



FTIR Profiles, Proximate Composition, and Microbial Counts of Tauco with Sensory and Amino Acid Profiles of Ultrafiltration Fractions

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Abstract— Twenty-four different tauco samples, available as commercial salt-fermented soybean products in Indonesia, were investigated for their microbial counts, proximate composition, as well as FTIR profiles obtained by attenuated total reflectance-fourier transform infrared (FTIR-ATR) instrument. The total and free amino acid profiles by UHPLC, as well as sensory characteristics by taste dilution analysis (TDA) of their ultrafiltration fractions (tauco fractions with molecular weights less than 3 kDa or <3 kDa) were also revealed. The objective of this study was to comprehensively characterize the commercial tauco products in Indonesia through integrated microbial, chemical, and sensory analyses of the products and their low molecular fractions. Tauco samples had total microbes reaching 4 to 9 log CFU/g. Lactic acid bacteria (LAB), which act in the second step of fermentation, were counted at much higher levels in all samples than molds/yeasts, which are important in the first step of fermentation. FTIR profiles of tauco samples are correlated with their protein content. Functional groups correlated with the proximate composition of tauco are identified as follows: protein with carbonyl (C=O) and aliphatic hydrocarbon (C-H), fat with carbonyl (C=O), and carbohydrate with hydroxyl (O-H) groups. Umami was identified as the predominant taste of their ultrafiltration fractions. The amino acid profiles of tauco fractions <3 kDa showed relatively high residual amino acid contents, suggesting abundant peptide content. However, multivariate orthogonal projections to latent structure (OPLS) analysis showed that free amino acids, including glutamic and aspartic acids (umami), glycine and serine (sweet), and phenylalanine and tyrosine (bitter), contributed significantly to the umami characteristic of tauco. This finding shows that free amino acids could contribute to the desirable taste of tauco. This study provides a comprehensive characterization of Indonesian tauco, offering a practical scientific reference for producers to understand product variability, improve fermentation control, and enhance the consistency and value of commercial products.

Keywords— Fermented soybean; multivariate analysis; taste dilution analysis; tauco; umami

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I. INTRODUCTION

Tauco is a fermented soybean paste used in Indonesian cuisine to impart a umami flavor [1]. Yellow soybeans, as the primary raw material, along with a small proportion of cereal flour (wheat/rice flour), are fermented with mold starters (*Aspergillus sp.* or *Rhizopus sp.*) for several days to obtain *koji* and subsequently fermented in brine solution (containing NaCl) for several months to obtain fresh tauco [2,3]. Fresh tauco may be cooked for several minutes to hours, with or without sugar/water additions, before marketing [4]. Tauco has unique

aroma and taste characteristics, resulting from biochemical transformations during fermentation [5,6]. During this process, molds serve as the primary decomposers during the initial fermentation stage (*koji* fermentation) by secreting extracellular enzymes, especially proteases and amylases, to hydrolyze soybean proteins into peptides and free amino acids. In contrast, soybean and cereal carbohydrates are hydrolyzed into simple sugars [7]. Yeasts and halophilic bacteria play a crucial role in the secondary fermentation stage (brine fermentation) by metabolizing sugars into alcohol, esters, organic acids, and other volatile compounds, thereby enhancing the product's

flavor [8]. This process leads to highly nutritious fermented soybean foods that contain essential amino acids and various bioactive compounds [9,10].

Research on tauco remains limited, particularly in terms of its comprehensive characterization. Previous studies have predominantly focused on its nutritional aspects [4], leaving significant gaps in the understanding of its chemical composition, microbiology, and sensory properties. As tauco is a protein-rich food, its amino acid content and profile are also essential from a nutritional perspective and warrant further investigation. The complexity of tauco's characteristics generates substantial analytical data, requiring multivariate analysis to derive meaningful insights. Principal component analysis (PCA) is commonly employed as an exploratory tool to investigate the characteristics of the original dataset. To construct classification models, several methods can be applied, such as soft independent modeling of class analogy (SIMCA), partial least squares discriminant analysis (PLS-DA), linear discriminant analysis (LDA), and orthogonal projections to latent structures (OPLS) [11].

Furthermore, amino acids have been reported for their contribution to the umami taste in many fermented soybean products, particularly in ultrafiltration fractions with molecular weights less than 3 kDa (<3 kDa) [12–14]. Free glutamic acid and aspartic acid, along with phenylalanine and tyrosine, in the presence of salt, have been shown to play an essential role in the umami taste of soy sauce and *oncom* [12,13]. Both alanine and serine have been reported to be present with glutamic acid and aspartic acid to contribute to the umami taste of shoyu and *tofiyo* [14,15]. The umami fraction of *doenjang* is also known to contain high concentrations of bound-type amino acids, particularly glutamate, aspartate, serine, lysine, and threonine [16]. Given these findings in other fermented soybean products, investigating the amino acid profiles and sensory characteristics of tauco, specifically in fractions <3 kDa, is crucial. Therefore, this study aims to characterize microbial counts and infrared spectral profiles of 24 different tauco samples, as well as amino acid profiles and sensory characteristics of ultrafiltration fractions, using a multivariate approach. Moreover, this study addresses the current knowledge gap by using multivariate approaches to elucidate the specific contributions of free and residual amino acids to the umami taste of tauco.

II. MATERIALS AND METHOD

A. Materials and chemicals

Twenty-four tauco samples were purchased from 24 tauco producers in Indonesia, and these samples were considered as fresh tauco samples. The abbreviated brand names were MIS, CKS, and SIM (solid tauco); CBC, CBM, MNB, SAP, PDP, TKP, MMP, CIP, LEP, and SKT (semi-solid tauco); CMC, SAB, CBB, CDS, KES, BMP, MIP, CGM, HAM, BEB, and SUM (liquid tauco). The main raw materials used in tauco are soybeans, flours, salt, and sugar.

Plate Counts Agar (PCA), Potato Dextrose Agar (PDA), and de Man Rogosa Sharpe Agar (MRSA) (Merck, Darmstadt, Germany) were used for microbial analyses. Sodium chloride (Merck), monosodium glutamate (Sigma-Aldrich, St. Louis, MO, USA), citric acid (Cica Kanto, Tokyo, Japan), caffeine (Fluka, Buchs, Switzerland), and glucose (Sigma-Aldrich) were used in sensory analysis. Chemicals for amino acid analysis by UHPLC were a solution of amino acid standard mixture, hydrochloric acid, alpha aminobutyric acid (AABA), AccQ fluor borrate buffer, and AccQ Tag Reagent 2A (6-aminoquinolyl-N-hydroxy succinimidyl carbamate). All the chemicals used for chemical analysis were of standard or analytical grade. Deionized water for sample solution and microbiological analysis, and redistilled water (API IPA Laboratories) for UHPLC analysis were used throughout this study.

B. Sample preparation

Taucu samples were collected in triplicate (due to the purchase of three different packages) and combined into a composite sample. Each sample was packaged in a plastic bottle, a glass bottle, or a bamboo container, and, for analysis, 3 packages of each were purchased and homogenized into a single mixture. Samples that arrived at the laboratory were stored at 4 °C in a refrigerator, and microbial analysis and infrared spectral profiling were conducted the following day. After sampling, the samples were stored in a freezer at -18 to -20 °C before undergoing ultrafiltration membrane fractionation, sensory characterization, and amino acid profile analysis.

C. Microbial analysis of tauco

The microbial analyses were conducted according to standard methods for total plate counts [17], total lactic acid bacteria (LAB) counts [18], and mold/yeast counts [19]. Briefly, 25 g of tauco samples were mixed with 225 mL of phosphate buffer and stomached for 1 min. Samples were serially diluted, and appropriate dilutions were poured onto plates, adding PCA for total microorganisms (total microbes), MRSA for LAB counts, and acidified PDA for molds/yeasts counts. The plates were incubated at 37 °C for 24 h to determine total microorganisms and LAB counts, and at 25 °C for 5 days to determine mold and yeast counts. Colony-forming units (CFU) per 1 g of sample (CFU/g) were the unit used to report the analytical results, determined from duplicate plate counts.

D. FTIR profile analysis of tauco by FTIR-ATR instrument

The FTIR profiles were analyzed for 24 tauco samples. A total of 0.5 g of each sample was placed on the Attenuated Total Reflectance (ATR) crystal of the FTIR instrument (FTIR-ATR). Measurements were conducted at 25 °C, and spectra were obtained over the wavenumber range 450–4,000 cm⁻¹. The resulting spectra were recorded as % transmittance, with 32 scans at a resolution of 8 cm⁻¹, using air as the reference spectrum.

E. Preparation of tauco extracts and ultrafiltration fractions

Tauco extracts were prepared by extracting composite samples with water. The amount of each tauco sample to be extracted was based on the protein concentration in the water extract (5 g proteins/L). The protein content of the tauco samples was determined from the proximate analysis results reported in the previous study [4]. Each tauco sample was homogenized with 1 L of water for 10 min. The homogenized samples were boiled for 10 minutes to inactivate enzymatic activities from tauco fermentation, and then cooled for 50 min at room temperature. The extracts were then filtered manually through cheesecloths, followed by centrifugation at 6,000 g, 4 °C, for 30 min (Hermle Z 383 K, Wehingen, Germany). The supernatants were then filtered using a 0.45 µm cellulose acetate membrane (Advantech Co., Ltd., Tokyo, Japan) under vacuum (Buchi V-700 vacuum pump, Flawil, Switzerland) to obtain tauco extracts.

To obtain ultrafiltration fractions containing low molecular components which contribute to sample taste, the extracts were ultrafiltered using a 3 kDa ultracentrifugal tube (Ultracel-3K, Amicon Ultra Centrifugal Filters, 15 mL Regenerated Cellulose 3,000 MWCO, Millipore, Ireland) and centrifuge unit (Hermle Z 383 K) at 6,000 g, 4 °C, for 60 min. The fractions that were collected had molecular weights less than 3 kDa (MW<3 kDa). The fractions were diluted with Aquadest to the initial volume of the tauco extracts (volume before ultrafiltration) to achieve comparable concentrations of low-molecular-weight compounds in the fractions. The tauco fractions (MW<3kDa) were further characterized for free and residual amino acids, as well as the sensory properties described below

F. Sensory analysis of tauco fractions

Ethical approval for the sensory evaluation was obtained from IPB University (No. 468/IT3).KEPMSM-IPB/SK/2021. Informed consent was obtained from each subject before participation in the study. The panel selection was performed with 59 untrained panelists following ISO 8586 [15], using sucrose solutions at 10 g/L and 3 g/L, citric acid at 0.3 g/L and 0.1 g/L, NaCl at 2.0 g/L and 0.7 g/L, caffeine at 0.3 g/L and 0.1 g/L, and MSG at 0.6 g/L and 0.5 g/L for matching and detection tests. The selected panel, consisting of 10 subjects (all women) aged 19 to 50 years from the Department of Food Science and Technology at IPB University, then participated in the training and sensory evaluation session using the taste dilution analysis (TDA) method [20].

During the training session, the tauco water extracts were used as samples and diluted according to 2ⁿ factors, with each dilution delivered in 2.00 mL self-lock tubes containing a blank solution (mineral water) for a triangle test. The same procedure was applied in the sensory analysis, except that tauco fractions (MW<3 kDa) were used as samples. Briefly, panelists assessed 2.00 mL of each diluted sample, following 2ⁿ factors in increasing concentration. Before testing and moving on to the subsequent dilution, panelists rinsed their taste buds with

mineral water. As part of the triangle test, panelists were asked to identify the different samples and describe their tastes. The maximum dilution value at which the test solution can still be distinguished from the two blanks is set as the dilution factor (DF). The DF value was determined by consensus among at least six panelists. The training and sensory analysis session was conducted in an air-conditioned room (20 ± 1 °C) with panelists in separate booths. Each sensory evaluation of the sample was done in *simplo* evaluation.

G. Free and residual amino acid composition of tauco fractions

Free and total amino acid profiles of tauco fractions were analyzed using a UHPLC instrument (WATERS UHPLC Acquity PDA Det H Class, Waters Corp., Massachusetts, USA), before and after hydrolysis, respectively [21]. The peptide amounts were calculated as amino acid residual amounts, i.e., the *difference* between the total and free amino acid amounts. Before injection, the test solution was filtered using a 0.2 µm syringe filter for all amino acid standards and fractions. For analysis of free amino acid composition, the filtered fractions were directly injected into the UHPLC instrument; for total amino acid composition, the fractions were first acid hydrolyzed before injection. Acid hydrolysis was performed with 6 N HCl at 110 °C for 22 h. The hydrolyzed samples were transferred quantitatively into a 50-mL volumetric flask and diluted with HPLC-grade water. The sample was then filtered with a 0.22 µm syringe filter to obtain the filtrate. For sample preparation, the internal standard alpha aminobutyric acid (AABA) was added to the hydrolyzed sample at a ratio of 1:1. The total of 10 µL of this mixture was further derivatized by AccQ fluor borrate buffer and AccQ Tag Reagent 2A (6-aminoquinoly1-N-hydroxy succinimidyl carbamate). The mixture was then incubated at 60 °C for 10 minutes and was ready for injection into the UHPLC instrument.

The determination by UHPLC was performed on an AccQ. Tag Ultra C-18 column (2.1×100 mm, 1.7 µm). The photodiode array detector (PDA) was set at 260 nm. UHPLC was run at 49 °C using an injection volume of 1 µL for the sample or standard. The mobile phase consisted of solvent A (AccQ. Tag Ultra Eluent A, 100%), solvent B (AccQ. Tag Ultra Eluent B: water, 10:90), solvent C (water), and solvent D (AccQ. Tag Ultra Eluent B, 100%), with a flow rate of 0.5 mL/min. Amino acid mixture standard solutions of 2.5 µmol/mL were also injected into the UHPLC under the same preparation and conditions as the samples for amino acid identification and quantification. Residual amino acids, which are amino acids still bound within the peptide sequences, were quantified by subtracting the concentration of free amino acids from the total amino acids. The amino acid composition was analyzed in duplicate.

H. Statistical analysis

The dataset resulting from microbial counts of tauco, total amino acid composition, total essential amino acids, and taste characteristics of tauco fractions was subsequently exported to

Umetrics SIMCA 17.0 (Umetrics, Sweden) for multivariate pattern recognition using principal component analysis (PCA). Before the development of the chemometric models, unit variance scaling was applied to the datasets. Briefly, PCA was performed to obtain a general overview of the tauco characteristics mentioned above. PCA summarized the original dataset by reducing its dimensionality. Subsequently, orthogonal projections to latent structure (OPLS) analysis was conducted using SIMCA 17.0 to determine amino acid markers associated with the umami taste of tauco. The model quality was assessed using the criteria of model accuracy (R^2) and predictive accuracy (Q^2). The model with good reliability was characterized by R^2 and Q^2 values greater than 0.5, and values closer to 1.0 indicated an excellent model. The robustness and significance of the generated model were examined through Cross-Validation Analysis of Variance (CV-ANOVA) at a 95% confidence level ($p < 0.05$) [22]. Potential amino acids of interest could be selected from the upper right quadrant of the S-plot, based on the positive value of Y-related coefficients and variable influence on projection (VIP) values above 0.8 [23]. This analysis provides new insights for future investigations into the identification and exploration of umami compounds in foods.

III. RESULTS AND DISCUSSION

I. Microbial counts of tauco

The microbial counts of tauco samples are summarized in **Table 1**. Total microbial load ranged from 3.16 to 9.29 log

CFU/g, with 17 tauco samples exhibiting counts greater than 7.00 log CFU/g. According to the Indonesian National Standard for tauco (SNI 01-4322-1996), the maximum allowable total microbial count is 4 log CFU/g. Based on this criterion, only two out of the 24 samples analyzed met the required microbial quality threshold (SIM 3.16 log CFU/g and CDS 3.62 log CFU/g). The remaining samples exceeded the permissible limit, indicating potential concerns regarding process hygiene or post-fermentation handling. The microbial counts observed in the tauco samples were higher than those reported for *doenjang*, a Korean fermented soybean paste, which ranged from 2.60 to 7.00 log CFU/g [24]. Lactic acid bacteria (LAB) were detected in 19 samples, with counts ranging from 1.00 to 8.22 log CFU/g, while the remaining five samples had LAB counts below 1.00 log CFU/g. Molds/yeasts were detected in 19 samples, with counts ranging from 1.00 to 8.22 log CFU/g, while the remaining five samples had LAB counts below 1.00 log CFU/g. Molds/yeasts were detected in 11 samples, with counts ranging from 1.00 to 6.40 log CFU/g. In contrast, the other 13 samples exhibited mold and yeast counts below 1.00 log CFU/g. Mold/yeast counts in tauco were comparable to those observed in *doenjang* (1.10–5.30 log CFU/g) [24]. Overall, LAB were more prevalent in tauco samples than molds/yeasts (**Table 1**). The differences in microbial abundance may be attributed to variations in raw material composition, inoculum use, fermentation time, and brine salt concentration, all of which can influence microbial growth during tauco production [25–27].

TABLE 1
 MICROBIAL CHARACTERISTICS OF TAUCO SAMPLES

Samples ^a	Type of tauco ^b	Salt content ^c (% ww)	Total microbes (log CFU/g)	Total LAB (log CFU/g)	Molds/yeasts (log CFU/g)
MIS	Solid	10.9 ± 0.1 ^o	8.39	4.05	<1.00 (0.00)
CKS	Solid	5.0 ± 0.1 ^c	4.07	<2.00 (1.18)	<1.00 (0.00)
SIM	Solid	9.6 ± 0.1 ^k	3.16	<1.00 (0.00)	<3.00 (2.30)
CBC	Semi-solid	6.3 ± 0.2 ^e	5.53	<1.00 (0.00)	<1.00 (0.00)
CBM	Semi-solid	11.9 ± 0.3 ^p	5.36	3.15	<1.00 (0.00)
MNB	Semi-solid	7.6 ± 0.1 ^{gh}	8.24	3.41	<1.00 (0.00)
SAP	Semi-solid	9.9 ± 0.1 ^l	9.04	4.57	<2.00 (1.00)
PDP	Semi-solid	10.2 ± 0.1 ⁿ	8.59	4.05	6.40
TKP	Semi-solid	7.6 ± 0.2 ^g	8.13	7.36	4.66
MMP	Semi-solid	7.8 ± 0.0 ^h	8.93	8.22	<1.00 (0.00)
CIP	Semi-solid	8.3 ± 0.1 ⁱ	8.33	6.22	2.36
LEP	Semi-solid	5.9 ± 0.1 ^d	7.91	3.61	3.41
SKT	Semi-solid	9.0 ± 0.1 ^j	7.29	<2.00 (1.44)	2.11
CMC	Liquid	4.4 ± 0.0 ^a	7.32	4.43	4.14
SAB	Liquid	10.2 ± 0.0 ^{mn}	5.36	<1.00 (0.00)	<1.00 (0.00)
CBB	Liquid	10.2 ± 0.0 ^a	9.29	3.79	<2.00 (1.56)
CDS	Liquid	4.6 ± 0.1 ^b	3.62	<2.00 (1.00)	<1.00 (0.00)
KES	Liquid	10.0 ± 0.1 ^{lm}	8.16	<1.00 (0.00)	<1.00 (0.00)
BMP	Liquid	9.0 ± 0.1 ^j	7.55	4.52	<1.00 (0.00)
MIP	Liquid	8.3 ± 0.1 ^f	5.28	<1.00 (0.00)	<1.00 (0.00)
CGM	Liquid	8.2 ± 0.2 ⁱ	8.40	3.00	<1.00 (0.00)
HAM	Liquid	18.7 ± 0.0 ^q	8.01	6.29	<2.00 (1.00)
BEB	Liquid	13.6 ± 0.1 ^r	8.99	2.20	<1.00 (0.00)
SUM	Liquid	15.4 ± 0.2 ^r	9.19	<2.00 (1.44)	<2.00 (1.66)

^aThe tauco samples were prepared as composite samples by combining equal portions from three different packages of tauco

^bThe physical appearance of tauco samples, as described by Herlina *et al.* (2022)

^cValues are presented as mean ± standard deviation with superscript letters in the same column that do not share the same alphabetical order represent significant differences ($p < 0.05$)

The highest total counts of LAB were observed in four samples, TKP, MMP, CIP, and HAM. This finding aligned with the study by Baroroh [28], which reported that LAB accounted for the majority of the bacterial population, comprising 72.80% of the total bacterial load in tauco. In contrast, the remaining 20 samples displayed relatively low counts of LAB and molds/yeasts, despite having high total microbial counts. This suggests the presence of other microbial communities that were not quantified in the present study. In line with these findings, Pauzi [29], also identified several bacterial genera in tauco, including *Staphylococcus* (17.69%), *Geobacillus* (16.62%), *Bacillus* (7.00%), *Cronobacter* (1.03%), *Kocuria* (0.53%), and *Brachybacterium* (0.49%). Additionally, other genera, such as *Corynebacterium*, *Oceanobacillus*, and *Clostridium*, have been reported in fermented soybean pastes, including *doenjang* [30] and *dajiang* [31].

As previously reported by Herlina et al. [4], tauco can be categorized as solid, semi-solid, and liquid types, each with distinct proximate compositions that contribute to their diverse microbial properties. A multivariate analysis of microbial

counts was also conducted to further explore these variations. Generally, semi-solid samples exhibited total microbial counts exceeding 7 log CFU/g, except for CBC and CBM, which had counts of 5 log CFU/g (Table 1). Principal component analysis (PCA) mapping (Figure 1A) revealed that these two samples were positioned at the far left of the projection, distinctly separated from the other semi-solid samples. Semi-solid samples with high LAB counts (TKP, MMP, and CIP) were grouped closely together. Additionally, molds/yeasts found in significant quantities in PDP and TKP displayed similar projections (Figure 1A). PCA mapping of the liquid tauco samples based on their microbial counts (Figure 1B), revealed that most liquid samples were positioned in the positive direction of PC2, characterized by high total microbial counts (>7 log CFU/g), except for SAB, CDS, and MIP. Liquid samples generally contained LAB, except for the SAB, KES, and MIP, which had LAB counts of less than 1.00 log CFU/g. Molds/yeasts were less prevalent in the liquid samples, except for CMC (4.14 log CFU/g), which was distinctly separated from the others in the PCA map.

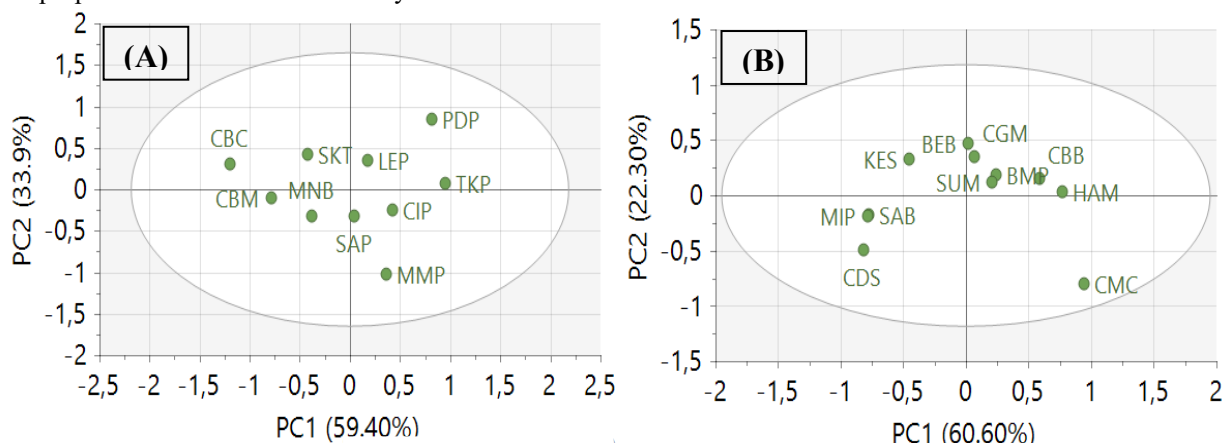


Fig. 1 The PCA score plot based on microbial counts for: (A) semi-solid tauco ($R^2 = 0.933$ and $Q^2 = 0.657$), and (B) liquid tauco ($R^2 = 0.829$ and $Q^2 = 0.693$)

J. Infrared spectrum profiles of tauco

The infrared spectrum profiles of 24 tauco samples, scanned over the wavelength range of 500–4,000 cm^{-1} , are presented in Figure S1. The spectra generally exhibited consistent absorption patterns, with notable variations in intensity. Several key functional groups were identified, including the hydroxyl (O-H) group of alcohols at 3,200–3,600 cm^{-1} , supported by C–O absorptions at 1,000–1,300 cm^{-1} . The hydroxyl (O-H) group of carboxylic acids was detected in the 2,500–2,700 cm^{-1} region. A prominent peak between 1,600 and 1,800 cm^{-1} indicated the presence of carbonyl (C=O) groups, likely originating from carboxylic acids (1,700–1,725 cm^{-1}) or amides (1,680–1,700 cm^{-1}). Additionally, the N–H group of amines or amides was observed at 3,350–3,500 cm^{-1} , with corresponding C–N absorptions at 1,056–1,059 cm^{-1} . These functional groups are characteristic of proteins, consistent with tauco’s

composition as a soybean-based fermented product rich in plant-derived proteins.

Multivariate analysis using orthogonal projections to latent structures (OPLS) further explored the relationship between tauco’s infrared spectrum profiles and its proximate composition. Protein was associated with specific absorption regions, particularly 1,700–1,750 cm^{-1} for the carbonyl (C=O) group and 2,850–2,950 cm^{-1} for aliphatic hydrocarbons (C–H). However, no specific absorption regions were directly linked to fat or carbohydrate (Figure 2A). The S-plot from the OPLS analysis further revealed associations between functional groups and the proximate composition of tauco. As depicted in Figure 2B, protein was linked to the absorption region at 2,850–2,950 cm^{-1} , corresponding to aliphatic hydrocarbons (C–H). Fat was correlated with the absorption region at 1,700–1,760 cm^{-1} , which represents carbonyl (C=O) groups (Figure

2C), while carbohydrates were associated with the absorption region at 3,200–3,600 cm^{-1} , characteristic of hydroxyl (O–H) groups (Figure 2D).

K. Taste characteristics of tauco fractions

Taste characteristics of tauco fractions, expressed as taste dilution factors (DF), are presented in Figure 3A. As the DF value increases, the detected taste intensity also increases. In Figure 3A, umami taste is found to be the most dominant taste in tauco fractions, with DFs ranging from 16 to 64. The umami taste is recognized in the tauco fractions with total soluble protein concentrations ranging from 2.41 mg/mL to 7.06 mg/mL. Umami taste was also identified as the dominant taste in the red oncom fraction with molecular weights less than 3 kDa. However, this taste was stronger than that of the tauco fractions, with a DF-umami value of 512 and total soluble protein concentrations 20-60 times higher (143.71 mg/mL) [12]. The observed total soluble proteins in tauco fractions are likely composed of amino acids and peptides resulting from protein hydrolysis.

The fractions with the highest umami intensity (DF-umami at 64), hereafter referred to as umami fractions, consisted of SIM

(solid tauco), LEP and MNB (semi-solid tauco), as well as MIP and SUM (liquid tauco). In addition to umami, salty and sour tastes were detected in 11 of 24 fractions. However, their intensity was lower than that of umami, with DF values for salty and sour tastes ranging from 8 to 16. The remaining fractions showed very little salty or sour taste, with DF values below 1. This variation in taste intensity could be attributed to differences in tauco production processes, including variations in the salt concentrations used [26,32]. Moreover, the sourness in some fractions could be influenced by the carbohydrate sources used during preparation, which promote bacterial growth [33] and lead to the production of acidic compounds, contributing to the final product's sour taste [34]. Most of the tauco fractions also showed very low sweetness (DFs less than 1), except for the LEP fraction (DF of 16) and the MMP fraction (DF of 8), which had slightly higher sweetness levels. Adding sugar to the final product is commonly used to balance the sourness produced during fermentation [35]. Meanwhile, all tauco fractions showed very minimal bitterness (DFs < 1), likely due to the masking effect of significant umami compounds [36].

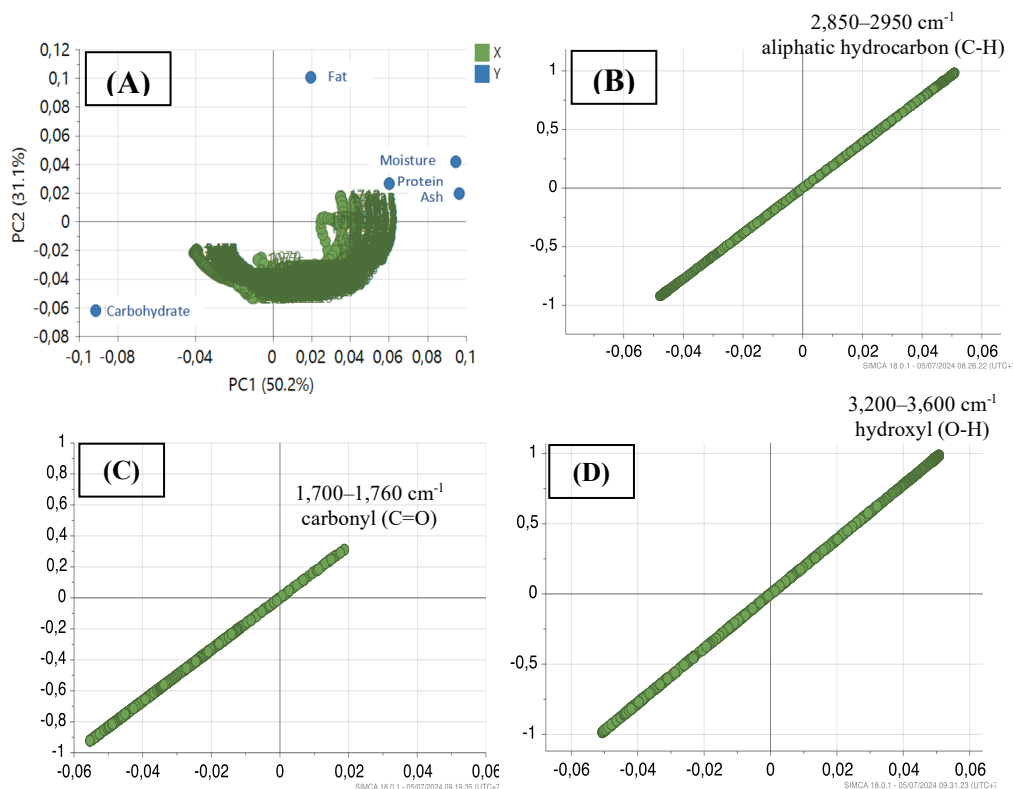


Fig. 2 The OPLS analysis results correlating infrared spectrum profiles of tauco samples with proximate composition, are presented as follows: (A) the loading plot, illustrating the relationship between spectral features and proximate composition, (B) the S-plot of protein, highlighting spectral regions associated with protein content, (C) the S-plot of fat, highlighting spectral regions associated with fat content, (D) the S-plot of carbohydrate, highlighting spectral regions associated with carbohydrate content.

PCA was further applied to investigate the similarity between tauco fractions based on their taste characteristics. This unsupervised chemometric technique simplifies the dataset by

highlighting the most critical information for preliminary classification, providing an overview of the data structure. The score plot (Figure 3B) provides an overview of group

clustering based on the taste characteristics of the tauco fractions to identify outliers and examine potential trends and patterns. The first principal component (PC1) accounts for 64.4% of the variance, while the second principal component (PC2) accounts for 23.7%. Together, these two components explain 88.1% of the total variance in the dataset. A cumulative variance greater than 70% is generally considered adequate [37]. Additionally, the robustness of the PCA model is demonstrated by the goodness-of-fit (R^2) and predictive ability (Q^2), which are 0.881 and 0.434, respectively. These results indicate that the two components fit the dataset well.

To enhance visual clarity and understanding, the score plot in the model was color-coded. The group of green-colored fractions on the negative PC2 axis showed a higher sour taste. In contrast, the group with the blue-colored fractions showed a higher salty taste. Moreover, some of the fractions in the red group overlapped with the blue group, indicating that, in addition to low umami taste, these fractions were also characterized by a salty taste (Figure 3B and 3C). The PCA model of the tauco fractions based on taste characteristics also

confirmed that the intense umami fractions, SIM, LEP, MIP, MNB, and SUM, showed projections consistent with umami taste (spots located to the right). These findings suggest that tauco typically has a desirable flavor profile, supporting its potential as a natural umami seasoning. Additionally, the results indicate that bitterness was generally absent in most tauco fractions. In line with previous studies, PCA multivariate analysis has also been used to summarize the sensory profiles of *doenjang* samples, reporting that sweet and umami tastes (taste and aftertaste) were strongly associated with modified and processed *doenjang*, while bitter and salty tastes were more prominent in traditional-style *doenjang* [38]. This demonstrates that PCA is a valuable tool for visualizing sensory patterns and can offer deeper insights into the complex sensory profiles of fermented products in future research.

L. Amino acid profiles of tauco fractions

Table 2 presents the total amino acid composition of tauco fractions with molecular weights below 3 kDa. The total amino acid concentrations ranged from 1.43 mg/mL (CBM) to 4.11 mg/mL (CIP).

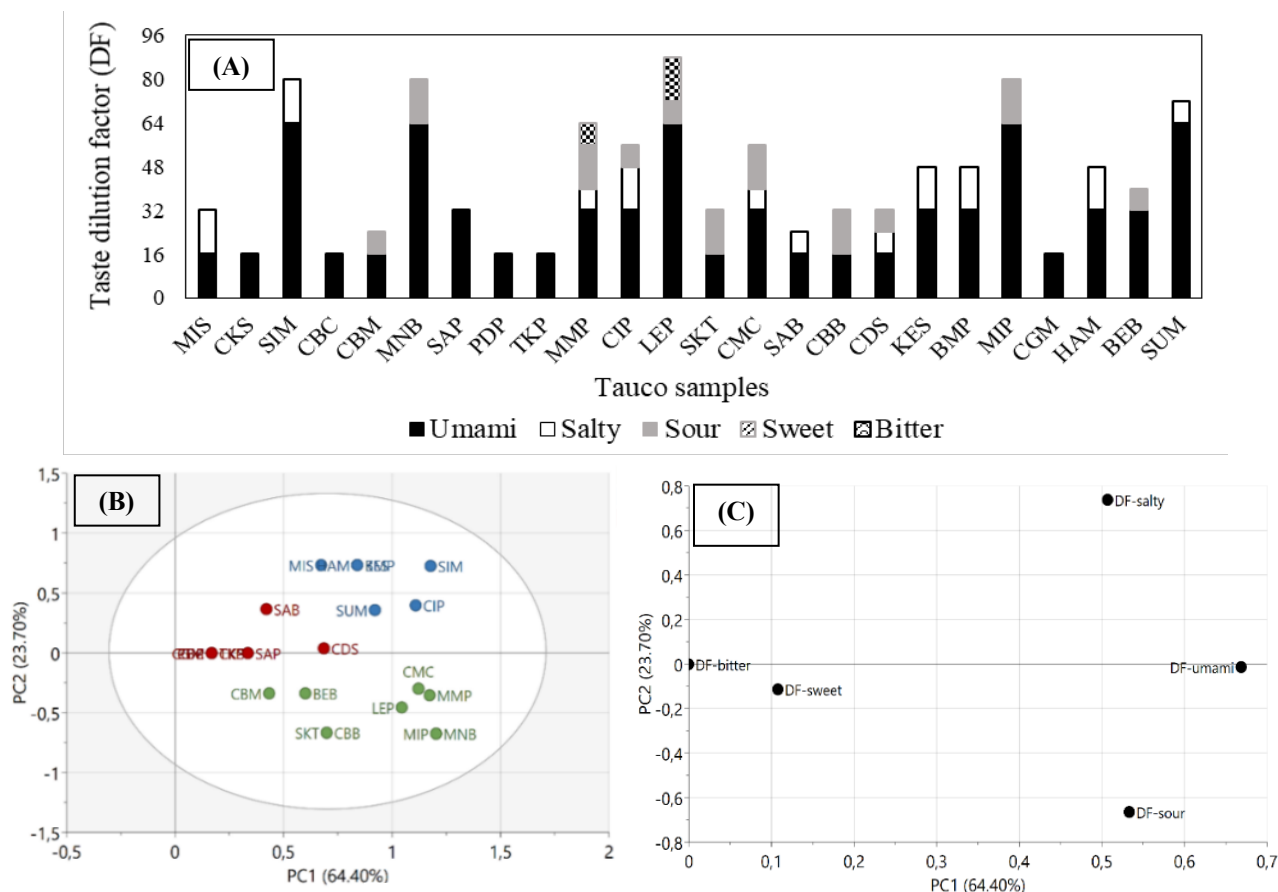


Fig. 3 Taste characteristics of tauco fractions with molecular weights less than 3 kDa: (A) Taste intensities expressed as taste dilution factors (DF); and their mapping by PCA multivariate analysis (B) the score plot and (C) the loading plot ($R^2 = 0.881$ and $Q^2 = 0.434$).

Glutamic acid was the predominant amino acid, with concentrations varying from 0.14 mg/mL (BMP) to 0.78 mg/mL (MIP). Notably, the total glutamic acid concentration was lower than that of the *tofiyo* fraction with a molecular weight of less than 3 kDa, which contained 2.2 mg/mL of glutamic acid [14]. However, these findings are consistent with previous studies that also identified glutamic acid as the dominant amino acid in fermented soybean pastes, such as raw tauco [4], *miso* [39], and *doenjang* [16]. In addition to glutamic acid, sweet and bitter-tasting amino acids were present in substantial amounts in the tauco fractions (Table 2). The sweet amino acids ranged from 0.26 mg/mL (BMP) to 1.45 mg/mL (CIP), and are known to contribute to the umami taste in other fermented products such as shoyu [15] and *tofiyo* [14]. The bitter amino acids in the tauco fractions ranged from 0.70 mg/mL (KES) to 1.51 mg/mL (CIP). Bitter amino acids in tauco fractions may reduce the umami taste, causing variations in umami intensity across the fractions. A similar effect has been

observed in other fermented soybean products, such as *tempe* [40].

The total amino acid in tauco fractions consists of both free and residual amino acid concentrations (for 15 amino acids) as presented in Table S1. Sixteen fractions, accounting for 67% of the total samples, exhibited higher concentrations of free amino acid compared to amino acid residues (Table 2), suggesting that these fractions contained a higher peptide content. Among the intense umami fractions, SIM, LEP, MIP, MNB, and SUM, there was a considerable amount of total amino acids (Table 2), with a notable predominance of free amino acids over residual amino acids (Table S1). Generally, the concentrations of free umami amino acids, specifically glutamic acid and aspartic acid, were higher than those of other amino acids. This observation is consistent with the fact that free glutamic acid and aspartic acid play a significant role in contributing to the umami taste intensity of fermented soybean products [14,15],[41].

TABLE 2
 TOTAL AMINO ACID COMPOSITION OF TAUCO FRACTIONS

Taste attributes	Amino acids	Total amino acids (mg/mL)											
		MIS	CKS	SIM	CBC	CBM	MNB	SAP	PDP	TKP	MMP	CIP	LEP
Umami	Asp	0.18	0.13	0.24	0.17	0.10	0.20	0.06	0.15	0.06	0.22	0.27	0.20
	Glu	0.37	0.25	0.50	0.44	0.21	0.42	0.16	0.27	0.18	0.32	0.60	0.55
	Sub total	0.55	0.38	0.74	0.61	0.31	0.62	0.22	0.42	0.24	0.54	0.87	0.75
Sweet	Ala	0.27	0.11	0.10	0.09	0.09	0.12	0.05	0.24	0.23	0.17	0.23	0.14
	Gly	0.09	0.09	0.12	0.11	0.06	0.11	0.03	0.07	0.07	0.09	0.17	0.16
	Ser	0.04	0.04	0.16	0.14	0.06	0.16	0.05	0.09	0.04	0.08	0.20	0.08
	Thr	0.16	0.07	0.69	0.52	0.06	0.12	0.56	0.18	0.16	0.10	0.85	0.72
	Sub total	0.56	0.31	1.07	0.68	0.27	0.51	0.69	0.58	0.50	0.44	1.45	1.10
	Val	0.08	0.09	0.10	0.08	0.04	0.09	0.04	0.07	0.06	0.09	0.16	0.13
Bitter	Met	0.10	0.08	0.10	0.11	0.06	0.10	0.04	0.07	0.04	0.11	0.14	0.13
	Ile	0.05	0.07	0.10	0.09	0.05	0.08	0.05	0.05	0.06	0.08	0.15	0.12
	Leu	0.09	0.10	0.16	0.13	0.06	0.15	0.05	0.09	0.07	0.15	0.23	0.18
	Tyr	0.18	0.18	0.25	0.23	0.18	0.18	0.19	0.18	0.19	0.18	0.31	0.25
	Phe	0.14	0.14	0.17	0.14	0.14	0.14	0.14	0.14	0.15	0.14	0.18	0.19
	His	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.08	0.09	0.09	0.12	0.09
	Arg	0.11	0.12	0.19	0.11	0.12	0.18	0.12	0.11	0.12	0.11	0.22	0.12
Sub total	0.84	0.87	1.16	0.98	0.74	1.01	0.72	0.79	0.78	0.95	1.51	1.21	
Other	Lys	0.10	0.10	0.17	0.15	0.11	0.12	0.11	0.10	0.10	0.15	0.28	0.20
Total		2.05	1.66	3.14	2.60	1.43	2.26	1.74	1.89	1.62	2.08	4.11	3.26
Taste attributes	Amino acids	Total amino acids (mg/mL)											
		SKT	CMC	SAB	CBB	CDS	KES	BMP	MIP	CGM	HAM	BEB	SUM
Umami	Asp	0.20	0.07	0.06	0.10	0.17	0.11	0.12	0.19	0.12	0.12	0.15	0.18
	Glu	0.39	0.30	0.16	0.23	0.44	0.23	0.14	0.78	0.25	0.25	0.38	0.41
	Sub total	0.59	0.37	0.22	0.33	0.61	0.34	0.26	0.97	0.37	0.37	0.53	0.59
Sweet	Ala	0.16	0.11	0.02	0.26	0.25	0.30	0.07	0.28	0.26	0.22	0.12	0.11
	Gly	0.09	0.08	0.02	0.07	0.10	0.06	0.07	0.10	0.04	0.08	0.11	0.11
	Ser	0.10	0.09	0.04	0.07	0.06	0.07	0.05	0.13	0.06	0.05	0.05	0.06
	Thr	0.23	0.07	0.83	0.17	0.16	0.19	0.07	0.19	0.35	0.21	0.12	0.12
	Sub total	0.58	0.35	0.91	0.57	0.57	0.62	0.26	0.70	0.71	0.56	0.40	0.40
	Val	0.09	0.07	0.04	0.07	0.08	0.04	0.08	0.08	0.16	0.10	0.11	0.10
Bitter	Met	0.10	0.09	0.04	0.08	0.09	0.04	0.05	0.10	0.05	0.04	0.11	0.11
	Ile	0.08	0.07	0.05	0.06	0.07	0.05	0.06	0.08	0.16	0.08	0.10	0.09
	Leu	0.13	0.11	0.05	0.11	0.11	0.05	0.09	0.13	0.24	0.12	0.14	0.14
	Tyr	0.18	0.18	0.18	0.18	0.19	0.18	0.18	0.18	0.18	0.18	0.19	0.18
	Val	0.09	0.07	0.04	0.07	0.08	0.04	0.08	0.08	0.16	0.10	0.11	0.10

Phe	0.14	0.14	0.14	0.15	0.14	0.14	0.15	0.14	0.14	0.14	0.14	0.15
His	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.08	0.09	0.09	0.09	0.09
Arg	0.11	0.12	0.12	0.11	0.12	0.11	0.12	0.12	0.12	0.11	0.12	0.12
Sub total	0.92	0.87	0.71	0.85	0.89	0.70	0.82	0.91	1.14	0.86	1.00	0.98
Other	Lys	0.14	0.10	0.10	0.10	0.10	0.10	0.12	0.21	0.14	0.10	0.10
Total	2.23	1.69	1.94	1.85	2.19	1.76	1.44	2.70	2.43	1.93	2.03	2.07

Glutamic acid, as the umami compound in tauco fractions, was confirmed by examining the umami taste activity value (TAV) [42]. In this case, the TAV for umami was calculated by assuming that the glutamic acid residue has the same umami intensity as free glutamic acid. A fraction with a TAV greater than one indicates the presence of umami taste. **Table S1** shows that the TAV of glutamic acid exceeds 1 in all tauco fractions, indicating its contribution to tauco's umami flavor, which is readily perceived.

PCA was conducted to determine the positioning of each tauco fraction based on its total amino acid composition. PCA transforms the original variables into a smaller set of new variables known as principal components (PCs). These PCs are designed to capture as much of the variance in the original data as possible. By plotting the PCs, one can visualize relationships among samples and assess potential groupings or clusters based on their similarities [43]. The PCA biplot is shown in **Figure 4**. This figure illustrates a total variability of 70.7%, with PC1 accounting for 58.6% of the total variability as the primary axis and PC2 accounting for 12.1% as the secondary axis. The model demonstrated strong reliability, as evidenced by high R^2 (0.707) and Q^2 (0.419), indicating excellent predictive capability and good model fit.

In **Figure 4A**, the general separation between fractions was not ideal. It is important to note that the variance explained by PC1 and PC2 was limited. However, the four fractions with the highest total amino acid content (CIP, LEP, SIM, CBC) were clearly separated in the positive PC1 and negative PC2 regions. These fractions also showed a higher concentration of bitter-eliciting amino acids, particularly tyrosine, phenylalanine, arginine, and histidine (**Figure 4B**). Tyrosine and phenylalanine have been shown to enhance umami taste, especially in the presence of salt, as demonstrated by Lioe et al. [44].

As a source of nutrition, the essential amino acid content of tauco fractions ranged from 0.55 mg/mL (CBM) to 1.97 mg/mL (CIP) and was dominated by threonine (**Table S2**). Smaller amounts of isoleucine, histidine, and valine were also detected. In comparison with other fermented soybean products, the essential amino acids in tauco fractions were lower than those of the red *oncom* fraction with molecular weights less than 3 kDa (8.76 mg/mL) [12]. Tauco fractions with a high total amino acid content also displayed higher levels of essential amino acids, and their mapping further indicated separation in the positive PC1 and PC2 regions, as illustrated in **Figures 4C and 4D**.

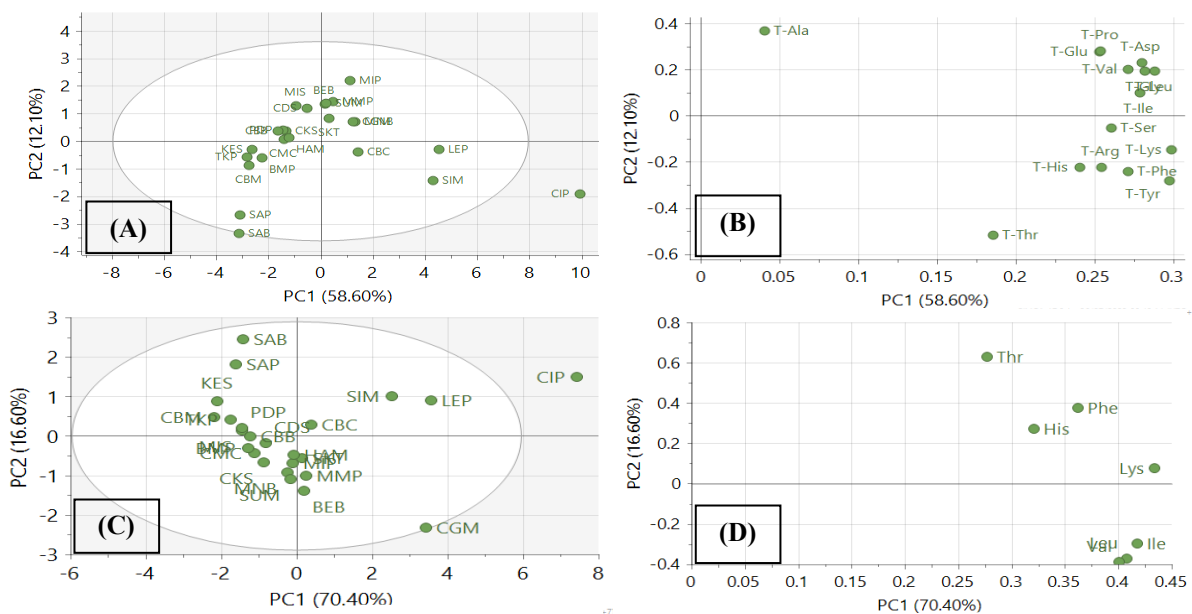


Fig. 4 The PCA mapping of tauco fractions (MW<3 kDa) based on amino acid profiles are presented as follows: (A) the score plot and (B) the loading plot for total amino acid composition ($R^2 = 0.707$ and $Q^2 = 0.419$); (C) the score plot and (D) the loading plot for total essential amino acid ($R^2 = 0.870$ and $Q^2 = 0.569$).

indicated that protein was associated with the carbonyl (C=O) group and aliphatic hydrocarbon (C-H) groups, fat with the carbonyl (C=O) group, and carbohydrates with the hydroxyl (O-H) group. The amino acid profiles of most tauco fractions <3 kDa revealed high levels of residual amino acids, with umami being the most dominant taste. Additionally, the OPLS multivariate analysis revealed that free amino acids, including glutamic acid and aspartic acid (umami), glycine and serine (sweet), phenylalanine and tyrosine (bitter), as well as residual threonine, were associated with the umami taste of tauco. This study delivers a detailed characterization of Indonesian tauco, establishing a technical foundation that may support producers in monitoring product variation and improving the overall quality of commercially produced tauco.

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CONFLICT OF INTEREST

The authors declare no conflict of interest to disclose.

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