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RESEARCH ARTICLE
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# Prediction of Active Proteolytic Enzymes in Tauco Fermentation based on Next Generation Sequencing

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## ABSTRACT

Tauco is a soybean that has undergone mould and salt fermentation and is commonly used as a flavor enhancer in Indonesia. During fermentation, enzymes from microorganisms catalyze the hydrolysis of proteins into peptides and amino acids, including those that contribute to umami flavors. The objective of this study was to ascertain the abundance of enzymes and predict their activity in tauco using the Metagenome Analyzer. The enzymes were predicted from the DNA sequences of microorganisms from tauco obtained from three regions in Indonesia: Cianjur, Pekalongan, and Singkawang. The most prevalent enzymes in all tauco were transferases, while hydrolases, which catalyze the hydrolysis of ester and peptide bonds, were the second most prevalent. The peptidases in tauco are predominantly endopeptidases, with significantly more assignments (3828 reads) than exopeptidases (3254 reads). A comparative analysis reveals that the number of peptidases assigned to tauco Singkawang (3257) is not significantly different from that of tauco Pekalongan (3177). However, the number of peptidases in tauco Cianjur is considerably lower (1520). This is also evidenced by the elevated level of peptides (14331.92mg/mL) in tauco Cianjur, as compared to the lower levels observed in the other two tauco. Both serine and metallo-carboxypeptidases in tauco are predicted to play a role in flavor production, as they have been shown to enhance umami and eliminate bitterness. The total activity of aminopeptidase, serine, and metallo-carboxypeptidase activity in tauco accounts for 2252 assignments (1.7%) out of the 135996 reads of the total enzyme activity.

**Keywords:** Fermentation, Enzyme prediction, Peptidases, Tauco, Umami.

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## INTRODUCTION

Food fermentation is a process that relies on microbial activity to produce a product distinct from its raw material. The process has been shown to enhance the sensory characteristics of products, yield vitamins, and prolong shelf life (Mannaa et al., 2021). This process is catalyzed by enzymes produced by the microorganisms involved in fermentation. During fermentation, microorganisms secrete enzymes such as amylases, proteases and/or lipases, which catalyze the hydrolysis of carbohydrates, proteins, and/or lipids, respectively, into smaller compounds (Robinson, 2015).

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Tauco is a traditional Indonesian fermented soybean commonly used as a seasoning. It is produced through a two-step fermentation involving mould and salt. Fermented soybean products similar to tauco are also found globally, such as miso, natto, doenjang, dajiang, and more (Elhalis et al., 2023). Soybean (*Glycine max*) contains an average of 36% protein, 35% carbohydrates, 17% fiber, 19% lipids, as well as minerals and other nutrients (Hassan, 2013). Soybeans are a food crop susceptible to pests and diseases (Khan et al., 2024); therefore, fermenting soybeans, such as to make tauco, is one way to preserve them. During tauco fermentation, the macromolecules are converted into their smaller compounds, especially those associated with its use as a flavor enhancer. Certain bacteria, such as *Bacillus*, and fungi, including Aspergillus, have the capacity to produce proteases, enzymes that hydrolyze proteins into peptides (Rawlings & Barrett, 2014). In addition, lactic acid bacteria can convert carbohydrates into lactic acid (Mannaa et al., 2021). Tauco fermentation involves a series of chemical reactions, one of which is the breakdown of protein-rich soybeans. Proteins are broken down into peptides and amino acids. This process is attributable to the action of proteolytic enzymes produced by either fungi, yeast, or bacteria, whether introduced through addition to the substrate or produced naturally during the process (Fernandes, 2010; Tamang et al., 2016).

Our previous studies indicated that Indonesian tauco produced in three distinct regions (Cianjur, Pekalongan, and Singkawang) exhibits notable diversity in its microbial composition (Seveline et al., 2025b). The diversity was thought to result in the distinct characteristics among the three types of tauco (Seveline et al., 2025a). Additionally, differences in microbial diversity among the three types of tauco were hypothesized to result in distinct enzyme profiles during tauco fermentation. The three types of tauco were processed differently and had different sensory characteristics (Seveline et al., 2025a).

Analysis of the enzymes involved in fermentation can be conducted by isolating the microorganisms that play a role in the process (Sharma et al., 2020). However, analyzing the enzyme's activity using this method is time-consuming. An alternative approach for exploring the enzymes during the fermentation process involves functional annotation of shotgun metagenomic data using automated bioinformatics tools such as MG-RAST, Prokka, Humann or MEGAN (Alderson et al., 2012; Nam et al., 2023). Enzyme annotation software and databases have been used to identify enzymes in the specimen. METAgenome ANalyzer (MEGAN) is a notable example of a tool that combines annotation and a database. One of MEGAN's modules, Enzyme Commission (EC) Viewer, integrates Enzyme Commission (EC) numbers into the enzyme database. This allows it to be manipulated to identify the dominant enzymes in a sample and estimate their roles. The MEGAN computational approach is used to analyze microbial sequences. This approach involves two fundamental steps. First, the sequences are aligned against protein databases provided by the National Center for Biotechnology Information (NCBI). Subsequently, the aligned sequences are classified and analyzed using functional annotation approaches. The objective of this study is to predict the abundance of enzymes and their peptidase types during tauco fermentation based on microbiome sequencing annotations of Indonesian tauco from three regions: Cianjur, Pekalongan, and Singkawang.

## MATERIALS & METHODS

### Materials

Materials for this research include samples of three types of Indonesian tauco obtained from Cianjur, Pekalongan, and Singkawang, and three sets of DNA sequencing data of the microorganisms extracted from tauco from the three regions. Tauco samples were analyzed for their peptide content. Raw data obtained from Next Generation Sequencing (NGS) machine results were processed for bioinformatic data processing after cleaning the short fragment data from adapters, processing to obtain the sequence base order, assembling the sequences into contigs (complete sequences), and finally obtaining data in the form of Read Mapping Annotation (RMA), which was then analyzed using Metagenome Analyzer (MEGAN).

### Peptide Analysis of Tauco Samples

Peptide analysis was performed according to Church et al. (1985). Preparation for peptide analysis was carried out by weighing 50 grams of tauco samples, grinding it with a mortar, mixing it with 400mL of distilled water, cooking it on a hotplate at 100 $^{o}$C for 10 minutes, cooling it for 30min until it reached room temperature, and then filtering it with a filter cloth. The filtered solution was then centrifuged at 6000rpm for 30 minutes and re-filtered through a 0.45µm cellulose acetate filter. o-phthalaldehyde (OPA) reagent (Sigma Aldrich, US) was prepared by dissolving 40mg of OPA in 1mL of methanol, then adding 25mL of 100mM sodium tetraborate (Merck, Germany), and 2.5mL sodium dodecyl sulphate (w/w) (Sigma Aldrich, US) was added to 50µL of the filtered sample solution. Next, 100mL of mercaptoethanol was added, mixed quickly, and incubated for 2min. The absorbance was then measured at 340nm (UV-Vis 1240, Shimadzu, Japan). Peptide concentrations were measured using a standard curve prepared with tryptone casein (Sigma Aldrich, US). The measured peptide concentrations were analysed using SPSS (ver. 23.0, SPSS Inc., USA) with a one-way ANOVA at the 5% significance level, and any significant differences were further tested using Duncan's multiple range test.

### Enzyme Prediction

The sequencing results, in the form of fastq datasets (raw data) from DNA extracts of tauco samples from Cianjur, Pekalongan, and Singkawang, were initially checked for base sequences (reads) using fastp 0.23.2 to remove excess content. The data was then entered into Knead Data version 0.10.0 to trim low-quality reads and remove adapter sequences. The filtered reads were assembled into contigs derived from the microorganism's genome. The contigs were then processed using the

Metaspades genome assembler version 3.15.5 to generate the assembled base sequence. After obtaining the contigs, Quast version 5.2.0 was applied (Gurevich et al., 2013). The process resulted in genome fractions of 73.66% (48486516bp) for Cianjur tauco, 51.78% (24972821bp) for Pekalongan tauco, and 69.89% (37627821bp) for Singkawang tauco.

Following assembly and quality assessment, taxonomic and functional annotations were performed using Diamond version 2.0.15.153, which was blasted against NCBI and then continued in Megan version 6.24.1 (Bağcı et al., 2021). Data analysis was performed by examining the number of enzymes assigned in the EC viewer in Megan for each sample. The results of the data analysis were then classified by enzyme class and further divided into specific enzyme types. The results from the EC Viewer were also confirmed using data from IUBMB (https://iubmb.qmul.ac.uk/enzyme) and EMBL-EBI (https://www.ebi.ac.uk/) and were further confirmed in KEGG (https://www.genome.jp/), which tabulated them. The annotation focuses on the hydrolase enzyme group, particularly peptidases associated with the umami taste of peptides.

## RESULTS & DISCUSSION

### Peptide Content of Tauco

The results show significant differences in the peptide content of tauco from Cianjur, Pekalongan and Singkawang. Cianjur tauco has the highest peptide content, which is significantly different ($p<0.05$) from those of Singkawang tauco and Pekalongan tauco (Table 1). Increase in peptide levels during fermentation is consistent with the research of peptides concentration in fermented soy milk, which showed an increase in peptide concentration in fermented soy milk (Fadlillah et al., 2023). Research on tempeh fermentation from various types of legumes also indicated an increase in peptide concentration with longer fermentation times (Indrati et al., 2021). Tauco from Pekalongan and Singkawang are suspected to undergo less protein hydrolysis due to their significantly shorter fermentation time, which are 30 days and 25 days, respectively, as compared to 90-100 days for Cianjur tauco (Seveline et al., 2025a).

**Table 1:** Concentration of tauco peptides from Cianjur, Pekalongan and Singkawang

| Tauco sample | Peptide concentration (mg/mL) |
|---|---|
| Cianjur | 14331.92±3408.95[a] |
| Pekalongan | 3906.95±176.97[b] |
| Singkawang | 5228.51±1539.42[b] |

Different letters with the same column mean significant ($P<0.05$) differences according to Duncan's multiple range test.

### Enzymes in Tauco

Analysis with Diamond and Megan show that the number of enzyme reads is 135996 for Cianjur tauco, 187240 for Pekalongan tauco, and 204519 for Singkawang tauco. Of the total reads, 26891 (24.65%) can be assigned to enzymes for tauco from Cianjur, 51366 (37.80%) for tauco from Pekalongan, and 59302 (40.84%) for tauco from Singkawang. These results indicate that the amounts of enzymes, from highest to lowest, are for tauco from Singkawang, Pekalongan, and Cianjur, respectively.

Food fermentation is a process associated with the activity of enzymes produced by microorganisms (Sharma et al., 2020). Fig. 1 shows the distribution of enzymes in tauco samples from Cianjur, Pekalongan, and Singkawang. The prediction indicates that the enzyme groups found in the three tauco samples are similar, with comparable percentages encompassing seven enzyme groups: transferase, hydrolase, oxidoreductase, ligase, lyase, isomerase, and translocase. Among the seven enzyme groups, transferases were the most abundant, while translocases were the least abundant. Transferase enzymes play a crucial role in transferring functional groups, including catalyzing the formation of new glycosidic bonds. This activity is particularly important in starch-converting enzymes, which modify carbohydrate structures during starch hydrolysis and restructuring processes (Franceus & Desmet, 2020; Miao & Bemiller, 2023). The second most abundant enzyme group is hydrolases, which are assumed to play a role in hydrolysis reactions involving water (Fernandes, 2010; Robinson, 2015).

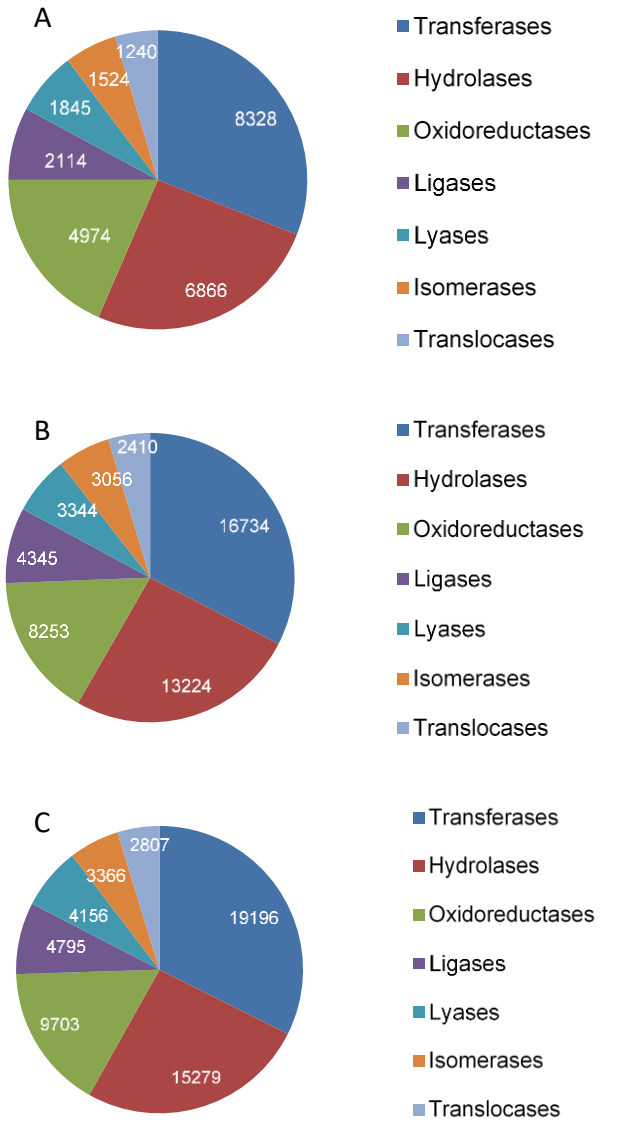


**Fig. 1:** Distribution of enzyme groups in Indonesian tauco samples from a) Cianjur (135996 reads), b) Pekalongan (187240 reads), and c) Singkawang (204519 reads).

**Hydrolase Enzymes**

During fermentation, macromolecules are broken down by many enzymes, including hydrolases. This enzyme group works by breaking the chemical bonds of substrates using water molecules (Okpara, 2022). Based on the total enzyme count, the hydrolase enzymes in Singkawang tauco (59302 reads, 28.99%) and Pekalongan tauco (51366 reads, 27.43%) have similar proportions, whereas tauco from Cianjur has the lowest proportion (26891 reads, 19.77%). Since the hydrolase enzymes in Singkawang tauco and Pekalongan tauco are significantly higher, hydrolysis of the substrate into simpler compounds is expected to occur more frequently as compared to that in tauco from Cianjur. The hydrolases may include amylases, lipases, and proteases, which are produced by *Aspergillus oryzae* in meju-doenjang and koji-miso (Kusumoto et al., 2021; Liu et al., 2024). Tauco fermentation involves mould fermentation, similar to meju fermentation in doenjang or koji fermentation in miso (Jung et al., 2014; Kusumoto et al., 2021) and is not much different from soy sauce fermentation (Setiani et al., 2024).

The types of hydrolase enzymes found in tauco from the three regions in Indonesia are presented in Fig. 2. The three most abundant hydrolase enzymes in all tauco samples are those acting on ester bonds, peptide bonds, and glycosylase bonds, with a total number of assigned reads of 11466, 7972 and 5892, respectively. Lipase enzymes break down ester bonds of triglycerides to produce glycerol and simple fatty acids, which play a significant role in the formation of aroma and texture in food (Day & Morawicki, 2018). The second most abundant enzymes are those that break down peptides or peptidases, which can be expected in fermented soybean products (Zhang et al., 2018; Xie et al., 2020) Due to the high protein content of soybeans. The role of peptidase is expected to be important, particularly the hydrolysis products that contribute to umami flavor, reduced bitterness and many other health functional properties such as bioactive peptides (Fernandes 2010; Ito & Matsuyama 2021; Kim et al. 2021). Glycosylases are enzymes that break glycosidic bonds between sugar residues, playing roles in DNA repair and in the degradation of polysaccharides, such as cellulase and hemicellulase. One of the functions of glycosylase is to hydrolyze carbohydrates in soybeans, which contain approximately 30–35% carbohydrates (Hassan, 2013; Elhalis et al., 2023).

**Peptidase Enzyme**

Peptidases are enzymes that act on peptide bonds and break down proteins into peptides and amino acids (Rawlings & Barrett, 2014). The annotation results show higher peptidase levels in tauco from Singkawang and Pekalongan than in Cianjur. These results align with the results of peptide analysis of tauco (Table 1). Singkawang tauco has the lowest peptide content (5228.51mg.mL$^{-1}$). Differences in peptidase activity may be due to the differences in the types of microorganisms present in tauco. Our previous study showed that the predominant microorganisms differed among the three types of tauco, with Cianjur tauco dominated by mould, while Singkawang and Pekalongan tauco were dominated by yeast and bacteria (Seveline et al., 2025b). A study on proteolysis in fermented soybean curd showed that the longer the fermentation period (90 days), the higher the number and diversity of peptides (Wei et al., 2021). Cianjur tauco fermentation is conducted for a longer period of 90–100 days, while Singkawang and Pekalongan tauco fermentations are 25 days and 30 days, respectively.

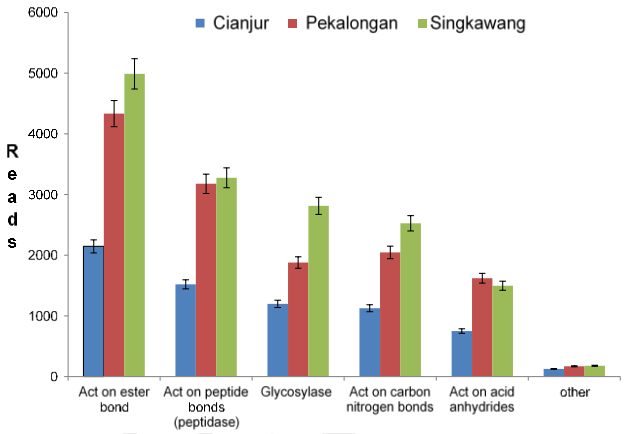


**Fig. 2:** Types of hydrolase enzymes in Indonesian tauco samples from three regions: Cianjur, Pekalongan, and Singkawang.

The results of this study indicate that the peptidase enzymes in the tauco collected from three regions in Indonesia consisted of endopeptidases (3828 reads) and exopeptidases (3254 reads) as depicted in Fig. 3. Endopeptidases typically act first to degrade proteins in food from within to produce peptides (Song et al., 2023). The resulting peptides from endopeptidase activity then serve as substrates for exopeptidases, which convert them into simpler forms, namely amino acids.

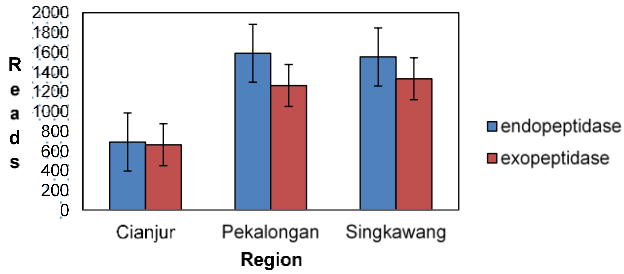


**Fig. 3:** Types of peptidase enzymes in tauco from Cianjur, Pekalongan, and Singkawang, Indonesia.

Our previous study showed that moulds *Lichtheimia* and *Aspergillus* are predominant in Cianjur tauco, while yeast *Pichia kudriavzevii* and lactic acid bacteria were predominant in Pekalongan and Singkawang tauco (Seveline et al., 2025b). *Aspergillus* has been reported to have high protease and peptidase activity, enabling it to break down proteins into peptides. *Aspergillus* is suspected to have a variety of peptidase types (Matsushita-Morita et al., 2017; Ito & Matsuyama, 2021; Zhang et al., 2023; Setiani et al., 2024). In addition, *Lichtheimia* exhibits proteolytic activity, degrading proteins into simpler compounds. Studies on both moulds have demonstrated their high protease activity (Kim et al., 2024). *Pichia* has

been reported to have the ability to produce hydrolase enzymes in the form of peptidases (Elkhairy et al., 2023), allowing the yeast to degrade organic acids and enhance flavor components (Chu et al., 2023). Chaves-López et al. (2012) also reported that *Pichia kudriavzevii* has relatively high peptidase activity. Bacteria from the genus of *Bacillus* were found in all tauco samples. *Bacillus* sp. is a bacterium that also produces relatively high levels of endopeptidase (Contesini et al., 2018; Nguyen et al., 2019; Yin et al., 2019).

In this study, seven exopeptidases and five endopeptidases were identified and are presumed to be involved in tauco fermentation. The seven exopeptidases found in tauco include aminopeptidase, serine carboxypeptidase, and metallo-carboxypeptidase, omega peptidase, dipeptidase, dipeptidyl peptidase, tripeptidyl peptidase, and peptidyl peptidase (Fig. 4). In soy sauce and doenjang, six of the above seven exopeptidases were also reported, namely aminopeptidase, carboxypeptidase (both serine and metallo-carboxypeptidase), omega peptidase, dipeptidase, and dipeptidyl-tripeptidyl peptidase. These six peptidases were reported to be responsible for the formation of simple protein compounds and flavor formation in soy sauce (Zhao et al., 2018; He et al., 2024) and meju-doenjang (Kim et al., 2024), and thus may have similar functions in tauco. Aminopeptidases, metallo-carboxypeptidase and serine carboxypeptidases, tripeptidyl peptidase are important for flavor production and will be discussed below.

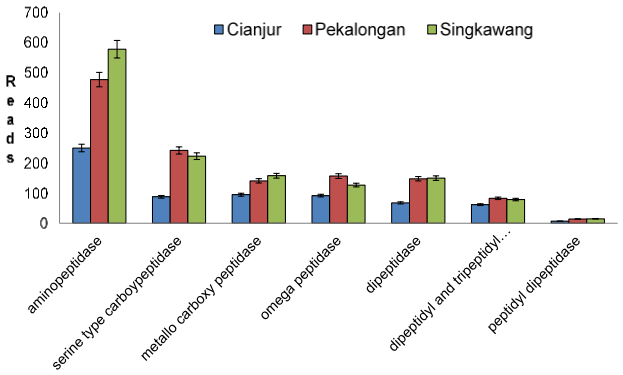


**Fig. 4:** Types of exopeptidases in tauco obtained from Cianjur, Pekalongan and Singkawang – Indonesia.

This study also showed that the two most abundant endopeptidases in all three types of tauco were serine and metallo-endopeptidases (Fig. 5). Microorganisms produce various endopeptidases, which are associated with their metabolic capabilities. In a study of fermented fish (chouguiyu), endopeptidases were more abundant than exopeptidases, with serine- and metallo-endopeptidases identified as the dominant types (Yang et al., 2022). Serine endopeptidases are commonly produced by genera such as *Acinetobacter*, *Enterococcus*, *Streptococcus*, *Lactococcus*, and *Carnobacterium*, while metallo-endopeptidases are produced by *Acinetobacter*, *Enterococcus*, *Streptococcus*, *Clostridium*, *Lactococcus*, *Fusobacterium*, and *Lysobacter* (Yang et al., 2022). Similarly, several predominant microorganisms have been identified in tauco, including moulds of Aspergillus and Lichtheimia, yeast Pichia, and lactic acid bacteria and *Bacillus* (Seveline et al., 2025b). The presence of these microbial groups suggests the potential for both serine- and metallo-endopeptidases in tauco fermentation, which may contribute to the release of amino acids associated with flavor development.

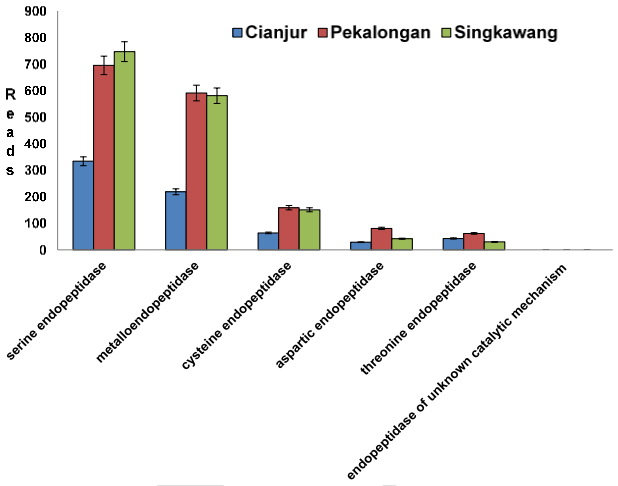


**Fig. 5:** Types of endopeptidases in tauco obtained from Cianjur, Pekalongan and Singkawang – Indonesia.

## Peptidases Involved in Umami Flavor Production

Endopeptidases such as serine peptidases catalyze the cleavage of internal polypeptide bonds by employing a catalytic triad, in which a serine residue acts as the nucleophile, assisted by histidine and aspartate. In contrast, metallo-peptidases require a divalent metal ion (e.g., $Zn^{2+}$) to activate a water molecule that serves as the nucleophile for protein hydrolysis into peptides (Rawlings & Bateman, 2019; Song et al., 2023). This supports the hypothesis that these enzymes play a crucial catalytic role in the release of amino acids that contribute to umami flavor. Other endopeptidases found in lower quantities in tauco include cysteine, aspartic, and threonine endopeptidases. Tauco from Pekalongan and Singkawang have similar read counts for endopeptidases, which are higher than that of tauco from Cianjur.

Glutamate and aspartate are the amino acids that form umami peptides produced by the action of peptidases in tauco (Herlina et al., 2024). Another study also found that enzymes for umami flavor production reported in soy sauce and doenjang such as aminopeptidase, serine carboxypeptidase, metallo-carboxypeptidase, dipeptidase, dipeptidyl or tripeptidyl peptidase and omega peptidase (Zhao et al., 2018; Jo et al., 2021; He et al., 2024; Kim et al., 2024) were also present in tauco. Aminopeptidase, serine and metallo-carboxypeptidase are the three most abundant enzymes found in tauco (Fig. 4). Aminopeptidase catalyzes the release of amino acid residues, often with a preference for hydrophobic amino acids, from the N-terminal of peptides, contributing to the accumulation of free amino acids associated with umami flavor in the final product (Nandan & Nampoothiri, 2020; Song et al., 2020). Previous research on soybean water extract has shown that aminopeptidase is optimally produced at neutral or alkaline pH. This aminopeptidase is capable of releasing glutamate and

aspartate residues from peptides (Chen et al., 2021).

Glutamate can be produced by the activity of glutamate carboxypeptidase enzyme, which belongs to the metallo-carboxypeptidase group. Metallo-carboxypeptidase is the third largest enzyme of the exopeptidase group, with a low number of reads in all three types of tauco (Fig. 4). Pekalongan tauco has the most assigned reads (30) for the glutamate carboxypeptidase belonging to the metallo-carboxypeptidases followed by tauco from Cianjur (20 reads) and Singkawang (11 reads).

In addition to these three enzymes, there is an enzyme that acts on carbon-nitrogen bonds: glutaminase. Glutaminase is an enzyme belonging to the linear amide enzyme group, which converts glutamine into glutamate and ammonium. Some foods, such as Dajiang and soy sauce, have an umami taste derived during fermentation (Cao et al., 2023; Ito & Matsuyama, 2021). Glutaminase is primarily produced by microorganisms, such as the mould *Aspergillus* (Ito et al., 2013; Liu et al., 2024). The genome of *Aspergillus sojae* used in soy sauce production contains 10 copies of the glutaminase gene (Ito et al., 2013). Additionally, microorganisms that produce glutaminase include *Escherichia* coli and *Bacillus subtilis* (Brown et al., 2005). In this study we found that glutaminase in Cianjur tauco and Pekalongan tauco accounts for 30 and 29 assigned reads, respectively, while tauco from Singkawang has the most reads of 40 (data not shown).

**Peptidases that Play a Role as Debittering Agent**

In addition to umami peptides, tauco also contain other amino acids such as serine, alanine, glycine, and threonine that contribute to sweet taste. Indonesian tauco from Cianjur, Pekalongan and Singkawang have less pronounced bitter taste than its umami flavor (Herlina et al., 2024). Several peptidases, such as aminopeptidase and carboxypeptidase, have been reported to reduce bitterness in fermented food products (He et al., 2024; Liu et al., 2024). In this study we found that all three types of tauco possess exopeptidases such as serine and metallo-carboxypeptidase. Carboxypeptidase enzymes can be produced by moulds from the genus *Aspergillus* (*A. oryzae*, *A. niger*, *A. sojae*) and *Bacillus* (Ito et al., 2013; Yin et al., 2019; Liu et al., 2024). Seveline et al. (2025b) reported that *Aspergillus* is the predominant microorganism in Cianjur tauco, while *Bacillus* was a common bacterium found in all samples. One study reported that during the early phase of koji fermentation, carboxypeptidases are extensively produced by *Aspergillus* (upregulated), which continues into the final phase of moromi fermentation with salt-tolerant carboxypeptidase (He et al., 2024). The continuous enzymatic activity of carboxypeptidase throughout fermentation facilitates prolonged peptide release, resulting in a higher overall peptide concentration in the final product.

Serine carboxypeptidase (Fig. 4) was reported to remove bitterness and degrade proteins into peptides and amino acids (Ito & Matsuyama, 2021). This enzyme produces a C-terminal residue consisting of two alanines and is active at pH 4.0–6.0 (Chen et al., 2021). Serine carboxypeptidase releases serine, threonine, and proline amino acids, while metallo-carboxypeptidase releases arginine, lysine, and tyrosine, depending on the type of enzyme (Ito & Matsuyama, 2021; He et al., 2024). Threonine, proline, alanine, and some amino acids have a sweet taste (Zhou et al., 2023). Fermented tauco did not have a bitter taste, as indicated by a bitterness value of less than 1 (Herlina et al., 2024). The combined amount of aminopeptidase and carboxypeptidase enzymes was very high, correlating with the low bitterness level in the tauco sample.

Serine carboxypeptidase can eliminate bitterness in hydrophobic residues by producing alanine, valine, and isoleucine (Zhou et al., 2023; Liu et al., 2024). The amount of serine carboxypeptidase in Pekalongan tauco (242) is not significantly different from Singkawang tauco (223) but is much higher than Cianjur tauco (88). Serine carboxypeptidase is active at low pH (Ito & Matsuyama, 2021), while Pekalongan tauco has the lowest pH of 4.38 (Seveline et al., 2025b) compared to the other two taucos, thereby enhancing the enzyme's activity.

Metallo carboxypeptidase produces two or three residues of histidine, aspartate, and glutamate (Gomis-Ruth, 2008). Metallo carboxypeptidase is an enzyme involved in the initial stages of soy sauce fermentation. During soy sauce production, in the moromi fermentation stage, metalloproteinase enzyme activity increases and rises by 16 times during the one to three-month fermentation period, but then decreases drastically after three months (Zhao et al., 2018). Singkawang tauco has the highest level of metalloproteinase, at 158, which is not significantly different from Pekalongan tauco at 141 but is markedly different from Cianjur tauco. Serine carboxypeptidase enzymes release serine, threonine, and proline amino acids, while metallo-carboxypeptidase enzymes release arginine, lysine, and tyrosine, depending on the activity of the enzyme (Ito & Matsuyama, 2021; Song et al., 2023; He et al., 2024).

Other exopeptidases found in tauco are the dipeptidase, tripeptidyl, and dipeptidyl peptidase groups. The highest number of reads in dipeptidases is Xaa pro peptidase, while in tripeptidyl and dipeptidyl peptidases are Xaa pro dipeptidyl peptidase is the highest, both of which can produce the amino acid proline (De et al., 2016). Proline is an amino acid with a sweet taste (Zhou et al., 2023). Xaa pro peptidase was most abundant in Singkawang tauco (67), followed by Pekalongan tauco (47) and Cianjur tauco (31), while Xaa pro dipeptidyl peptidase was most abundant in Pekalongan tauco, followed by Singkawang tauco and Cianjur tauco (57, 41 and 21) (data not shown).

**Peptidases Involved in the Production of Bioactive Compounds**

Bioactive peptides such as ACE inhibitors, antimicrobials, antihypertensives, and others can be produced during fermentation by the activity of peptidase enzymes (Sanchez & Vazquez, 2017; Chai et al., 2020). In tauco, two exopeptidases may be useful in producing health-beneficial compounds.

The peptidyl dipeptidase enzyme, which belongs to the exopeptidase family and has been reported to play a role in biological processes, such as the conversion of angiotensin I to angiotensin II, which is beneficial in vasoconstrictor activity of blood vessels, thereby increasing blood pressure (Rawlings & Barrett, 2014). This enzyme is present only in Pekalongan and Singkawang tauco, albeit in very small amounts (1 read). The peptidyl dipeptidase A, an enzyme that acts as an angiotensin-converting enzyme, is not the dominant enzyme in all tauco samples, and it is not present in Cianjur tauco. Another important enzyme in body metabolism and capable of treating type 2 diabetes is dipeptidyl peptidase IV (Roppongi et al., 2018). This enzyme is present in all three types of tauco in varying amounts: Cianjur tauco (7 reads), Singkawang tauco (4 reads), and Pekalongan tauco (2 reads).

**Conclusion**

The fermentation process of tauco is enhanced by the enzymatic activity of the microorganisms involved. While tauco from Cianjur, Pekalongan, and Singkawang are processed distinctly and contain different predominant microorganisms, they have similar types and proportions of the enzymes in the products. Seven enzyme groups, i.e., transferases, hydrolases, oxidoreductases, ligases, lyases, isomerases, and translocases were found in the three tauco with the same proportion. However, the total enzymes in the three tauco was different and the highest to the lowest amount of enzymes was found in Singkawang, Pekalongan dan Cianjur tauco, respectively.

Of the seven enzyme groups, the second most abundant group is hydrolases which primarily hydrolyze protein as the largest component in soybean. The amount of peptidases is higher in tauco from Pekalongan and Singkawang which correlates with the lower amount of peptides in the two tauco as compared to that in tauco from Cianjur.

Both endopeptidases and exopeptidases are present in tauco. The seven exopeptidases in tauco are similar to those found in other soy fermented products. Umami flavor resulting from peptidases that cleave peptides into glutamate and aspartic acid. Aminopeptidases, as well as serine and metallo-carboxypeptidases, do not directly synthesize glutamate and aspartate, but rather facilitate their release by cleaving peptide bonds involving these residues. Umami flavor is also produced extensively by enzymes that act on carbon-nitrogen bonds, specifically linear amide enzymes, particularly glutaminase, which produces glutamate. Two endopetidases, serine-endopeptidase and metallo-endopeptidase, are enzymes reported to reduce bitterness by producing amino acids with sweet taste. Aminopeptidases, serine-carboxypeptidases, and metallo-carboxypeptidases are the most abundant enzymes in Singkawang and Pekalongan tauco, with similar quantities, and are believed to play a role in reducing bitterness.

Additionally, enzyme activity during fermentation can produce bioactive peptides, such as peptidyl dipeptidase A, an angiotensin-converting enzyme that helps regulate blood pressure, and dipeptidyl peptidase IV, which is involved in the treatment of type 2 diabetes.

## DECLARATIONS

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**Ethics Statement:** This study did not involve live animals or humans, thus it does not require ethical approval/statement.

**Author's Contribution:** SS investigated the study, collected data, analyzed, prepared manuscript (writing and editing); RDH contributed in conceptualization, writing, reviewing and editing; LN contributed to writing, reviewing and editing; WAK contributed to bioinformatic analysis and editing.

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