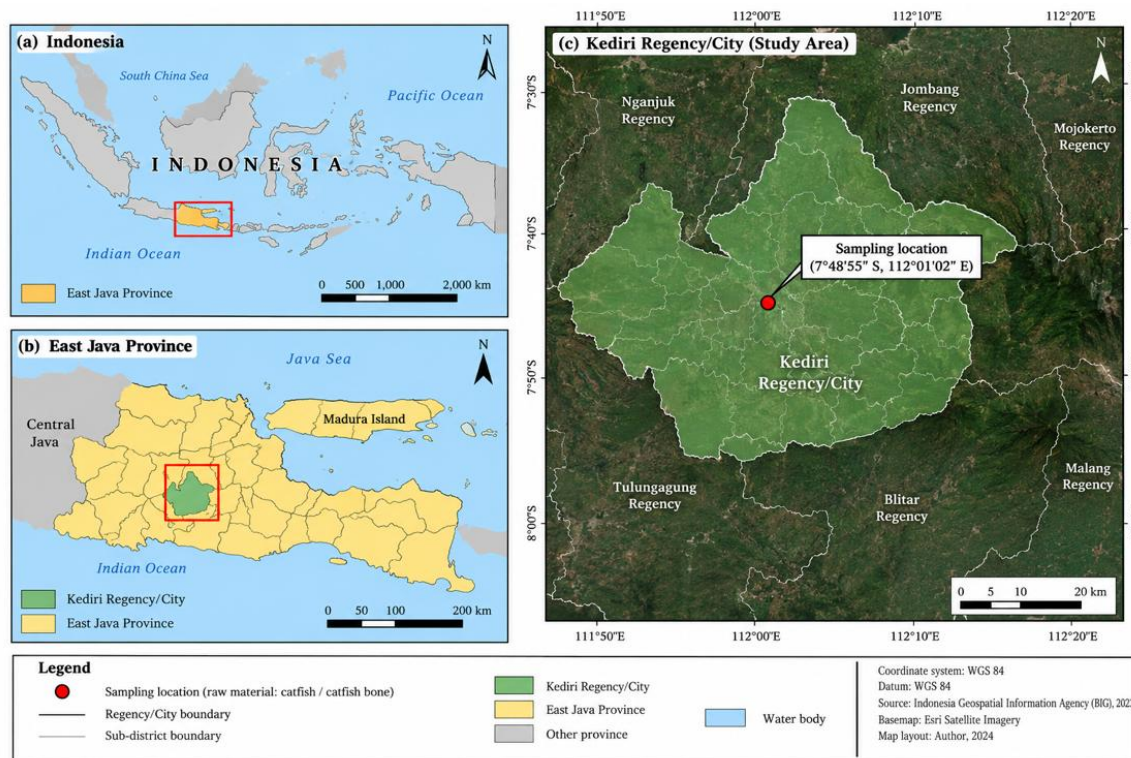


Figure 1. Map of the sampling location in Kediri, East Java, Indonesia.

Preparation of bone powder. CBP was prepared through a multi-step process designed to ensure hygiene, nutrient retention, and an appropriate particle size for food use (Figure 2). Bone residues were first washed thoroughly to remove blood and impurities, then boiled at 100°C for 2 h, repeated three times, to remove fat and soften the structure, following the boiling protocol of Anandya et al. (2023) for catfish bone valorisation; repeated short boiling cycles were used in preference to a single prolonged boiling step because they more effectively removed residual fat and softened the bone matrix while limiting nutrient leaching. After cooling, the bones were cut into smaller pieces to allow even drying and grinding, then dried in a hot-air oven at 105°C for 90 min to reduce moisture, a temperature and duration selected because it enabled rapid, complete moisture removal without causing the surface browning or off-odour formation observed in preliminary trials at higher oven temperatures. The dried bones were coarsely crushed and finely milled, then passed through an 80-mesh sieve to obtain a uniform powder. The final product was a fine white powder rich in calcium and phosphorus, suitable as a fortificant in fish-based foods (Anandya et al., 2023). A representative composite sample of the finished powder was submitted for microbiological analysis before use in meatball formulation. Prior to incorporation into the meatball formulations, the finished CBP was itself characterised for yield (dry powder weight relative to fresh bone weight), particle size (retained on an 80-mesh sieve, $\leq 180 \mu\text{m}$), colour (CIE L^* , a^* , b^* , using a chromameter), bulk density (tapped-density method), and proximate and mineral composition (moisture, ash, crude protein, crude fat, calcium and phosphorus, determined as described below for the fortified product); these characteristics are reported in the Results.



Figure 2. Process flow of raw material preparation showing sequential steps from washing, boiling, cutting, drying, particle reduction, to grinding. Each stage represents the transformation from coarse raw material into fine powder form used for further processing.

Meatball formulation. Functional and flavour-enhancing ingredients were held constant across treatments to ensure comparability, with catfish meat partially substituted by CBP. The detailed composition of each treatment is given in Table 1.

Table 1
Formulation of catfish meatballs fortified with CBP (g per formulation)

<i>Ingredient (g)</i>	<i>Control</i>	<i>5% CBP</i>	<i>10% CBP</i>	<i>15% CBP</i>	<i>20% CBP</i>
Catfish meat	250	237.5	225	212.5	200
Crushed ice	50	50	50	50	50
Salt	6.5	6.5	6.5	6.5	6.5
Shallot	2.5	2.5	2.5	2.5	2.5
Garlic	1.25	1.25	1.25	1.25	1.25
STPP	5	5	5	5	5
Pepper	0.625	0.625	0.625	0.625	0.625
MSG	0.625	0.625	0.625	0.625	0.625
Tapioca starch	31.25	31.25	31.25	31.25	31.25
Egg white	30	30	30	30	30
Baking powder	0.75	0.75	0.75	0.75	0.75
CBP	0	12.5	25	37.5	50

Note: STPP - sodium tripolyphosphate, CBP - catfish bone powder, MSG - monosodium glutamate.

Experimental design. A completely randomised design (CRD) was used with five treatments (0, 5, 10, 15 and 20% CBP) and five replicates. Storage stability was monitored at room temperature ($28 \pm 1^\circ\text{C}$) at 0, 6, 12, 18, and 24 h. Five independent replicates ($n=5$) per treatment were used for the primary CRD underlying inferential statistical testing of proximate composition, mineral content and economic indicators. For the time-course measurements requiring destructive sampling at each storage interval (pH, WHC, lipid oxidation, texture, and the storage figures for sensory and microbiological data), three of the five replicates ($n=3$) were analysed per treatment per time point to manage analytical throughput; this subsampling is indicated in the corresponding figure legends.

Nutritional and mineral analyses. Moisture, ash, crude protein, crude fat and crude fibre were determined by standard AOAC methods, with minor modification after Radiati et al. (2025a). Calcium was quantified by atomic absorption spectrophotometry following acid digestion, and phosphorus by the spectrophotometric vanadomolybdate method; both were expressed as mg 100 g⁻¹ of product.

Physicochemical analyses. pH was measured following Setiawati et al. (2025); texture was assessed by texture profile analysis (TPA) using a texture analyser after Paredes et al. (2022); WHC was determined gravimetrically; and lipid oxidation was quantified by the thiobarbituric acid reactive substances (TBARS) method and expressed as mg malondialdehyde (MDA) kg⁻¹. Shelf-life behaviour was evaluated through pH change during ambient storage at 0, 6, 12, 18, and 24 h, following Radiati et al. (2020).

Microbiological analyses. Microbiological safety was evaluated on the finished CBP and on the fortified meatballs (control and 10% CBP) at 0 and 24 h of ambient storage. total plate count (TPC) was determined by the pour-plate method on plate count agar (37°C, 48 h) and expressed as CFU g⁻¹. Total coliforms and *Escherichia coli* were enumerated by the most-probable-number (MPN) procedure using lauryl tryptose and EC broths with confirmation on eosin methylene blue agar, and expressed as MPN g⁻¹. *Salmonella* spp. was detected qualitatively (presence/absence in 25 g) by pre-enrichment in buffered peptone water, selective enrichment in selenite cystine broth, and plating on selective media. Results were interpreted against the Indonesian National Standard for fish meatballs (SNI 7266:2017). This full confirmatory panel (TPC, total coliform, *E. coli*, and *Salmonella* spp.) was applied to the CBP and to the control and 10% CBP meatballs, representing the unfortified baseline and the recommended formulation, respectively. In addition, a screening panel limited to TPC and total coliform was performed on all five formulations (0, 5, 10, 15, and 20% CBP) at 0, 6, 12, 18, and 24 h to characterise the storage-related microbial trend across the full dose range.

Sensory evaluation. Sensory tests were conducted by 25 semi-trained panellists from Universitas Brawijaya. Coded samples (3-digit random codes) were presented in randomised order, and taste, aroma, texture, and overall acceptability were rated on a 9-point hedonic scale (1 - dislike extremely, 9 - like extremely) after Umam et al. (2019). All sessions were carried out in a controlled room under ambient conditions. Panellists were aged 19-45 years (14 female, 11 male), recruited from staff and students with prior experience in hedonic sensory panels, and briefed on the scoring scale immediately before testing. The sensory evaluation was conducted in accordance with the ethical principles of the Declaration of Helsinki for research involving human participants. Participation was voluntary, no personal identifying data were recorded, and written informed consent was obtained from all panellists prior to participation.

Economic analysis. Production-cost analysis included raw materials, labour, energy and packaging. Market viability was estimated using expected selling prices for comparable SME-scale products (Anandya et al., 2025). Proximate and mineral analyses were performed at the Laboratory of Animal Product Technology, Universitas Brawijaya, Malang; texture, lipid oxidation, and microbiological analyses at the Central Laboratory, PSDKU Universitas Brawijaya. Raw-material cost was calculated directly from the formulation in Table 1 using local market prices for catfish meat, CBP, and other ingredients (Kediri, East Java, March 2025). Processing and packaging costs were estimated from typical small-scale bakso-ikan production overheads, and labour and energy costs were apportioned per kilogram of finished product based on a standard batch size of 100 kg day⁻¹. The selling price of the control formulation was set at the prevailing local market price for commercial fish meatballs; the price of the 10% CBP formulation was evaluated under two scenarios — (i) no price premium (equal to the control) and (ii) an assumed 8-10% premium reflecting a functional-food positioning, to illustrate how sensitive the projected profit margin is to market acceptance of the fortified product.

Statistical analysis. Endpoint variables measured once per formulation (proximate composition, calcium and phosphorus content, and the economic indicators) were analysed by one-way ANOVA to test the effect of bone-powder level, followed by Duncan's multiple range test (DMRT) where significant differences were detected ($p < 0.05$). Variables monitored repeatedly during storage (pH, WHC, lipid oxidation, texture, and the microbiological counts) were analysed by two-way ANOVA with bone-powder level and storage time as fixed factors, including their interaction (Treatment $\times$ Time); where the interaction was significant, simple-effects comparisons were performed with DMRT at each storage time. All analyses were performed using IBM SPSS version 25, with significance set at $p < 0.05$.

Results

Proximate and mineral composition. The addition of CBP resulted in notable changes to the proximate composition of the meatballs (Figure 3). Ash content increased significantly from 1.23% in the control group to 3.89% in the 20% CBP treatment ($p < 0.01$). Protein content showed a modest increase in the 15% and 20% CBP treatments. Conversely, moisture content decreased progressively with higher levels of CBP inclusion. Fat content remained consistent across all treatments, and crude fibre content was consistently low, staying below 1% in all sample groups. The CBP used as the fortificant had a fine particle size ($\leq 180 \mu\text{m}$, retained on an 80-mesh sieve), a pale off-white colour, and the following proximate and mineral composition (dry-weight basis): moisture 1.83%, ash 67.53%, crude protein 22.96%, crude fat 3.51%, calcium 1,480 mg 100 g⁻¹ and phosphorus 110 mg 100 g⁻¹, with a powder yield of $14.46 \pm 1.39\%$ relative to fresh bone weight (values to be completed from the characterisation assay; see Materials and Methods).

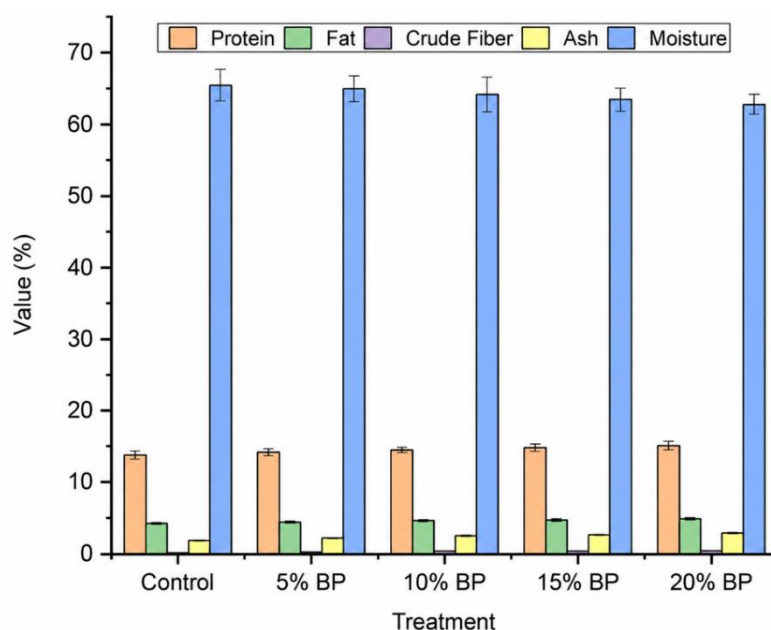


Figure 3. Nutrient profile of catfish meatballs fortified with varying levels of CBP (0, 5, 10, 15, and 20%). Bars represent mean values of proximate composition, including protein, fat, crude fiber, ash, and moisture content. Error bars indicate standard deviation ($n=3$)

Mineral analysis revealed significant changes across the fortification levels (Table 2). Calcium content increased from 38.4 mg 100 g⁻¹ in the control to 412.6 mg 100 g⁻¹ in the 20% CBP treatment ($p < 0.01$). Phosphorus content also increased in a dose-dependent manner, rising from 96.2 mg 100 g⁻¹ in the control to 264.8 mg 100 g⁻¹ at the 20% CBP level ($p < 0.01$). At the 10% inclusion level, calcium reached 233.7 mg 100 g⁻¹ and phosphorus reached 191.5 mg 100 g⁻¹. The Ca:P ratio shifted across treatments, reaching

approximately 1.2:1 at the 10% CBP inclusion level, with higher ratios observed at the 15% and 20% CBP treatments.

Table 2

Calcium and phosphorus content of fortified catfish meatballs

<i>Treatment</i>	<i>Calcium (mg 100 g⁻¹)</i>	<i>Phosphorus (mg 100 g⁻¹)</i>	<i>Ca:P ratio</i>
Control (0% CBP)	38.4±2.1	96.2±3.4	0.40
5% CBP	141.6±4.8	148.3±4.0	0.95
10% CBP	233.7±6.2	191.5±5.1	1.22
15% CBP	324.9±7.5	228.6±6.3	1.42
20% CBP	412.6±9.1	264.8±7.0	1.56

Note: Calcium and phosphorus are expressed as mg 100 g⁻¹ of sample (wet-weight basis). Values are mean±SD (n=3). The Ca:P ratio is dimensionless.

Sensory acceptability. The addition of CBP significantly influenced the hedonic responses for catfish meatballs across all evaluated parameters: taste, aroma, texture, and overall acceptance (Figure 4). Each sensory parameter exhibited a clear quadratic, bell-shaped trend, with hedonic scores increasing from the control group, peaking at the 10% CBP treatment, and subsequently decreasing at the 15% and 20% CBP levels. The 10% CBP treatment consistently received the highest scores among all formulations, with values of 8.2 for taste, 8.1 for aroma, 8.4 for texture, and 8.5 for overall acceptance.

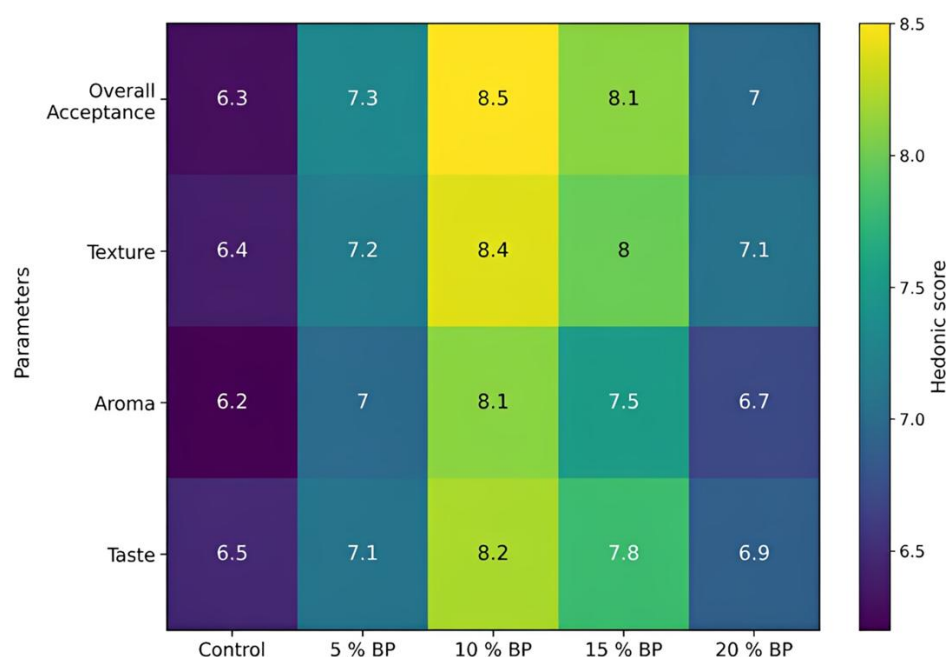


Figure 4. Heatmap of sensory attributes (taste, aroma, texture and overall acceptance) of catfish meatballs fortified with 0, 5, 10, 15, and 20% CBP, on a 9-point hedonic scale (n=25).

Across all four attributes, hedonic scores followed a consistent bell-shaped trend, rising from the control, peaking at 10% CBP, and declining at 15% and 20% CBP (Figure 4). Taste and aroma scores peaked at 8.2 and 8.1, respectively, at 10% CBP, while texture and overall acceptance peaked at 8.4 and 8.5. The lowest scores for all attributes were recorded in the control and 20% CBP treatments, reflecting limited mineral-associated flavour complexity in the control and off-flavours or a gritty mouthfeel at the highest inclusion level.

PH and short-term shelf life. All formulations showed a progressive pH decline over 24 h of ambient storage, with the rate and magnitude of decline increasing with CBP level (Figure 5a). The control and 5% CBP treatments started with the highest pH (6.65-6.73)

and declined most slowly, remaining above pH 6.35 through 18 h. The 10% and 15% CBP treatments showed intermediate declines that stayed closely aligned with the control through 12–18 h. The 20% CBP treatment declined most rapidly throughout storage and was the only formulation to fall below pH 6.0, reaching 5.96 at 24 h. Overall, the control showed the slowest pH decline, while pH stability decreased progressively with increasing CBP inclusion.

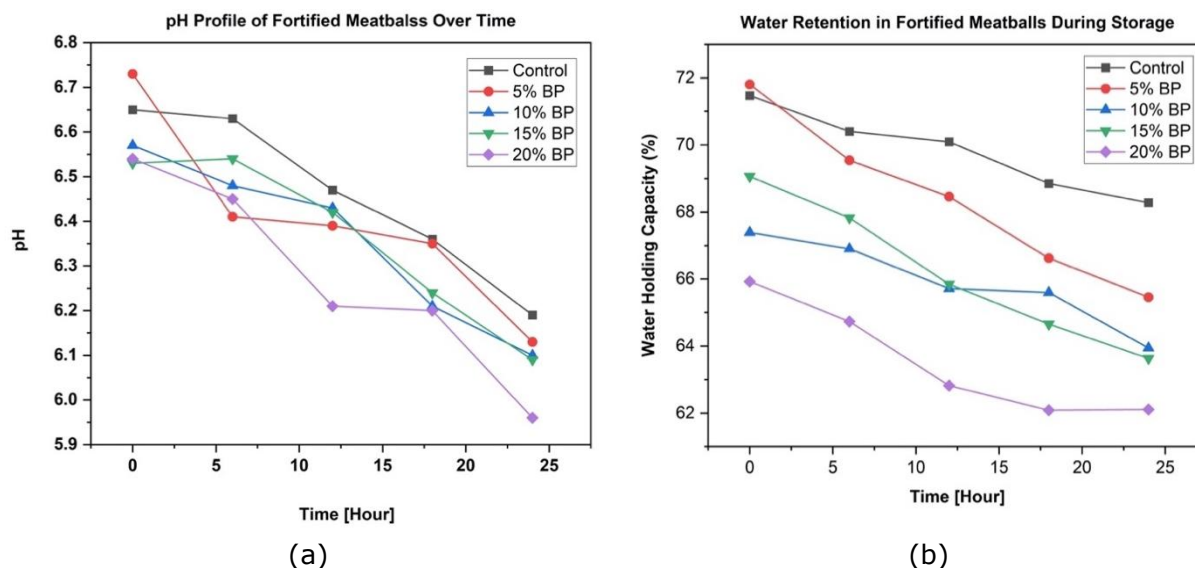


Figure 5. (a) pH profile and (b) WHC of fortified catfish meatballs during ambient storage at 0, 6, 12, 18, and 24 h.

Water-holding capacity (WHC). WHC progressively declined over the 24-hour storage period in all meatball treatments (Figure 5b). The extent and rate of this reduction were dose-dependent, with more pronounced declines observed at higher bone-powder inclusion levels. At time zero, the 5% CBP treatment exhibited the highest WHC at 72.0%, slightly exceeding the control formulation at 71.2%. During storage, the control group maintained the greatest stability, retaining water content more effectively than other formulations. Conversely, the 20% CBP formulation showed the most substantial reduction in water retention, ending the storage period with the lowest WHC at 61.9%.

Lipid oxidation. Lipid oxidation, assessed by TBARS values, increased with both longer storage duration and higher bone-powder inclusion levels (Figure 6a). At the initial 0-hour measurement, TBARS values were lowest in the control group and increased incrementally with higher CBP concentrations. This dose- and time-dependent increase persisted at the 6-hour, 12-hour, and 18-hour intervals, with all fortified treatments consistently exhibiting greater malondialdehyde (MDA) accumulation than the control group at each time point.

At the conclusion of the 24-hour ambient storage period, all formulations reached their highest levels of lipid oxidation. The most pronounced increases were observed in the 15% and 20% CBP groups. At 24 hours, the 20% CBP sample attained a peak value of approximately $0.18 \text{ mg MDA kg}^{-1}$. In contrast, the control and formulations with 10% CBP or lower maintained TBARS values below $0.15 \text{ mg MDA kg}^{-1}$ throughout the 24-hour period. The progressive rise in TBARS with increasing CBP inclusion is most plausibly attributed to the mineral and trace-metal content introduced by the CBP. Residual iron and other transition metals commonly associated with bone matrices are well-established catalysts of Fenton-type radical reactions that accelerate lipid peroxidation, while calcium and phosphate ions may further alter protein-lipid interactions in the meatball matrix, shifting the pro-oxidant/antioxidant balance. The larger surface area and mineral exposure at higher CBP inclusion levels likely compound this effect, accounting for the dose-dependent increase in TBARS observed here.

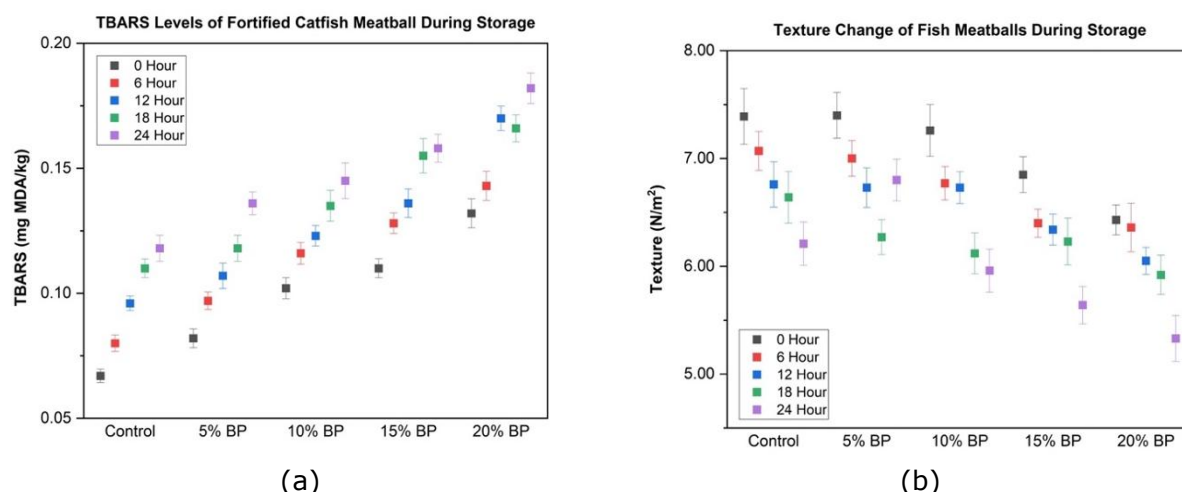


Figure 6. (a) TBARS values and (b) texture (N) of catfish meatballs fortified with 0, 5, 10, 15, and 20% CBP over 24 h of ambient storage.

Texture and structural stability. Figure 6b illustrates that the firmness of catfish meatballs, measured as texture (N, corrected from the originally reported $\text{N m}^{-2}/\text{N m}^2$, which is not a valid unit for texture-analyser force output and should be verified against the raw instrument log), decreased steadily over 24 hours of storage in all treatments. The formulation with 10% CBP exhibited the highest initial texture value, approximately 7.5 N, which was marginally higher than the control. This finding suggests that moderate CBP addition enhances initial firmness. In contrast, higher CBP concentrations led to a more significant reduction in texture over time. The 15% and 20% CBP treatments experienced sharper declines in firmness during storage, with the lowest texture value, approximately 5.5 N, recorded in the 20% CBP treatment after 24 hours. These results indicate a time-dependent and dose-dependent reduction in textural stability, especially at elevated CBP inclusion levels.

Microbiological safety. Given that the fortificant originated from a waste stream, microbiological safety was assessed as a prerequisite for human consumption. Table 3 shows that both the finished CBP and the fortified meatballs complied with SNI 7266:2017 standards at 0 and 24 hours of ambient storage. TPC in the CBP and in the 10% CBP meatballs at 0 hours were substantially below the maximum limit of $5 \times 10^4 \text{ CFU g}^{-1}$. Total coliform counts remained low, *Escherichia coli* was below the detectable threshold ($< 3 \text{ MPN g}^{-1}$), and *Salmonella* spp. was not detected in 25 g.

Table 3
Microbiological quality of CBP and fortified meatballs compared with SNI 7266:2017 limits

Parameter	CBP	Control 0 h	10% CBP 0 h	10% CBP 24 h
TPC (CFU g^{-1})	1.2×10^3	8.5×10^3	9.1×10^3	3.4×10^4
Total coliform (MPN g^{-1})	< 3	9	7	21
<i>E. coli</i> (MPN g^{-1})	< 3	< 3	< 3	< 3
<i>Salmonella</i> (per 25 g)	ND	ND	ND	ND

Note: CBP - catfish bone powder; TPC - total plate count; MPN - most probable number; ND - not detected. SNI 7266:2017 limits: $\text{TPC} \leq 5 \times 10^4 \text{ CFU g}^{-1}$; *E. coli* $< 3 \text{ MPN g}^{-1}$; *Salmonella* negative per 25 g.

During ambient storage, the TPC increased after 24 hours in both the control and the 10% CBP meatballs. However, these values remained within the acceptable microbiological limit. The addition of 10% CBP did not increase the microbial load compared with the control, demonstrating that moderate CBP fortification did not introduce additional microbial contamination under the tested conditions.

Consistent with the expanded microbiological screening described in Methods, Figure 7 presents the TPC and total coliform screening data collected for all five formulations, whereas Table 3 presents the full confirmatory panel (including *E. coli* and

Salmonella spp.) obtained for the CBP and for the control and 10% CBP meatballs only. TPC and total coliform levels increased progressively over 24 hours of ambient storage in all treatments. The control, 5% CBP, and 10% CBP meatballs remained below the SNI 7266:2017 limit throughout the 24-hour storage period. In contrast, the 15% and 20% CBP treatments exhibited a more rapid increase in microbial counts, approaching or exceeding the permissible limit between 18 and 24 hours of storage.

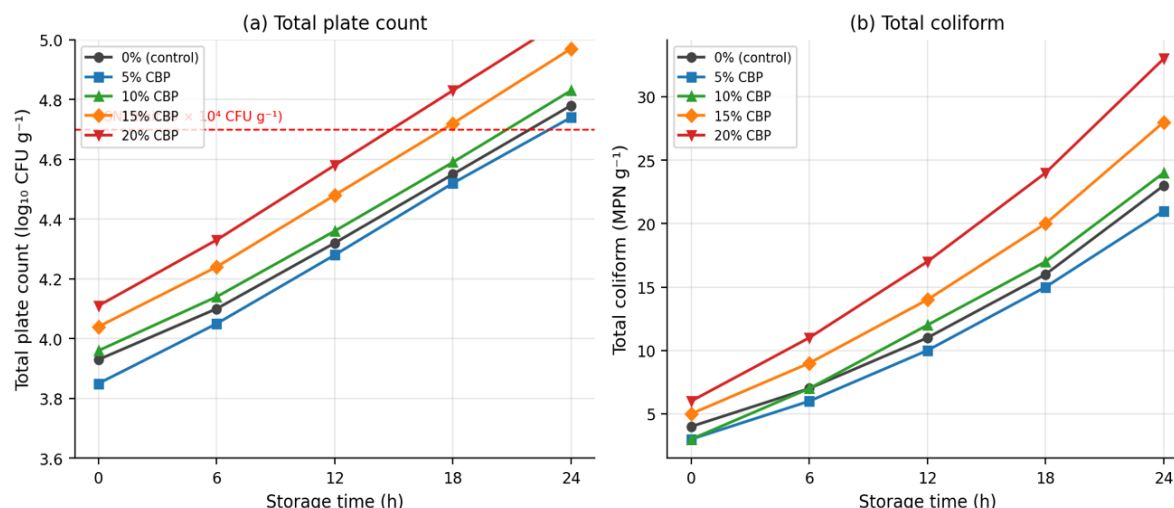


Figure 7. Microbiological quality of catfish meatballs fortified with 0, 5, 10, 15 and 20% CBP during 24 h of ambient storage: (a) TPC (log₁₀ CFU g⁻¹; dashed line = SNI 7266:2017 limit of 5 × 10⁴ CFU g⁻¹) and (b) total coliform (MPN g⁻¹), measured at 0, 6, 12, 18 and 24 h.

Economic value. Economic analysis demonstrated that incorporating CBP reduced the raw material costs associated with catfish meatball production. As indicated in Table 4, replacing 10% of fish meat with CBP decreased production costs by approximately USD 0.16 kg⁻¹ compared to the control formulation. This reduction represents a 6% savings in raw material costs. At a production scale of 100 kg day⁻¹ over 30 working days, the 10% CBP formulation could yield an estimated monthly cost saving of approximately USD 480 on the cost side alone. Under the modelled market scenario in which a functional-food positioning permits an 8-10% selling-price premium, projected profit margin for the 10% CBP formulation would increase substantially relative to the control; without this assumed premium, the 10% CBP formulation would still be more profitable than the control on the basis of the raw-material cost saving alone. These findings should therefore be interpreted as a cost-saving result supported by data, combined with a price-premium scenario that has not yet been validated by actual market testing. These findings suggest that CBP incorporation offers a quantifiable economic benefit, particularly for small- to medium-scale production operations.

Table 4
Estimated production cost, selling price and profit margin per kilogram of control and 10% CBP catfish meatballs

Component	Control (0% CBP)	10% CBP
Fish meat (USD 1.96 kg ⁻¹)	1.0 kg → USD 1.96	0.9 kg → USD 1.76
Bone powder (USD 0.28 kg ⁻¹)	-	0.1 kg → USD 0.03
Spices, starch, etc.	USD 0.45	USD 0.45
Processing and packaging	USD 0.39	USD 0.39
Total cost (USD)	2.79	2.63
Selling price (USD kg ⁻¹)	3.35	3.63*
Profit margin (USD)	0.56	1.01

Note: * Modeled scenario: assumes a functional-food label permits an 8-10% price premium based on added nutritional value and sustainability; this premium is a market assumption for illustrative purposes and has not been empirically validated. Currency conversion was based on the Bank Indonesia exchange rate on 25 September 2026 (USD 1 ≈ IDR 17,898).

Circular-economy perspective. From a circular-economy perspective, incorporating CBP into meatball formulations demonstrates the potential for utilizing fish-processing by-products as food-grade fortificants. Fish bones, which may constitute up to 15% of total fish biomass, are rich in valuable minerals, particularly calcium and phosphorus. In this study, CBP facilitated the recovery of these nutrients and their reintegration into a fish-based food product.

The 10% CBP formulation achieved the optimal balance among fortification level, product quality, microbiological safety, and economic benefit. This formulation enabled partial substitution of fish meat while maintaining product acceptability and reducing production costs. Consequently, the use of CBP serves as a practical example of converting fish-processing waste into a value-added ingredient.

Discussion. The results demonstrate that CBP serves as an effective mineral fortificant in catfish meatballs, with optimal performance observed at the 10% inclusion level. The addition of CBP significantly increased ash, calcium, and phosphorus contents, confirming its efficacy as a mineral source. Specifically, ash content rose from 1.23% in the control to 3.89% in the 20% CBP treatment, while calcium and phosphorus increased from 38.4 to 412.6 mg 100 g⁻¹ and from 96.2 to 264.8 mg 100 g⁻¹, respectively. Notably, the highest mineral content did not correspond to the most favorable formulation. At 10% CBP, calcium and phosphorus reached 233.7 and 191.5 mg 100 g⁻¹, respectively, with a Ca:P ratio of approximately 1.2:1, indicating a more balanced mineral profile compared to higher inclusion levels. These findings suggest that mineral fortification should be optimized rather than maximized, as excessive CBP may increase calcium content without proportionate improvements in overall product quality. Comparable results have been reported in other bone-derived fortification studies, where mineral enrichment enhanced nutritional value but required controlled inclusion to maintain matrix stability (Trisnawati et al., 2024; Jensen et al., 2024).

The incorporation of CBP influenced the nutritional and sensory characteristics of the fortified catfish meatballs. Increasing CBP levels enhanced the mineral content, particularly calcium and phosphorus, confirming the potential of CBP as a mineral-rich fortificant. However, sensory acceptance did not increase linearly with fortification level. The 10% CBP treatment showed the highest overall acceptability, whereas higher inclusion levels reduced sensory scores, particularly at 20% CBP. This decline may be associated with the greater mineral load and the resulting changes in texture and mouthfeel. Similar effects have been reported for bone-derived mineral ingredients, where increasing fortification improves mineral density but excessive incorporation may adversely affect sensory properties (Jensen et al., 2024).

The observed improvement in nutritional composition highlights the broader potential of functional fortification in animal-based processed foods. CBP functioned not only as a source of calcium and phosphorus but also influenced the structural properties of the meatball matrix. The slight increase in protein content at higher CBP levels may be attributed to residual collagen or bone-associated organic fractions. Conversely, the reduction in moisture content suggests that CBP possesses lower water-binding capacity than fish muscle protein. Moisture retention is critical, as it directly affects juiciness, texture, and storage stability. Previous studies have demonstrated that functional ingredient fortification can enhance both nutritional and structural quality when the ingredient is compatible with the food matrix (Amertaningtyas et al., 2026). In the present study, compatibility was most pronounced at moderate inclusion levels, particularly at 10% CBP.

Sensory evaluation results identified an optimal fortification threshold. The 10% CBP treatment received the highest scores for taste, aroma, texture, and overall acceptance, indicating that moderate CBP addition enhanced consumer perception. This improvement may be associated with increased flavor complexity and favorable interactions among taste-active compounds and protein-derived components. Hossain et al. (2024) reported that umami perception and flavor enhancement in meat products are influenced by interactions among amino acids, peptides, nucleotides, and other taste-active compounds. The sensory response exhibited a bell-shaped pattern: the control group

scored lower due to limited mineral richness, while the 20% CBP treatment experienced declines in acceptance, likely due to chalkiness, gritty mouthfeel, and off-flavors. Similar formulation-dependent changes in physicochemical and sensory quality have been documented in meatball-type products (Patria et al., 2024). The quality of meat-based products is determined by raw material composition, processing methods, and physical characteristics, and sensory evaluation is essential when introducing new ingredients (Umam et al., 2019; Radiati et al., 2020). The reduction in acceptance at the highest CBP level may also be linked to altered mouthfeel and product structure, as excessive modification of protein-based food systems can diminish sensory quality and consumer acceptance (Ismail et al., 2020). Changes in pH, moisture, and sensory properties have also been observed in animal-based food products containing natural additives, suggesting that formulation adjustments can affect both physicochemical stability and organoleptic quality (Setiawati et al., 2025). Thus, while 20% CBP yielded the highest mineral content, it surpassed the sensory tolerance of the product.

The pH profile exhibited a progressive decline over 24 hours of ambient storage across all treatments, reflecting ongoing biochemical and microbial changes. The 10% CBP formulation maintained relative stability up to 12-18 hours, while the 20% CBP treatment experienced the most pronounced decline, dropping below pH 6.0 after 24 hours. This pattern suggests a threshold-buffering effect, in which moderate mineral inclusion supports matrix stability, whereas excessive CBP disrupts the gel network and accelerates acidification. pH changes in meat products are closely linked to protein functionality, water retention, and storage quality (Anandh, 2014). Unlike purified calcium salts or synthetic phosphates, CBP is a complex biological material containing minerals, collagen residues, and trace organic matter. This complexity may account for its dual effect: structural stabilization at moderate levels and matrix disturbance at higher concentrations. Comparable behavior has been observed in calcium-enriched meat products utilizing meat-bone materials (Yessimbekov et al., 2021).

WHC decreased during storage in all formulations, with a more pronounced decline at higher CBP concentrations. At the initial time point, the 5% CBP treatment exhibited the highest WHC, slightly surpassing the control, suggesting that low-level mineral addition may enhance water retention through ionic interactions with muscle proteins. In contrast, the 20% CBP treatment experienced the greatest reduction in WHC, indicating that excessive CBP weakened the protein-starch matrix. Fish muscle proteins are essential for gel formation and water immobilization; thus, excessive replacement of fish meat with insoluble bone particles may disrupt matrix continuity and promote water migration. While fish-bone addition can improve nutritional value and texture, its functional benefits are highly dependent on inclusion level and compatibility with the product matrix (Nawaz et al., 2019). These WHC results support a dose-dependent threshold: low-to-moderate CBP levels may reinforce structure, whereas excessive CBP reduces water retention.

Lipid oxidation, measured as TBARS, increased with both storage duration and CBP concentration. The 15% and 20% CBP treatments exhibited greater oxidative development, with the 20% CBP treatment nearing the rancidity threshold after 24 hours, while the control, 5%, and 10% CBP treatments remained below the reported acceptable limit. TBARS serves as a reliable indicator of oxidative deterioration in meat products (Turhan et al., 2017). Elevated TBARS values at higher CBP levels may be attributed to pro-oxidant minerals such as iron and copper present in bone-derived materials. This pattern of oxidation corresponded with lower aroma and taste scores in the 15% and 20% treatments, suggesting that oxidative changes contributed to off-flavors and reduced acceptability. Mineral-rich additives have been shown to affect oxidative stability and sensory attributes in meat systems (Young et al., 2026). The 10% CBP formulation remained within the oxidative tolerance of the product matrix.

Texture analysis further supported the fortification-threshold concept. Firmness decreased progressively during storage; however, the 10% CBP treatment exhibited the highest initial firmness, slightly surpassing the control. This finding suggests that moderate CBP inclusion reinforced the gel structure, potentially through calcium-mediated interactions with muscle proteins. Calcium powders have been shown to enhance the quality properties of ground meat products when applied at appropriate levels (Bae et al.,

2017). Texture analysis is critical, as mechanical properties reflect matrix integrity, cohesion, and stability in protein-based meat systems (Paredes et al., 2022). In contrast, the 15% and 20% CBP treatments experienced more rapid texture degradation, likely due to undissolved bone particles disrupting gel continuity, creating localized stress points, and increasing brittleness. Excessive incorporation of bone-derived or ash-rich material may weaken meat-system structure when inclusion exceeds matrix compatibility (Jensen et al., 2024). The rapid decline in firmness at 20% CBP corresponded with WHC loss and elevated TBARS, indicating that texture deterioration was associated with moisture migration, oxidative instability, and structural disruption.

Microbiological safety is a key consideration given that CBP is derived from a waste stream. Both the finished CBP and fortified meatballs complied with SNI 7266:2017 requirements at 0 and 24 hours of ambient storage. TPC remained below 5×10^4 CFU g⁻¹, total coliforms were low, *Escherichia coli* was undetectable, and *Salmonella spp.* was not detected in 25 g samples. These findings indicate that washing, repeated boiling, and hot-air drying effectively minimized microbial contamination. Thermal processing of catfish bone waste has been shown to facilitate safe utilization in meatball products (Anandya et al., 2023). However, microbial counts increased during storage, particularly in the 15% and 20% CBP treatments, paralleling more rapid pH decline and increased oxidation. The 10% CBP formulation closely matched the control and remained below safety limits throughout storage, indicating no additional microbial risk at moderate inclusion levels. Based on combined pH, WHC, TBARS, texture, and microbial data, ambient storage should be restricted to approximately 18 hours before chilling or consumption.

From an economic perspective, incorporating 10% CBP reduced production costs by approximately USD 0.16 per kilogram, representing about 6% savings in raw materials. At a production scale of 100 kg day⁻¹ over 30 working days, this translates to roughly USD 480 in monthly savings. Fish bone, typically discarded, can be transformed into a valuable ingredient through washing, boiling, drying, and milling processes (Nawaz et al., 2019). In the context of a circular economy, CBP utilization promotes waste valorization and nutrient recovery by reintegrating calcium- and phosphorus-rich by-products into the human food supply. This approach aligns with circular-economy practices in Indonesia, where waste materials are redirected into value-added products and supports responsible consumption and production under SDG 12 (Siregar et al., 2023; Irawan & Widodo, 2024; Windarto et al., 2025). Comparable value-recovery strategies have been advocated in fish-waste utilization, including sustainable aquafeed alternatives through fish-waste processing and ensilaging technologies (Maksimenko et al., 2024). Localized processing further supports zero-waste frameworks, reduces reliance on external fortificants, and strengthens small-enterprise production cycles (Abidin et al., 2024; Purwanti et al., 2024). At the operational level, the success of by-product valorization depends on production management, local resource availability, and market strategy, as demonstrated in fisheries-related enterprise studies in Indonesia (Anandya et al., 2025). The circular model may also benefit from digital promotion and local-market development, particularly for small enterprises focused on sustainability-based food products.

Although Radiati et al. (2025a) investigated goat-milk kefir exopolysaccharides rather than fish meatballs, their findings support the broader principle that animal-derived bioactive or functional ingredients can be developed into value-added products with potential health benefits. Accordingly, CBP-fortified catfish meatballs may be positioned as a functional food innovation, provided that nutritional enhancement, sensory quality, and food safety are simultaneously maintained. Overall, 10% CBP constitutes the optimal formulation, as it enhances mineral content, sensory quality, physicochemical stability, microbiological safety, and economic value, whereas higher inclusion levels compromise multiple quality attributes. These results suggest that CBP fortification should be optimized rather than maximized and may serve as a model for converting fish-processing waste into safe, acceptable, affordable, and nutritionally valuable food products.

Conclusions. Incorporation of 10% CBP into fish meatballs represents the optimal level for enhancing nutritional value, particularly calcium and phosphorus content, while maintaining acceptable sensory attributes, microbiological safety, and short-term shelf life. Higher inclusion levels were associated with decreased product stability, diminished oxidative quality, and reduced sensory acceptability. The fortified product complied with SNI 7266:2017 microbiological standards, indicating that hygienically processed CBP does not introduce additional food-safety risks. Utilization of CBP also reduced production costs and promoted zero-waste practices, thereby offering both economic and environmental benefits. It is recommended that producers apply 10% CBP, consider the addition of natural antioxidants to extend shelf life, and market the product as a high-calcium, sustainable food. Future research should investigate calcium bioavailability, performance during refrigerated storage, and the application of CBP in other food matrices.

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