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ANALYSIS OF AMINO ACID PROFILE OF ANCHOR SAUCE (*Stolephorus sp.)* WITH THE ADDITION OF PAPAIN ENZYME

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ABSTRACT

Anchovy *(Stolephorus sp.)* is a protein-rich fish with considerable potential for the production of fermented products such as fish sauce. This study aimed to analyze the amino acid profile and organoleptic characteristics of anchovy fish sauce with the addition of papain enzyme. The experiment was conducted from July to September 2025 using two treatments consisting of fish sauce without papain enzyme (control) and fish sauce supplemented with 1% papain enzyme. Amino acid composition was determined using high-performance liquid chromatography (HPLC), while sensory properties, including taste, aroma, texture, and color, were evaluated using a hedonic test. Statistical analysis was performed using the Mann–Whitney U test. The results showed that the control treatment exhibited a higher total amino acid content (19.49% w/w) than the papain-treated sample (11.54% w/w). Glutamic acid was the dominant amino acid in both treatments, followed by aspartic acid and lysine. However, papain enzyme supplementation did not significantly affect amino acid composition ($p = 0.110$). Similarly, no significant differences were observed in taste, aroma, texture, or color between treatments ($p > 0.05$). These findings indicate that the addition of 1% papain enzyme did not improve the nutritional or sensory quality of anchovy fish sauce. Therefore, natural fermentation remained more effective under the experimental conditions employed in this study.

INTRODUCTION

Indonesia is recognized as one of the world's largest maritime countries, possessing extensive coastal resources and abundant fishery commodities. Among these resources, anchovy (*Stolephorus* spp.) represents one of the most economically important small pelagic fish species because of its high nutritional value and widespread availability. According to the Ministry of Marine Affairs and Fisheries of Indonesia (2022), anchovies contribute significantly to the national capture fisheries sector and serve as an important source of protein for coastal communities. In addition to their relatively low market price and year-round availability, anchovies are characterized by high protein content, making them suitable raw materials for value-added fishery products. However, due to their high moisture content and enzymatic activity, anchovies are highly perishable and require immediate processing to maintain their quality and extend shelf life (Wahyuningsih et al., 2019).

Fish processing technologies have been continuously developed to improve the economic value and utilization efficiency of fishery resources. One of the traditional fish-based products widely consumed in Asian countries is fish sauce. Fish sauce is a fermented liquid condiment characterized by its distinctive aroma and umami taste, resulting from the hydrolysis of fish proteins into peptides and free amino acids (Mouritsen et al., 2019). In countries such as Vietnam, Thailand, and the Philippines, fish sauce has become an integral component of culinary culture and represents an important fermented food industry (Park et al., 2019).

Nevertheless, compared with soy sauce, fish sauce production and consumption in Indonesia remain relatively limited despite the country's vast fishery potential and diverse marine resources (Moniharapon et al., 2024).

The sensory characteristics and nutritional quality of fish sauce are strongly influenced by the biochemical transformations that occur during fermentation. As reported by Li et al. (2021), the hydrolysis of proteins during fermentation produces free amino acids, especially glutamic acid and aspartic acid, which are responsible for the characteristic umami flavor of fermented products. In addition to their contribution to taste, free amino acids also determine the nutritional quality and biological value of fish sauce. Therefore, amino acid composition is considered one of the most important indicators for evaluating the quality of fermented fish products (Zhao et al., 2020).

Conventional fish sauce production relies on natural fermentation processes that generally require six to twelve months to achieve the desired quality characteristics. Although traditional fermentation produces unique sensory attributes, the prolonged processing time represents a major limitation for industrial-scale production. Extended fermentation periods increase storage requirements, production costs, and the risk of microbial contamination, thereby reducing production efficiency (Tran et al., 2021). Consequently, efforts to accelerate protein hydrolysis without compromising product quality have become an important area of research in food biotechnology.

One promising approach to shortening fermentation time involves enzymatic hydrolysis using proteolytic enzymes. According to Bhaskar et al. (2018), enzymatic hydrolysis offers several advantages over traditional fermentation, including faster processing, better control of hydrolysis conditions, and higher yields of bioactive compounds. Proteolytic enzymes facilitate the cleavage of peptide bonds, resulting in the release of peptides and free amino acids that contribute to nutritional and sensory properties. Among various proteases, papain has attracted considerable attention because of its broad substrate specificity, thermal stability, and effectiveness under neutral and slightly alkaline conditions (Noman et al., 2020).

Papain is a cysteine protease derived from the latex of immature papaya (*Carica papaya*) fruits and has been widely utilized in food processing applications. According to Arshad et al. (2021), papain effectively hydrolyzes muscle proteins, thereby increasing protein digestibility and improving the release of essential amino acids. In addition, papain-assisted hydrolysis has been shown to enhance flavor development and generate bioactive peptides with antioxidant and antimicrobial properties. Similar findings were reported by Chalamaiah et al. (2019), who demonstrated that enzymatic hydrolysis significantly improves the functional characteristics and nutritional quality of protein-rich foods.

Anchovies possess considerable potential for fish sauce production because of their high protein content and small body size, which facilitate enzyme penetration and accelerate hydrolysis reactions. Kim et al. (2020) reported that anchovy proteins contain numerous peptides and amino acids that can be transformed into valuable bioactive compounds during enzymatic hydrolysis. Furthermore, hydrolyzed anchovy products exhibit antioxidant, antihypertensive, and antimicrobial activities, thereby increasing their functional value beyond their role as flavor enhancers. According to Liu et al. (2022), the production of protein hydrolysates from marine organisms represents a promising strategy for developing nutritionally enhanced and health-promoting food products.

The amino acid profile of fish sauce is closely related to both nutritional quality and sensory acceptance. Essential amino acids such as lysine, leucine, valine, and threonine are important for human growth and metabolism, whereas non-essential amino acids, particularly glutamic acid and aspartic acid, contribute significantly to umami flavor and consumer preference (Zhao et al., 2020). Furthermore, free amino acid composition is widely used as an indicator of fermentation efficiency and protein degradation. As emphasized by FAO and WHO (2020), amino acid composition is an essential criterion for evaluating the quality of dietary protein and determining its contribution to human nutrition.

Besides nutritional characteristics, sensory quality is another critical parameter determining the acceptance and marketability of fish sauce products. Color, aroma, taste, and overall acceptability are influenced by amino acid composition and the formation of volatile compounds during fermentation. Moniharapon et al. (2024) reported that sensory evaluation using hedonic scales remains one of the most effective methods for assessing consumer acceptance of fermented fish products. The Indonesian National Standard (SNI 01-2346-2006) also provides guidelines for sensory evaluation to ensure that fermented products meet acceptable quality standards.

Despite numerous studies investigating fish protein hydrolysis, comprehensive information regarding the amino acid profile of anchovy fish sauce produced with papain enzyme supplementation remains limited, particularly in Indonesia. Most previous studies have focused on traditional fermentation processes or general physicochemical characteristics, while detailed evaluations of amino acid composition following enzymatic hydrolysis are still scarce. Given the importance of amino acids in determining nutritional value and sensory properties, a thorough understanding of amino acid profiles is necessary for optimizing fish sauce production and enhancing product quality.

Therefore, this study is important because it provides scientific information regarding the effect of papain enzyme addition on the amino acid profile of anchovy (*Stolephorus* sp.) fish sauce. The findings are expected to contribute to the development of more efficient fish sauce processing technologies, improve the nutritional quality of fermented fish products, and support the diversification and value addition of Indonesia's fishery resources.

METHOD

This study was conducted from July to September 2025. The preparation of anchovy fish sauce samples was carried out at the Integrated Laboratory of the Institute of Technology and Maritime Business Balik Diwa, Makassar, while amino acid profile analysis was performed at the Integrated Laboratory of IPB University. Fresh anchovies (*Stolephorus* sp.) were used as the primary raw material, whereas commercial papain enzyme and salt were employed to facilitate protein hydrolysis and preservation. Additional ingredients, including lemongrass, bay leaves, coriander, garlic, galangal, turmeric, and palm sugar, were incorporated to enhance flavor characteristics. The fish sauce preparation procedure was adapted from Briani et al. (2014) with modifications. Briefly, anchovies were washed, weighed, and mixed with papain enzyme solutions prepared in phosphate buffer according to the treatment levels. The mixtures were subjected to enzymatic hydrolysis and fermentation at room temperature for 30 days. Subsequently, enzyme activity was terminated by heating the mixtures at 90°C for 60 min, followed by filtration to separate the liquid hydrolysate from solid residues. The resulting filtrate was collected as hydrolyzed fish sauce. Enzymatic hydrolysis has been widely employed to accelerate protein degradation and enhance the release of free amino acids in fish-based products (Arshad et al., 2021; Noman et al., 2020), thereby improving both nutritional and sensory characteristics (Li et al., 2021).

An experimental laboratory approach was employed using two treatment groups consisting of A = 0% papain enzyme (control) and B = 1% papain enzyme based on fish weight, with each treatment conducted once. The resulting fish sauce samples were evaluated for free amino acid composition and sensory characteristics, including color, aroma, taste, and texture. Amino acid analysis was performed using high-performance liquid chromatography (HPLC) following derivatization procedures and standard analytical protocols described by AOAC (2006). Samples were filtered and transferred into HPLC vials before analysis. Sensory evaluation was conducted using hedonic methods according to the Indonesian National Standard (SNI 01-2346-2006). Data obtained from amino acid and sensory analyses were statistically analyzed using the Mann-Whitney U test. This non-parametric method was selected because the data did not satisfy the normality assumption required for parametric

tests and is considered appropriate for comparing differences between two independent groups (Sriwidadi, 2011). Similar statistical approaches have been recommended for food quality studies involving small sample sizes and non-normally distributed data (Kim, 2017; Mishra et al., 2019).

RESULT AND DISCUSSION

Amino Acid Profile of Anchovy Fish Sauce (*Stolephorus* sp.)

The amino acid composition of anchovy fish sauce with and without papain enzyme supplementation is presented in Table 1. The analysis identified eighteen amino acids consisting of essential and non-essential amino acids. These amino acids are important indicators of nutritional quality and contribute to the characteristic flavor of fermented fish products.

Table 1.
Amino acid composition of anchovy fish sauce (*Stolephorus* sp.) with and without papain enzyme supplementation

Amino Acid	Treatment A (0% Papain)	Treatment B (1% Papain)	Unit
Aspartic acid	2.96	1.22	% w/w
Threonine	0.71	0.51	% w/w
Serine	0.68	0.48	% w/w
Glutamic acid	4.06	2.18	% w/w
Proline	0.67	0.50	% w/w
Glycine	1.38	0.76	% w/w
Alanine	1.65	0.95	% w/w
Cystine	0.09	0.07	% w/w
Valine	0.95	0.66	% w/w
Methionine	0.41	0.25	% w/w
Isoleucine	0.66	0.48	% w/w
Leucine	1.05	0.71	% w/w
Tyrosine	0.33	0.10	% w/w
Phenylalanine	0.55	0.39	% w/w
Histidine	0.60	0.44	% w/w
Lysine	1.80	1.15	% w/w
Arginine	0.78	0.59	% w/w
Tryptophan	0.16	0.10	% w/w
Total Amino Acids	**19.49**	**11.54**	**% w/w**

Source: data processing results, 2025

The results demonstrated that the control treatment (A, without papain enzyme) exhibited a higher total amino acid content (19.49% w/w) than treatment B containing 1% papain enzyme (11.54% w/w). Mann–Whitney U analysis indicated that papain enzyme addition did not significantly affect amino acid content ($p = 0.110 > 0.05$). Interestingly, all amino acids detected in the control treatment were present at higher concentrations than those observed in the papain-treated sample. This finding suggests that the addition of papain under the present experimental conditions did not enhance amino acid accumulation and may have promoted further degradation reactions.

Among the amino acids identified, glutamic acid represented the dominant component, accounting for 4.06% w/w in treatment A and 2.18% w/w in treatment B. Aspartic acid and lysine were also present in relatively high concentrations. Glutamic acid and aspartic acid are recognized as the primary contributors to the umami taste characteristic of fermented fish products, whereas lysine represents an important essential amino acid associated with protein

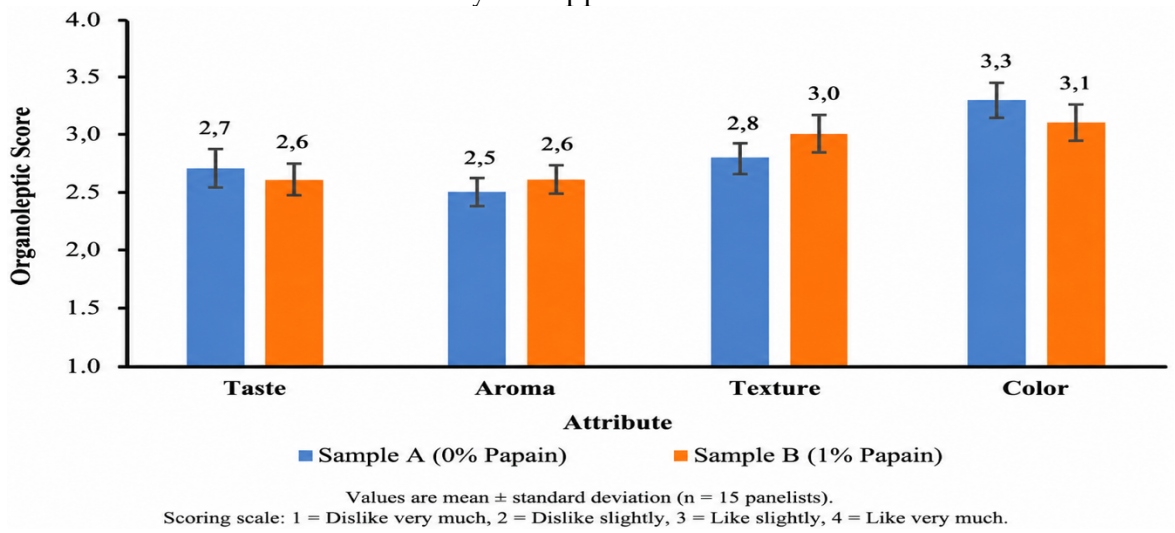
quality (Li et al., 2021). According to Zhao et al. (2020), the amino acid profile of fermented foods reflects both the nutritional value and the extent of protein hydrolysis during processing. Therefore, the relatively high glutamate concentration observed in the control treatment suggests that natural fermentation was sufficient to produce desirable flavor compounds without the need for enzymatic supplementation.

Essential amino acids, including threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine, and tryptophan, were consistently higher in the control sample than in the papain-treated sample. Similar trends were observed for non-essential amino acids such as aspartic acid, serine, glutamate, proline, glycine, alanine, cystine, tyrosine, and arginine. The lower concentrations detected in treatment B may be attributed to excessive hydrolysis and secondary reactions occurring during fermentation, resulting in the conversion of amino acids into peptides or other metabolites. Korkmaz and Tokur (2022) reported that the amino acid profile of fish protein hydrolysates is strongly influenced by enzyme type, salinity, pH, and hydrolysis conditions. Likewise, Noman et al. (2020) emphasized that prolonged enzymatic hydrolysis may lead to degradation or transformation of free amino acids, thereby reducing their measurable concentrations. Consequently, the present findings indicate that the application of 1% papain enzyme was not effective in increasing amino acid availability in anchovy fish sauce.

Organoleptic Properties of Anchovy Fish Sauce (*Stolephorus* sp.)

Sensory evaluation is an important aspect in determining consumer acceptance of fermented products. The organoleptic characteristics of anchovy fish sauce produced with and without papain enzyme supplementation are presented in Figure 1.

Figure 1.
Organoleptic characteristics of anchovy fish sauce (*Stolephorus* sp.) with and without papain enzyme supplementation



Source: data processing results, 2025

The sensory evaluation revealed that both treatments were generally categorized as moderately acceptable. Treatment A achieved slightly higher scores for taste (2.7), color (3.3), and overall preference, whereas treatment B obtained marginally higher scores for aroma (2.6) and texture (3.0). However, Mann–Whitney U analysis showed that papain enzyme supplementation had no significant effect on taste ($p = 0.568$), aroma ($p = 0.856$), texture ($p = 0.477$), or color ($p = 0.348$). These results indicate that the addition of papain enzyme at the concentration used in this study did not produce perceptible changes in the sensory quality of anchovy fish sauce.

The similarity in sensory scores between treatments suggests that the hydrolysis process

induced by papain did not substantially alter the formation of flavor-active compounds. Although proteolytic enzymes can accelerate the release of amino acids and volatile compounds, the resulting changes may not necessarily be reflected in sensory perception. Nakano et al. (2017) reported that sensory quality is not always directly proportional to free amino acid concentration because taste perception depends on the interactions among multiple compounds. Similarly, Yimdee and Wang (2016) demonstrated that volatile compounds may exert masking effects, thereby reducing differences in aroma intensity perceived by panelists.

Texture scores tended to increase slightly in the papain-treated sample, suggesting that protein hydrolysis improved product homogeneity and fluidity. However, excessive protein degradation may also reduce the structural integrity of the product, resulting in only minor improvements in texture. In terms of color, treatment A showed slightly higher preference scores than treatment B. This phenomenon may be associated with differences in Maillard reactions and pigment formation during fermentation. Ritthiruangdej and Suwonsichon (2006) reported that protein degradation and oxidation reactions influence melanoidin formation and consequently determine the color characteristics of fermented products.

Overall, the results of the present study indicate that papain enzyme supplementation at a concentration of 1% did not significantly improve either the amino acid composition or the organoleptic properties of anchovy fish sauce. The control treatment exhibited higher total amino acid content and comparable sensory acceptability, suggesting that natural fermentation remained more favorable under the experimental conditions employed in this study.

CONCLUSION

This study demonstrated that the addition of papain enzyme at a concentration of 1% did not significantly affect the amino acid composition or organoleptic properties of anchovy fish sauce (*Stolephorus* sp.). The control treatment without papain enzyme exhibited a higher total amino acid content (19.49% w/w) than the papain-treated sample (11.54% w/w), although the difference was not statistically significant based on the Mann–Whitney U test ($p > 0.05$). Glutamic acid was the predominant amino acid in both treatments, followed by aspartic acid and lysine, indicating that anchovy fish sauce possesses considerable nutritional value and potential as a natural source of umami compounds. Sensory evaluation showed that both treatments were moderately acceptable to panelists, and papain supplementation did not significantly influence taste, aroma, texture, or color. Overall, natural fermentation without papain enzyme produced fish sauce with superior amino acid composition and comparable sensory characteristics under the conditions applied in this study.

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