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Effect of Bromelain and Papain on the Nutritional and Sensory Quality of Anchovy (Stolephorus indicus) Fish Sauce

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ABSTRACT

Traditional fish sauce fermentation relies on endogenous and microbial proteases and typically requires 6–12 months, which limits production efficiency. Adding plant proteases is a practical strategy to accelerate protein hydrolysis. This study compared the effects of bromelain and papain on the proximate composition and sensory acceptability of fish sauce made from Indian anchovy (Stolephorus indicus). A completely randomised design was applied with three treatments and three replicates: A, no enzyme (control); B, papain at 1% (w/w; 10 g kg⁻¹ fish); and C, bromelain at 1% (w/w). Anchovies were mixed with 20% salt and the enzyme, fermented for 30 days at ambient temperature, boiled, filtered and seasoned. Proximate composition was determined, and taste, odour, texture and colour were evaluated by 30 untrained panellists and ranked with the Analytical Hierarchy Process (AHP). Enzyme addition significantly increased moisture (74.39% in A; 76.34% in B; 76.20% in C; $p = 0.027$) and significantly decreased ash (4.10%, 2.58% and 2.05%; $p = 0.027$) and fat (2.95%, 1.88% and 1.68%; $p = 0.001$). Protein content was highest with papain (18.50%) and lowest with bromelain (15.98%), but the differences were not significant ($p = 0.298$); carbohydrate also did not differ significantly ($p = 0.382$). Taste was the most important sensory criterion (AHP weight 0.516), followed by odour (0.317), texture (0.111) and colour (0.056). Bromelain-treated sauce received the highest scores for taste, texture and colour and the highest overall AHP priority (0.342), whereas the control was preferred for odour. Both enzymes are suitable for accelerated anchovy fish sauce production, and bromelain gave the most acceptable product after 30 days of fermentation.

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INTRODUCTION

Anchovies *(Stolephorus spp.)* are among the most abundant small pelagic fishes in Indonesian waters and support an important small-scale fishery. Besides being eaten fresh or dried, anchovies are a traditional raw material for fish sauce, a clear, amber to brown liquid condiment obtained by fermenting fish with salt. Fish sauce is widely used in Southeast Asian cuisine as a seasoning and dipping sauce, and its production is mainly based on anchovies, mackerel and herring (Lopetcharat et al., 2001). Fish sauce is valued not only for its salty and savoury taste but also for its nitrogen-rich composition, which includes free amino acids and peptides derived from fish protein. Demand for fish sauce is growing both in domestic markets and in international trade.

Traditional fish sauce is produced by mixing fish with salt, usually in a ratio of two or three parts fish to one part salt, and allowing the mixture to ferment at ambient temperature for 6 to 12 months or longer (Lopetcharat et al., 2001). During this period, fish proteins are broken down by endogenous muscle and digestive enzymes and by proteases from halotolerant microorganisms. The high salt concentration prevents spoilage but also slows enzyme activity and microbial growth, which is why fermentation takes so long. Traditional methods also vary widely between regions and

producers, leading to inconsistent quality and safety (Li et al., 2024). Shortening the fermentation period can reduce production time and costs and allow faster product turnover (Li et al., 2024).

The flavour and nutritional value of fish sauce derive from the breakdown of fish macromolecules. During fermentation, proteins, lipids and carbohydrates are degraded into free amino acids, peptides and volatile compounds such as aldehydes, ketones, alcohols and esters (Li et al., 2024). In a traditional Italian anchovy sauce, glutamate was the predominant free amino acid and, together with aspartate, was responsible for the umami taste (Corona et al., 2023). Prolonged maturation improved the safety, nutritional value and volatile profile of that sauce (Corona et al., 2023). The degree of protein hydrolysis is therefore a key determinant of fish sauce quality, and any method that accelerates fermentation must reach a comparable level of hydrolysis.

Safety is another reason to control fish sauce fermentation carefully. Histamine and other biogenic amines can accumulate in fermented fish products when raw material quality or process conditions are poor. Histamine is the only biogenic amine with regulatory limits for fishery products (Visciano et al., 2020). Accelerated processes that reduce the time fish spend in conditions favouring amine-forming bacteria may help to limit these hazards, provided that the raw material is fresh and hygienically handled.

Several strategies have been developed to accelerate fish sauce fermentation. Yongsawatdigul et al. (2007) hydrolysed anchovy with commercial proteinases before adding halophilic starter cultures and obtained, within 4 months, a fish sauce with sensory characteristics similar to a commercial product fermented for 12 months. Starter cultures can also improve flavour: inoculation with Tetragenococcus halophilus increased total amino acids, particularly glutamic acid, and reduced dimethyl disulfide, a compound associated with a faecal note (Udomsil et al., 2011). These studies show that controlling proteolysis is central to producing good fish sauce in a shorter time.

The addition of exogenous proteases is the most direct way to speed up protein hydrolysis. In saline solutions, proteolysis of sardine and anchovy decreased as salt concentration increased, but commercial proteases still helped to liquefy the fish and cleave peptide bonds, with anchovy showing higher liquefaction and degree of hydrolysis than sardine (Le et al., 2015). In anchovy fish sauce fermented for 180 days, a commercial protease improved total nitrogen and amino acid contents compared with the control (Chau et al., 2021). These findings confirm that anchovy is a suitable raw material for enzyme-assisted fish sauce production.

Plant proteases are particularly attractive for this purpose because they are widely available, relatively inexpensive and accepted by consumers. Bromelain is a group of protein-digesting enzymes obtained from the fruit or stem of pineapple (Ananas comosus) and consists of a mixture of thiol endopeptidases and other components (Pavan et al., 2012). As a cysteine protease, it has been used commercially in the food, beverage, meat tenderisation, cosmetic and pharmaceutical industries (Arshad et al., 2014). Papain, a cysteine protease from the latex of papaya (Carica papaya), is one of the most studied and widely used proteases in the food industry (Fernández-Lucas et al., 2017). Papain, bromelain and ficin are the plant proteases most commonly used for meat tenderisation (Mohd Azmi et al., 2023).

Bromelain has already been tested in fish sauce production. Rabie et al. (2019) accelerated the fermentation of mackerel mixed with 20% salt by adding 0.2% and 0.4% bromelain at 37 °C. The 0.4% bromelain treatment produced an acceptable fish sauce within 90 days, and this sauce was organoleptically preferred at the end of fermentation

(Rabie et al., 2019). However, information on the use of papain and on the direct comparison of the two enzymes in anchovy fish sauce is limited, particularly for tropical anchovy species fermented at ambient temperature.

Comparative studies on fish protein hydrolysates suggest that the two enzymes do not behave identically. Enzymatic hydrolysis changes the solubility, water-holding capacity, emulsification and other functional properties of fish proteins (Kristinsson & Rasco, 2000). When papain and bromelain were used to prepare protein hydrolysates from threadfin bream frame waste, bromelain was relatively more effective in producing hydrolysates with desirable bioactive and functional properties (Gajanan et al., 2016). Differences in cleavage specificity, pH and temperature optima, and salt tolerance between the two enzymes could therefore lead to differences in the composition and sensory properties of fish sauce.

Uncontrolled protease activity can also have negative effects. In meat, excessive action of plant proteases may cause over-tenderisation, a mushy texture and poor quality (Mohd Azmi et al., 2023). In fish sauce, excessive or unbalanced hydrolysis may produce bitter peptides or change the characteristic aroma. Selecting the enzyme that gives the best balance between hydrolysis, nutritional composition and sensory acceptability is therefore important for producers who want to shorten fermentation without compromising quality.

Indian anchovy (Stolephorus indicus) is abundant and inexpensive in South Sulawesi, where it is commonly sold in traditional markets. Converting it into fish sauce with a shortened, enzyme-assisted process could add value to this resource and provide small producers with a product that can be made within weeks rather than months. However, no study has compared bromelain and papain in fish sauce made from S. indicus under the ambient conditions typical of small-scale production in the region.

This study therefore aimed to compare the effects of bromelain and papain, each added at 1% (w/w), on the proximate composition (moisture, ash, fat, protein and carbohydrate) and sensory acceptability (taste, odour, texture and colour) of Indian anchovy fish sauce fermented for 30 days at ambient temperature. A further aim was to determine which treatment produced the most preferred product, using the Analytical Hierarchy Process to weight the sensory criteria according to panellists' priorities.

METHODS

The study was conducted from June to September 2025. Fresh Indian anchovies (S. indicus) were purchased from Paotere traditional fish market, Makassar, washed three times with clean water and divided into 1 kg portions. A completely randomised design was used with three treatments and three replicates (nine experimental units): A, control without enzyme; B, papain at 10 g kg^{-1} fish (1%, w/w); and C, bromelain at 10 g kg^{-1} fish (1%, w/w). Each portion was mixed with 200 g of salt (20% of fish weight) and the respective enzyme, placed in a clean, sterilised container, sealed and fermented for 30 days at ambient temperature, following the principle of enzyme-assisted fish sauce production (Lopetcharat et al., 2001; Rabie et al., 2019; Chau et al., 2021). After fermentation, the mixture was boiled for 1 h and filtered to separate the liquid from the residue. The filtrate was cooked again for 20 min with lemongrass and garlic, filtered, bottled in sterilised glass bottles and stored at ambient temperature until analysis. Moisture, ash, fat and protein contents were determined by standard proximate methods at the Fishery Products Biochemistry Laboratory, Politeknik Pertanian Negeri Pangkajene dan Kepulauan, and carbohydrate was calculated by difference.

Sensory evaluation was carried out at Institut Teknologi dan Bisnis Maritim Balik Diwa by 30 untrained panellists, who scored the taste, odour, texture and colour of each sample on a hedonic scale. Proximate data were tested for normality with the Shapiro–Wilk test (Shapiro & Wilk, 1965). Normally distributed data were analysed by one-way ANOVA followed by Tukey's test (Tukey, 1949), and non-normally distributed data by the Kruskal–Wallis test (Kruskal & Wallis, 1952), at a significance level of 0.05, using SPSS. Sensory data were analysed with the Analytical Hierarchy Process (AHP) to derive the relative weight of each sensory criterion from panellists' pairwise judgements and the local priority of each treatment within each criterion (Saaty, 1990). The overall priority of each treatment was calculated as the sum of its local priorities multiplied by the corresponding criterion weights.

RESULTS AND DISCUSSION

Proximate composition

Enzyme addition significantly changed the moisture, ash and fat contents of anchovy fish sauce, but not its protein or carbohydrate contents (Table 1). Both enzymes increased moisture and reduced ash and fat relative to the control, with bromelain producing the lowest ash and fat values.

Table 1.
Proximate composition (%) of Indian anchovy fish sauce produced without enzyme (A), with 1% papain (B) and with 1% bromelain (C).

Parameter	A (control)	B (papain)	C (bromelain)	Test	p
Moisture	74.39	76.34	76.20	Kruskal–Wallis	0.027
Ash	4.10	2.58	2.05	Kruskal–Wallis	0.027
Fat	2.95[a]	1.88[b]	1.68[c]	ANOVA, Tukey	0.001
Protein	17.60	18.50	15.98	Kruskal–Wallis	0.298
Carbohydrate	0.95	0.68	0.75	ANOVA	0.382

Different superscript letters in the fat row indicate significant differences between treatments (Tukey, $p < 0.05$)

Moisture. Moisture was highest in the papain treatment (76.34%), followed by bromelain (76.20%) and the control (74.39%). The two enzyme treatments differed only slightly, indicating that both enzymes had a similar effect. The higher moisture of enzyme-treated sauces reflects more extensive liquefaction of fish tissue. Exogenous proteases accelerate the liquefaction of anchovy and sardine even in saline solutions, converting solid muscle into a liquid phase (Le et al., 2015). Hydrolysis also produces peptides and free amino acids that are more soluble and have greater water-holding capacity than intact muscle proteins (Kristinsson & Rasco, 2000). As a result, a larger proportion of the fish is recovered as liquid after filtration, which raises the moisture content of the final sauce.

Ash. Ash decreased from 4.10% in the control to 2.58% with papain and 2.05% with bromelain. Ash in fish sauce comes mainly from salt and from minerals in bones and other tissues. One explanation for the decrease is dilution: enzyme treatments released more liquid, so the same amount of soluble minerals was distributed in a larger volume. In addition, insoluble mineral-rich fractions, such as bone fragments, remain in the residue removed during filtration, and more complete liquefaction of the soft tissue may

separate them more cleanly from the liquid phase (Le et al., 2015). Hydrolysis also changes the partitioning of fish components between soluble and insoluble fractions, which affects the composition of the recovered liquid (Kristinsson & Rasco, 2000).

Fat. Fat decreased significantly from 2.95% in the control to 1.88% with papain and 1.68% with bromelain, and all three treatments differed from each other. During enzymatic hydrolysis, lipids are released from the disrupted muscle matrix and tend to separate from the aqueous protein fraction, either floating as an oil layer or remaining with the insoluble residue (Kristinsson & Rasco, 2000). Removing these fractions during boiling and filtration lowers the fat content of the sauce. Lipids are also degraded into fatty acids and volatile compounds during fermentation (Li et al., 2024); Rabie et al. (2019) found higher levels of total fatty acids in mackerel sauce treated with bromelain. The lower fat content of enzyme-treated sauces is desirable because it reduces the risk of lipid oxidation and rancid off-flavours during storage.

Protein. Protein was highest with papain (18.50%), intermediate in the control (17.60%) and lowest with bromelain (15.98%), but the differences were not significant ($p = 0.298$). The absence of a significant effect may reflect the high salt concentration, which reduces protease activity; proteolysis of anchovy and sardine decreased as salt content increased (Le et al., 2015). The slightly higher protein with papain is consistent with its broad specificity and good stability, which make it one of the most widely used proteases in food processing (Fernández-Lucas et al., 2017). The lower value with bromelain does not necessarily indicate weaker hydrolysis. Total protein measured by nitrogen content includes peptides and free amino acids, and extensive hydrolysis can convert part of the nitrogen into volatile compounds or into forms lost during boiling. In comparative studies, bromelain produced fish protein hydrolysates with more desirable bioactive and functional properties than papain (Gajanan et al., 2016). Nitrogen fractions such as amino nitrogen and total nitrogen, which are the usual quality indicators for fish sauce, would better describe the extent of hydrolysis (Chau et al., 2021).

Carbohydrate. Carbohydrate content was low in all treatments (0.68–0.95%) and did not differ significantly. Fish contains little carbohydrate, mainly glycogen, which is rapidly depleted after death. The small amounts present are consumed by microorganisms or participate in Maillard reactions with amino acids during fermentation and heating (Li et al., 2024). Because carbohydrate was calculated by difference, small variations also reflect the accumulated error of the other analyses.

Sensory acceptability

Taste was by far the most important sensory criterion for panellists (AHP weight 0.516), followed by odour (0.317), texture (0.111) and colour (0.056). Bromelain-treated sauce obtained the highest scores for taste, texture and colour, while the control obtained the highest score for odour (Table 2). Combining local priorities with criterion weights, the bromelain treatment had the highest overall priority (0.342), followed by the control (0.331) and the papain treatment (0.327).

Table 2.
Mean sensory scores and AHP local priorities of Indian anchovy fish sauce, criterion weights and overall priority of each treatment.

Criterion (AHP weight)	A score	B score	C score	A priority	B priority	C priority
Taste (0.516)	2.4	2.4	2.6	0.324	0.324	0.351
Odour (0.317)	2.4	2.3	2.3	0.343	0.329	0.329

Texture (0.111)	2.6	2.7	2.7	0.325	0.338	0.338
Colour (0.056)	2.9	2.8	3.0	0.333	0.322	0.345
Overall priority				0.331	0.327	0.342

Taste. The bromelain treatment received the highest taste score (2.6) and local priority (0.351), whereas the control and papain treatments scored equally (2.4; 0.324). Because taste carried more than half of the total weight, this difference largely determined the overall ranking. The savoury taste of fish sauce depends on free amino acids and peptides rather than on total protein; glutamate, together with aspartate, is responsible for the umami taste of anchovy sauce (Corona et al., 2023). Bromelain may have released a profile of peptides and amino acids that was more favourable to umami perception, despite the slightly lower total protein content. This agrees with the finding that mackerel fish sauce made with 0.4% bromelain was organoleptically preferred at the end of fermentation (Rabie et al., 2019). Starter cultures that increase glutamic acid content have also been shown to improve fish sauce flavour (Udomsil et al., 2011).

Odour. The control received the highest odour score (2.4) and local priority (0.343), while both enzyme treatments scored 2.3. The characteristic aroma of fish sauce develops mainly through microbial metabolism and chemical reactions during long fermentation, rather than through protein hydrolysis alone (Lopetcharat et al., 2001). Accelerated processes may therefore produce sauces with less developed aroma; in a 180-day trial, anchovy sauces made with or without a commercial protease had a weaker smell than commercial products (Chau et al., 2021). Prolonged maturation improves the volatile profile of anchovy sauce (Corona et al., 2023), and halophilic starter cultures can produce a fish sauce with sensory characteristics similar to traditionally fermented products in a shorter time (Yongsawatdigul et al., 2007). Combining enzymes with suitable starter cultures could therefore improve the aroma of enzyme-assisted fish sauce.

Texture. All sauces were thin liquids, as expected for fish sauce. The enzyme treatments scored slightly higher (2.7; local priority 0.338) than the control (2.6; 0.325). More complete liquefaction and the removal of fat and insoluble particles probably produced a clearer and more homogeneous liquid in the enzyme treatments (Le et al., 2015). Although uncontrolled protease activity can cause undesirable texture in meat products (Mohd Azmi et al., 2023), no negative effect on the fluidity of the sauce was observed at the enzyme level used here.

Colour. Colour scores were similar across treatments (2.8–3.0) and colour carried the lowest weight (0.056). The bromelain treatment had the highest score (3.0) and local priority (0.345), followed by the control (0.333) and papain (0.322). The colour of fish sauce develops through Maillard reactions between free amino acids and carbonyl compounds during fermentation and heating (Li et al., 2024). In mackerel fish sauce, colour saturation increased significantly with bromelain addition and fermentation time (Rabie et al., 2019), which is consistent with the slightly higher colour preference for the bromelain-treated sauce.

Implications and limitations

Both enzymes accelerated the conversion of anchovy into a liquid sauce within 30 days, a fraction of the 6–12 months required for traditional fermentation (Lopetcharat et al., 2001). Bromelain produced the most acceptable product overall, mainly because of its better taste, while papain gave a slightly higher protein content. For small-scale producers, bromelain has the additional advantage that it can be obtained from

pineapple processing by-products, and its use in food is well established (Arshad et al., 2014; Pavan et al., 2012).

This study has several limitations. Fermentation lasted only 30 days, and the aroma of enzyme-assisted sauces was still less preferred than that of the control. Amino nitrogen, total nitrogen, salt content, pH and free amino acids were not measured, so the extent of hydrolysis and compliance with fish sauce quality standards could not be fully assessed. Histamine was not determined, although it is the biogenic amine with regulatory limits in fishery products (Visciano et al., 2020). The enzymes were added on a weight basis without standardising their activity. Future studies should compare enzyme doses on an activity basis, monitor nitrogen fractions, free amino acids, volatile compounds and histamine during fermentation, and evaluate combinations of enzymes and halophilic starter cultures to improve aroma (Udomsil et al., 2011; Yongsawatdigul et al., 2007).

CONCLUSION

Adding bromelain or papain at 1% (w/w) shortened the production of Indian anchovy (Stolephorus indicus) fish sauce to 30 days and significantly changed its proximate composition. Both enzymes increased moisture (74.39% in the control; 76.34% with papain; 76.20% with bromelain) and significantly reduced ash (4.10%, 2.58% and 2.05%) and fat (2.95%, 1.88% and 1.68%), reflecting more extensive liquefaction of fish tissue and separation of lipid and insoluble fractions during filtration. Protein content was highest with papain (18.50%) and lowest with bromelain (15.98%), but these differences, like those in carbohydrate content, were not statistically significant. Taste was the dominant sensory criterion (AHP weight 0.516), followed by odour (0.317), texture (0.111) and colour (0.056). The bromelain-treated sauce received the highest scores for taste, texture and colour and achieved the highest overall AHP priority (0.342), ahead of the control (0.331) and the papain treatment (0.327). The control was preferred for odour, indicating that the characteristic aroma of fish sauce requires longer fermentation or microbial activity in addition to enzymatic hydrolysis. Bromelain is therefore the more suitable enzyme for producing acceptable anchovy fish sauce in a short time. Future work should standardise enzyme activity, monitor amino nitrogen, free amino acids, volatile compounds, and histamine, and test combinations of enzymes with halophilic starter cultures to improve aroma.

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