Nutritional Composition, Amino Acid Profiles, and Protein Digestibility of Indonesian Smoked Eel, Shirayaki, and Kabayaki

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Abstract

Eel (*Anguilla* sp.) is a catadromous fish with high nutritional value, originating in freshwater and migrating to spawn, producing glass eel larvae. In Indonesia, processed eel products such as kabayaki, shirayaki, and smoked eel, which are popular in Japan, have not been widely developed. Comprehensive data on the nutritional composition and protein quality of these products are essential for product development and nutritional improvement. This study aims to determine the nutritional composition, amino acid profiles, and in vitro protein digestibility of Indonesian smoked eel, shirayaki, and kabayaki. Analyses included nutritional composition, amino acid profiles using high-performance liquid chromatography (HPLC), and in vitro protein digestibility for processed eel products. The results indicated that the processing method affected the nutritional value, albumin, minerals, and amino acids. Fresh eel had the highest protein content, followed by smoked eel, while there was no significant difference between shirayaki and kabayaki. The protein digestibility was highest in smoked (67.59%), but there was no significant difference among the others (63.30-64.44%). Calcium (Ca), magnesium (Mg), and potassium (K) contents were highest in smoked eel compared to fresh eel and decreased significantly in shirayaki and kabayaki, but for zinc (Zn), shirayaki and kabayaki tend to be higher than smoked eel. An analysis of essential amino acids showed no significant difference between the processing methods. Leucine had the highest concentration, followed by valine, threonine, and isoleucine, with concentrations ranging from 4.02% to 7.83%. The research found that the best processing method was smoked, and there was no significant difference between kabayaki and shirayaki.

Keywords: nutritional composition, amino acid profile, in vitro protein digestibility, processed eel products

Introduction

Eel (*Anguilla* sp.) is a catadromous fish that originates from freshwater and then migrates to spawn and produce larvae (glass eels) in warm seas (Durif et al., 2023). Eel (*Anguilla* sp.) is highly nutritious and known as an export commodity. In 2023, the global market value of eel is estimated at USD 4.45 billion and is projected to reach USD 6.17 billion by 2033, with a compound annual growth rate (CAGR) of around 3.32% (Spherical Insights, 2025). Total eel production in Indonesia in 2020-2022 ranged from 3,400 to 3,544 tons, with the export volume in the range of 1,688 to 2,070 tons and the export value from USD 1,701 million to USD 1,952 million (Ministry of Marine Affairs and Fisheries Republic of Indonesia, 2025; UN Comtrade Database, 2025). According to Noor & Abidin (2019), Indonesian eel exports include live, fresh, and frozen products, with frozen eel exhibiting the most significant export potential. In the period 2012-2020, there was a change in the share of eel product exports. In 2012, the export share of eel products was dominated by live products (66.38%) and frozen products (30.53%), while in 2018, it was dominated by frozen products (65.92%) and live products (32.79%).

Eel has a high nutritional content, especially protein, vitamins A and E, and unsaturated fatty acids (EPA and DHA) (Seo et al., 2013; Kusharto et al., 2014; Nafsiyah et al., 2018). The proximate content of Korean eel *A. bicolor pacifica* and *A. marmorata* is dominated by water content of 71.1% and 71.8%, respectively, and crude protein of 17.4% and 17.7%, with EPA content of 3.28% and 9.6%, and DHA 2.93% and 9.14% (Ahn et al., 2015). Kim & Setiyorini (2018) reported that *A. bicolor* from cultivation originating from the Cilacap area (Indonesia) with an average weight of 300 g/head contained 17.51% protein, 17.72% fat, 62.36% moisture content, and 1.33% ash content.

Research into developing new products is needed to create higher-value products, since eel is currently sold only in frozen form. Processed eel products in Japan, among others, in the form of kabayaki (product marinated in kabayaki sauce, with added salt and vinegar), shirayaki (marinated, with added salt), and smoked products, have not been widely developed in Indonesia. This product is expected to increase the variety of eel products and increase the selling value of eel products already on the market. Eel is rich in macronutrients, especially protein, which plays a vital role in determining its nutritional quality (Cheng et al., 2020). Analysis of amino acid profiles and protein digestibility can simultaneously provide comprehensive information on the nutritional quality of processed eel products, enabling their use for product development and nutritional improvement, and the further development of more nutritionally optimal processed eel products (Gomez-Limia et al., 2021a). A complete and balanced amino acid profile generally has a high protein digestibility value; conversely, an unbalanced amino acid profile or the presence of limiting amino acids can reduce protein digestibility value (Sun et al., 2022). Knowing the amino acid profile and protein digestibility value of processed eel products allows evaluation of the nutritional quality and potential for protein absorption by the body.

Several researchers have conducted in vitro studies on the protein digestibility of processed eel products (*Anguilla* sp.). Huang et al. (2019) examined the value of in vitro protein digestibility in smoked eel products using a simulated gastrointestinal digestion method with pepsin and pancreatin. The results showed that the in vitro protein digestibility of smoked eel was around 86-89%. While research by Mengyin et al. (2022) showed that the digestibility of eel proteins can vary significantly depending on the processing method, for instance, heating eel muscle proteins with eel muscle oil significantly increased the levels of free amino acids in the digested products, indicating enhanced digestibility. Another study by Bhat et al. (2021) compared the in vitro protein digestibility value between fresh, fried, and steamed eel using the gastrointestinal digestion simulation method. The results showed that the in vitro protein digestibility values for fresh eel, fried eel, and steamed eel were 90%, 87%, and 88%, respectively. Roasting has been widely used as a culinary technique to improve the digestibility of eel (Chu et al., 2024). Various cooking methods, including microwaving, roasting, and frying, decreased water content and increased protein and fat content. Overall, processing techniques significantly affected the fatty acid composition of eel, potentially altering its nutritional value.

Research on the amino acid profile and digestibility has been conducted for various cooking methods; however, no studies have been conducted on processed eel products, such as kabayaki and shirayaki. The purpose of this study was to determine the nutritional value, amino acid profile, and digestibility of processed eel products.

Material and Methods

Materials and Equipment

The eels (*Anguilla* sp.) were obtained from farmers in Yogyakarta, Indonesia, for the production of smoked eel, shirayaki, and kabayaki. Shirayaki sauce includes vinegar, salt, and soy sauce. Commercial kabayaki sauce consists of soy sauce (water, soybeans, wheat, salt), sugar, water, glucose syrup or corn syrup, salt, vinegar, modified starch, caramel-colored yeast extract, xanthan gum as a thickener, citric acid, and sodium benzoate.

Materials used for in vitro protein digestibility analysis included HCl 0.1 N (Merck Germany), NaOH 0.1N (Merck Germany), pepsin enzyme (Sigma), and TCA (Trichloroacetic acid) 20% (Merck Germany). Materials for HPLC analysis include methanol, sodium acetate, and triethylamine in the ratio of 3:3:4, acetonitrile at 60%, and 1 M sodium acetate buffer (Merck, Germany).

Equipment used for the preparation of eels included an electric oven (EYELA Windy Oven WFO-601SD, Japan), glassware, a stopwatch, a vacuum sealer (Maksipack), and digital scales (OHAUS). Equipment used for in vitro protein digestibility analysis included protein assay kits, drop pipettes, micropipettes, waterbath shaker (PRIO DSW-30), and centrifuge (Gemmy 12 Hole PLC-05). The equipment used for amino acid analysis were High Performance Liquid Chromatography (HPLC, Thermo Dionex UltiMate 3000), and the equipment used for mineral analysis were the Perkin-Elmer 3110 Atomic Absorption Spectrophotometer and spectrophotometer UV-Vis (Genesys 10S UV-Vis) for iron and phosphorus analysis.

Product Preparation

Product preparation used the Tomo (2021) method with modifications. Preparation of smoked eel began by cutting the skinless eel into small pieces, soaking it in a 5% salt solution for 2 hours, and draining it for 2 hours. The fish was soaked in a 10% liquid smoke solution for 1 minute, then drained for 30 minutes, baked at 90°C for 2 hours, stored at room temperature, and ready to serve.

Kabayaki preparation began with filleting, followed by cutting and marinating in vinegar and salt for 30 minutes. All kabayaki sauce ingredients were mixed and cooked over medium heat until boiling. The fish was grilled on a non-stick pan for 5 minutes at 150°C until medium-cooked, then evenly coated with kabayaki sauce. The kabayaki sauce was poured over the fish, which was left to rest for 10 minutes, then served.

The shirayaki preparation started with skinless fish cut into small pieces, which were then marinated in salt for 30 minutes. Thereafter, the fish was grilled in a non-stick pan until cooked, for about 15 minutes at 150°C.

Nutritional Composition Analysis

Moisture content was measured according to AOAC (2005) with modifications, using the moisture analyzer. Ash content analysis was carried out in accordance with the Indonesian National Standard [Standar Nasional Indonesia] (SNI 01-2354.1-2010) procedures from National Standardization Agency of Indonesia [BSN] (2010). Protein content analysis based on the Indonesian National Standard (SNI 01-2354.4-2006), determines protein content using the total nitrogen method in fishery products (BSN, 2006). Fat analysis was carried out using the Soxhlet extraction method according to Indonesian National Standard SNI 01-2354.3-2017 (BSN, 2017). Albumin determination was conducted according to Sudarmadji et al. (1989).

In Vitro Protein Digestibility Testing (Hadiwiyoto & King, 1994; SNI 01-2354.4-2006)

In vitro protein digestibility testing was carried out in two stages, namely total N analysis using the Micro-Kjeldahl method and in vitro protein digestibility analysis. The total N analysis is the same as the protein test. The next step is to analyze the in vitro protein digestibility value. First, 200 mg samples were pulverized and dissolved in 9 ml of 0.2 N Walpole Buffer, pH 2, and then 2% pepsin enzyme was added. Then incubated in a 100 rpm waterbath shaker at 37°C for 5 hours. The sample was then centrifuged at 3000 rpm for 20 minutes, and 5 ml of supernatant was transferred to a test tube containing 5 ml of 20% TCA. The mixture was allowed to stand for 16 hours, and the filtrate was analyzed for total N content. The following formula can calculate protein digestibility:

$$\text{Digestibility (\%)} = \frac{\text{total N of filtrate}}{\text{(total N of material)}} \times \text{fp} \times 100\%$$

Where: fp is the dilution factor, total N of filtrate is the protein content after the digestion reaction (%), and the total N of the material is the protein content of the material before the digestion reaction (%).

Mineral Content Testing with Atomic Absorption Spectroscopy (AAS) (Perkin Elmer, 1994)

Testing of Mg, Zn, and Ca mineral content was carried out using the Atomic Absorption Spectroscopy (AAS) method with a Perkin-Elmer 3110 Atomic Absorption Spectrophotometer. First, prepare the standard, sample, and blank solutions using several dilution steps. Then,

the Hollow Cathode Lamp (HCL) for the element to be measured is installed on the device, and the current is adjusted according to the settings on each HCL. Then, the wavelength is set according to the element to be measured (Mg = 285 nm, Zn = 215 nm, and Ca = 422 nm). Next, set the energy mode to the maximum energy (visible on the display), then press the Gain button to save the set energy. In addition, the integration time (reading time distance in seconds) and replicate (repeat reading) are set according to the conditions of the element to be measured in the "options" section. Another factor to consider is the nebulizer system on the tool, including the passage of HCL rays, burner height, capillary channels, and burner cleanliness. The gas and air conditions are set to gas = 2 L/min and air = 4 L/min. Then, the oxidant button is turned to the air position, and the ignite button is pressed until the burner ignites. Next, press the continue button to display the measured solution's absorbance. Next, a standard solution was prepared for absorption optimization. Optimization is performed by adjusting the air and gas flow rates, slit width, burner height, and injection flow rate, which are then reflected in several uptakes. Of the several uptakes, there is a condition (uptake) that is considered the most ideal for analysis. Then, the data button is pressed to start the measurement, the injection hose is inserted into the blank solution, and the A/Z (auto zero) button is pressed. Next, the Read button is pressed to measure the solution and sample, obtaining standard concentration and absorbance data that can then be used to determine the regression equation and sample concentration.

Iron Analysis (Skoog et al., 2013)

The method used to analyze the iron (Fe) content in the sample is the spectrophotometric method described by Skoog et al. (2013). This method can determine iron levels by forming colored complexes. The test step first involves burning a 5-gram sample that has been mashed in a muffle furnace. Then, the resulting ash was dissolved in HNO_3 1:3 up to 50 ml while being crushed in a porcelain mortar. The mixture was then filtered through filter paper. Next, 1 ml of the clear filtrate obtained was placed in an Erlenmeyer flask, and 2 ml of 1.5 M ammonium thiocyanate was added. If the mixture changed color to red, it showed the sample contains iron (Fe). The solution was diluted to 10 ml with distilled water, and the absorbance was measured with a wavelength of 510 nm using a spectrophotometer (Genesys 10S UV-Vis). An iron standard curve was made to obtain a regression equation.

Phosphorus Analysis (AOAC, 2005; Skoog et al., 2013)

The method used to analyze the phosphorus content in fish samples was the Vanadate-Molybdate

spectrophotometric method. This method could determine phosphorus levels by forming colored complexes. This test began by preparing a vanadate-molybdate solution first. A total of 20 grams of ammonium molybdate was dissolved in distilled water. In addition, 1 gram of ammonium metavanadate was dissolved in 300 ml of hot distilled water, and 140 ml of concentrated HNO3 was added.

Furthermore, the ammonium molybdate solution was mixed with the ammonium vanadate solution, and the mixture was diluted to 1 liter. A 5-gram sample was placed in a porcelain crucible and heated in a furnace until ash formed. Then, the ash was dissolved in a 1:3 HNO3 solution to a volume of 50 ml while being crushed in a porcelain mortar. The solution was filtered through filter paper until a clear filtrate was obtained. Next, 1 ml of the clear filtrate was put in a test tube, and 3 ml of vanadate-molybdate solution was added. In the process, there will be a color change; if the mixture is yellow, then it contains phosphorus. Then, the discolored mixture was diluted to 10 ml with distilled water. Then, the solution was vortexed, and the absorbance was measured using a spectrophotometer (Genesys 10S UV-Vis) with a wavelength of 410 nm. A standard curve of phosphorus was made to obtain a regression equation. Phosphorus levels are known with the data obtained from the absorbance results, according to the following formula:

$$\text{Posphorus Content (\%)} = \frac{(X \times fp)}{m} \times 100\%$$

Where: fp is the dilution factor, X is obtained from the regression equation (x=((y-a))/b), and m is the sample weight (mg).

Potassium Analysis (Thompson, 1964)

Analysis of potassium content in fish samples was carried out using the gravimetric method. The first step of the test is to put a sample weighing 5-10 grams into a 100 ml Erlenmeyer flask, dilute to 100 ml with distilled water, and then filter through filter paper. Then, 10 ml of filtrate is placed in an Erlenmeyer flask heated in the oven, and 5 ml of a 1:2 perchloric acid solution is added. Next, the mixture was heated in a fume hood until a white precipitate formed. The white precipitate formed was then washed with 20 ml of ethanol 3 times, each time slowly. Furthermore, the precipitate in the Erlenmeyer was heated in an oven at 105°C until constant weight, then weighed. The following formula calculates the determination of potassium content:

$$\text{Potassium content (\%)} = \frac{(b-a) \times fp \times 0.3399}{m} \times 100\%$$

Where: B is the final constant weight (g); a is the empty weight of the Erlenmeyer (g); and fp is the dilution factor, 0.3399 is a constant, and m is the weight of the sample (g).

Amino Acid Content Testing (Settle, 1997)

Amino acid content in fish was analyzed by HPLC (High-Performance Liquid Chromatography) using a Thermo Dionex UltiMate 3000. The first step of the test is to add 60 mg of the sample to 4 ml of 6 N HCl, heat at 110 °C for 24 hours, and then cool to room temperature. Then, the mixture was neutralized to pH 7 with 6N NaOH, and aquabides were added to a volume of 10 ml, then filtered with a Whatman 0.2 μm filter paper. Next, 50 μL of the filtration result was combined with 300 μL of OPA (Ortho-phthalaldehyde) solution and stirred for 5 minutes. The mixture was injected into the HPLC injector at 10 μL, and data on amino acid types will be obtained. The column used was LiChrospher 100 RP-18 (5 μm), with mobile phase A consisting of a mixture of CH_3O: 50 mM sodium acetate: tetrahydrofuran in a 2:96:2 ratio at pH 6.8, and mobile phase B being 65% CH_3OH. The flow rate was 1.5 ml/min, with elution gradients at 0.1, 15, and 30 minutes for pump A (100, 0, 0) % and pump B (0, 35, 100) %, respectively, and the process stopped at 35 minutes. The detector used was the Thermo Dionex UltiMate 3000 RS Fluorescence Detector, with an excitation wavelength of 300 nm and an emission wavelength of 500 nm.

Data Analysis

The nutritional content and in vitro protein digestibility testing were repeated three times. The results from the nutritional content and protein digestibility tests were checked for normality using the Shapiro-Wilk test and then analysed using one-way analysis of variance (ANOVA) at the 95% confidence level. If significant differences were found, further analysis was conducted using Duncan's Multiple Range Test (DMRT) at the 95% confidence level.

Results and Discussion

In this study, eels were processed into kabayaki, shirayaki, and smoked eel. Unagi Shirayaki is an intermediate product used as an ingredient for making Unagi Kabayaki. Shirayaki is made with salt, with the expectation that it will be easier to further modify before roasting. Unagi Kabayaki is one of the Japanese eel products. The process involves splitting the eel, removing its entrails and bones, followed by grilling. Kabayaki is one way to cook fish in Japanese cuisine. After splitting and removing the guts and bones, the fish is skewered with iron/bamboo skewers and served with a sauce made from a mixture of soy sauce, mirin, sugar, and sake. In this study, we modified the processing by incorporating halal Indonesian sauce. Kabayaki, shirayaki, and smoked eel are presented in Figure 1.
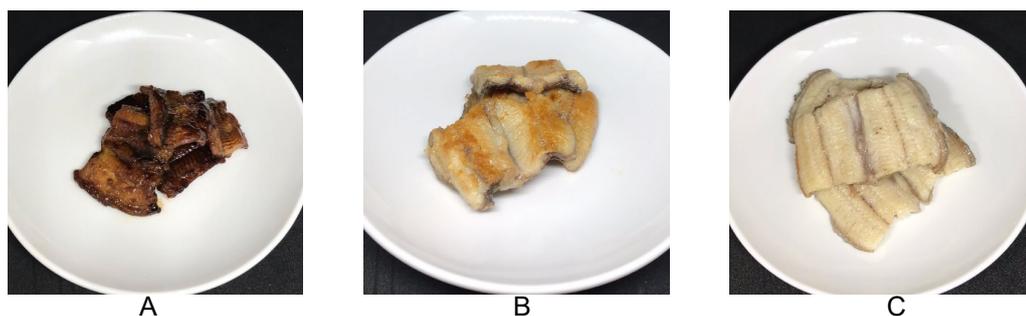
Figure 1: Various processed eel A. Kabayaki, B. Shirayaki, C. Smoked

The results in Table 1 show that processing affects (P<0.05) the nutritional composition of eel. Processing of eel into smoked eel, shirayaki, and kabayaki significantly affects the proximate composition, which includes water content, fat, protein, and ash. This result is consistent with research by Widyasari et al. (2014), which shows that processing decreases water, fat, and protein content.

Smoking followed by baking at 90°C for 2 hours in this study generally results in a decrease of moisture content. Baking generally leads to a decrease in moisture content in fish, one study on Asian sea bass (*Lates calcarifer*) reported a decrease in moisture content after baking (Marimuthu et al., 2014). Different cooking methods, including baking, consistently reduce moisture content, with greater decreases at higher temperatures and longer exposure (Biandolino et al., 2023). The grilling process in shirayaki generally results in moisture loss, similar to smoking. Research indicated that direct heating caused water to evaporate, potentially resulting in a drier product than raw eel. The kabayaki method tends to retain more moisture content than shirayaki, as there is a process of adding liquid seasoning before grilling.

Based on the data in Table 1, it can be concluded that processing methods such as smoking, shirayaki, and kabayaki decrease protein content in eel compared to fresh eel. The highest protein content was found in fresh eel, followed by smoked eel, and there was no significant difference between shirayaki and kabayaki. These results are in line with fat content, but in contrast with ash content, where shirayaki and kabayaki tend to have higher ash content.

Fresh eel has the highest protein content (38.47 ± 0.25% db). Smoking reduced the protein content to 34.95 ± 0.36% db. This decrease can be caused by protein denaturation and degradation due to exposure to high temperatures, as well as by smoke, which can induce structural changes, protein degradation, changes in amino acid profiles, and possible loss of some water-soluble proteins (Yunika et al., 2023). Shirayaki experienced a further decrease in protein content to 33.29 ± 0.07% db. Shirayaki involves roasting without sauce, which can lead to further protein denaturation and protein loss due to the Maillard reaction and dehydration (Cheng et al., 2020). Kabayaki involves grilling with sweet seasonings, which can accelerate the Maillard reaction, leading to more protein degradation than in shirayaki (Han et al., 2022).

Smoking did not significantly affect ash content, as the mineral content remained relatively stable. After smoking, the ash content did not change significantly, at 3.30 ± 0.14% (db). This indicates that the smoking process does not affect the mineral content of eel, despite the decrease in moisture content. Some studies have shown that smoking affects sensory characteristics and fat content more than ash content (García-Arias et al., 2003). The ash content of eels increases after being processed into shirayaki and kabayaki, mainly because heat removes water, causing minerals to concentrate

Table 1. Nutritional composition and albumin of eel preparations, mean ±SD (n=3, p<0.05)

Type of Processed	Water (%)	Protein (db)	Ash (db)	Fat (db)	Albumin (db)
Fresh eel	65.31 ± 0.19[d]	38.47 ± 0.25[c]	3.48 ± 0.26[a]	58.75 ± 0.29[d]	3.60 ± 0.02[d]
Smoked eel	47.21 ± 0.09[c]	34.95 ± 0.36[b]	3.30 ± 0.14[a]	53.73 ± 0.19[c]	1.37 ± 0.01[b]
Shirayaki	38.39 ± 0.14[a]	33.29 ± 0.07[a]	10.69 ± 0.17[c]	45.76 ± 0.22[a]	0.86± 0.01[a]
Kabayaki	41.22 ± 0.02[b]	33.12 + 0.19[a]	9.79 ± 0.11[b]	46.23 ± 0.19[a]	2.25 ± 0.01[c]

Notes: Different letters in the same column indicate not significantly different at p<0.05.

in the tissue. Shirayaki, which is grilled without sauce, loses more water, resulting in a higher ash content than kabayaki. Conversely, the sweet sauce of kabayaki helps retain moisture and slightly reduces the concentration of minerals or ash. The use of salt in shirayaki also affects the mineral content, so shirayaki generally has a higher ash content than kabayaki (Ersoy & Özeren, 2009; Bastías et al., 2017; Marimuthu et al., 2012).

In shirayaki, the ash content increased significantly to $10.69 \pm 0.17\%$ (db). This increase is likely due to the drastic reduction in water content due to the direct roasting process. With less water, the mineral content becomes more concentrated, resulting in higher ash content (Rochima & Damayanti, 2020; Ciptawati et al., 2021). In kabayaki, the ash content was slightly lower than in shirayaki, at $9.79 \pm 0.11\%$ (db). This could be due to adding sauce during roasting, which can cause some minerals to dissolve in the sauce or undergo a Maillard reaction.

All processing methods reduced fat content compared to fresh eel. Fat content decreased to $53.73 \pm 0.19\%$ (db) after smoking. This decrease is likely due to the heating process, which releases fat from fish tissue, primarily through melting and evaporation during smoking (Linus-Chibuezeh et al., 2022). Shirayaki had lower fat content than smoked eel, at $45.76 \pm 0.22\%$ (db). The direct roasting process in shirayaki results in greater fat loss, mainly due to the high temperature and direct exposure to heat, which accelerates fat release (Ersoy, 2011). Kabayaki had a fat content of $46.23 \pm 0.19\%$ (db), slightly higher than shirayaki. This increase compared to shirayaki is likely due to the use of sauce during the grilling process. The caramelized coating of kabayaki sauce prevents rendered fat from escaping during grilling, resulting in a higher fat content than shirayaki, which is grilled plain and allows oil to drip off (Biandolino et al., 2023).

From Table 1, it can be seen that albumin levels decreased significantly after processing, either through smoking, shirayaki, or kabayaki. Albumin is a water-soluble protein that is essential in the structure and function of fish tissues (Nurfaidah et al., 2021). Albumin

levels vary between eel species, with *Anguilla marmorata* having higher levels than *Anguilla bicolor* (Putri et al., 2016; Jamaluddin et al., 2020). Fresh eel has the highest albumin content, which is $3.60 \pm 0.02\%$ (db). After smoking, the albumin content dropped dramatically to $1.37 \pm 0.01\%$ (db). This decrease is likely due to protein denaturation due to heating, as well as the possible dissolution of some albumin into the dripping liquid during smoking (Fuentes et al., 2010). Shirayaki had the lowest albumin content, which was $0.86 \pm 0.01\%$ (db). This could be due to the direct roasting process at high temperatures, which causes greater protein denaturation, as well as possible albumin loss from liquid release during cooking. Kabayaki had higher albumin levels than shirayaki, which was $2.25 \pm 0.01\%$ (db). This difference is likely due to the sauce used in kabayaki, which can reduce evaporation and protein loss during the cooking process (Haq et al., 2018)

In general, processing methods alter mineral content due to reduced moisture, altered tissue structure, and possible mineral loss during heating or liquid release. Phosphorus content was highest in smoked eel (267.88 mg/100 g), higher than in fresh eel (220.72 mg/100 g), and decreased in shirayaki (207.36 mg/100 g) and kabayaki (183.30 mg/100 g). The increase in P content in smoked eel is likely due to the reduction in moisture content, which causes the phosphorus concentration to increase (Linus-Chibuezeh et al., 2022). Roasting (shirayaki and kabayaki) may cause phosphorus loss due to the release of liquid during heating (Kiczorowska et al., 2019).

The Fe content of smoked eel (4.49 mg/100 g) was not significantly different from shirayaki (4.32 mg/100 g), with higher concentrations than fresh eel (3.32 mg/100 g). In comparison, Fe levels decreased dramatically in kabayaki (2.19 mg/100 g). Iron in fish is predominantly present as heme iron, which is tightly bound within proteins such as myoglobin and hemoglobin. These protein-iron complexes are extremely stable and do not degrade when cooked. Therefore, medium- to high-heat cooking methods such as smoking and grilling don't significantly alter the fish's iron content. Regardless of

Table 2. Mineral Content and Protein Digestibility of Processed Eel, mean ±SD (n=3, p<0.05)

Type of Processed	Minerals						Protein Digestibility Value (%wb)
	P (mg/100 g)	Fe (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)	Zn (mg/100 g)	K (mg/100 g)	
Fresh	220.72 ± 0.25[c]	3.32 ± 0.17[b]	41.10 ± 0.58[c]	36.92 ± 1.40[c]	4.84 ± 0.10[a]	197.16 ± 1.46[c]	64.49 ± 0.77[a]
Smoke	267.88 ± 0.15[d]	4.49 ± 0.71[c]	45.38 ± 0.48[d]	40.92 ± 0.33[d]	5.95 ± 0.46[b]	282.28 ± 1.99[d]	67.59 ± 0.78[b]
Shirayaki	207.36 ± 2.82[b]	4.32 ± 0.17[c]	30.88 ± 0.67[b]	34.00 ± 0.16[b]	6.76 ± 0.50[c]	126.30 ± 1.88[a]	64.44 ± 0.31[a]
Kabayaki	183.30 ± 0.67[a]	2.19 ± 0.40[a]	27.88 ± 1.66[a]	32.61 ± 0.45[a]	6.82 ± 0.51[c]	180.71 ± 2.26[b]	63.60 ± 1.10[a]

Notes: Different letters in the same column indicate a significantly different at p<0.05.

the cooking method, this stability maintains the iron content (Turhan et al., 2004). Smoking can increase Fe levels by reducing water content and increasing mineral concentration (Linus-Chibuezeh et al., 2022). Roasting of processed kabayaki causes Fe loss due to the release of liquid during heating (Islam et al., 2020). In addition, Charles et al. (2011) reported that Fe is soluble in water, so the roasting process, which releases liquid, can reduce the iron content in the final product. Islam et al. (2020) also explained that overheating can convert Fe to a form that is more difficult for the body to absorb.

Calcium (Ca), magnesium (Mg), and potassium (K) contents were highest in smoked eel compared to fresh eel, and decreased significantly in shirayaki and kabayaki. Smoking increases the levels of Ca, Mg, and K due to reduced moisture content (Linus-Chibuezeh et al., 2022). Roasting can cause loss of Ca, Mg, and K due to the release of liquid during the process, especially K, which is highly soluble in water. Ca and Mg are generally more stable during roasting and less likely to be lost compared to potassium (Marimuthu et al., 2012; Uran & Gokoglu, 2014).

Zinc (Zn) content was highest in kabayaki (6.82 mg/100 g) and shirayaki (6.76 mg/100 g), and tended to decrease in smoked eel (5.95 mg/100 g) compared to fresh eel (4.84 mg/100 g). Kabayaki marinated with sauce may increase Zn retention due to complex reactions with sauce components. Some minerals may be better bound in protein tissues after roasting with seasonings, resulting in more stable Zn in the final product. Most Zn in fish exists as metalloenzymes or Zn-protein complexes that are not disrupted by moderate heating or surface caramelization. Although kabayaki and shirayaki are processed with different sauces, they are exposed to similar heat and retain minerals well. Zinc is a stable and protein-bound element, so the Zn content did not significantly change after grilling or baking, but a longer smoking process tends to reduce the zinc content (Ersoy & Ozeren, 2009). Marimuthu et al. (2012) explained that heating at high temperatures can increase the bioavailability of Zn in food by reducing the complex of Zn with certain proteins, making more Zn available in the free form.

The results in Table 2 show that the highest protein digestibility value was found in smoked eel. Research shows that fish processing methods, such as smoking and roasting, can affect the protein structure and the rate of digestion in fish. Thermal denaturation can alter protein structure by breaking hydrogen and hydrophobic bonds (Swastawati et al., 2013; Atowa et al., 2014). In addition, relatively long high-temperature cooking during smoking causes protein denaturation, which can open the tertiary structure and expose peptide bonds to protease enzymes

(Abraha et al., 2018). Shirayaki and Kabayaki have lower protein digestibility values and are not significantly different from fresh eel, as over-roasting can cause a decrease in protein solubility, making it more difficult to digest by digestive enzymes (Atowa et al., 2014). In addition, processing Shirayaki and Kabayaki eel resulted in excessive protein denaturation at high temperatures. The Maillard reaction, combined with roasting and sugar-containing sauces, especially in Kabayaki, can form complex compounds that reduce protein digestibility, resulting in a lower protein digestibility value (Rochima & Damayanti, 2020). In the smoking process, a moderate Maillard reaction occurs, making it easier to digest than roasting. The duration of roasting affects protein levels, thereby altering amino acid composition and digestibility. Over-roasting fish (≥100°C, for a long time) reduces protein solubility through hydrophobic aggregation and disulfide cross-linking, while oxidation makes the protein resistant to enzymatic cleavage. These structural and chemical changes collectively reduce nutrient bioavailability, as evidenced by in vitro digestion models (Hu et al., 2018). Optimal roasting should balance cooking safety with minimal heat exposure to maintain protein quality.

Table 3 shows that there was no significant difference among processing treatments, with the highest concentration being leucine, followed by valine, threonine, and isoleucine. The amino acid composition in this study showed a pattern consistent with previous research. Heating eels tends to raise the levels of stable amino acids like valine and isoleucine, which is due to concentration or partial hydrolysis during heating. Similar results were reported by Gomez-Limia et al. (2020) in canned European eels (*Anguilla anguilla*), where frying and sterilization increased the levels of these amino acids. Conversely, heat-sensitive amino acids such as lysine and glycine showed a decrease, in line with the observations of Gomez-Limia et al. (2021b), who found that prolonged heating and increased body weight in European eels reduced the content of these amino acids. Glycine and lysine are also altered by processing methods like smoking and roasting (Gómez-Limia et al., 2021b; Swastawati et al., 2023). The results of this study are consistent with those of Akintola (2015), who showed that smoking can affect protein structure and essential amino acid content, but, in general, smoking does not significantly reduce amino acid content. In some fish, smoking can increase the content of certain amino acids such as alanine, threonine, tyrosine, and cysteine. Roasting, on the other hand, can cause the Maillard reaction, which involves amino acids and reducing sugars. This reaction can reduce the availability of some essential amino acids, such as lysine and arginine, in the kabayaki process, as

Table 3. Amino acid composition (%) of processed eel products (mean ±SD (n=3, p<0.05))

Amino Acids	Fresh	Shirayaki	Kabayaki	Smoke
Essential Amino Acids				
Valin	5.29 ± 0.56[a]	6.24 ± 0.34[b]	5.96 ± 0.63 [ab]	5.76 ± 0.27 [ab]
Leucine	7.05 ± 0.49[a]	7.83 ± 0.48[a]	7.56 ± 0.81[a]	7.48 ± 0.18[a]
Lysine	2.27 ± 0.32[b]	1.83 ± 0.47[a]	1.84 ± 0.23[a]	1.75 ± 0.12[a]
Histidine	1.72 ± 0.26[a]	2.02 ± 0.21[a]	1.98 ± 0.19[a]	2.02 ± 0.08[a]
Threonine	5.51 ± 0.66[a]	5.95 ± 0.79[a]	6.32 ± 0.69[a]	5.92 ± 0.10[a]
Methionine	2.59 ± 0.51[a]	2.54 ± 0.52[a]	1.53 ± 0.68[a]	2.57 ± 0.46[a]
Phenylalanine	2.69 ± 0.15[a]	2.80 ± 0.18[a]	2.71 ± 0.09[a]	2.71 ± 0.01[a]
Isoleucine	4.02 ± 0.48[a]	5.16 ± 0.32[b]	4.72 ± 0.69 [ab]	4.90 ± 0.26 [ab]
Conditionally Essential Amino Acids				
Arginine	6.02 ± 0.26[b]	6.25 ± 0.43[b]	5.39 ± 0.45[a]	5.86 ± 0.11 [ab]
Glycine	13.71 ± 2.89[b]	8.93 ± 2.51[a]	11.13 ± 6.10[a]	9.87 ± 0.87[a]
Non-Essential Amino Acids				
Aspartic Acid	11.17 ± 0.20[a]	12.38 ± 1.09[a]	12.12 ± 2.30[a]	12.63 ± 0.26[a]
Glutamic Acid	18.64 ± 0.64[a]	19.55 ± 1.17[a]	19.48 ± 2.01[a]	19.53 ± 0.35[a]
Serin	5.91 ± 0.40[a]	5.89 ± 0.43[a]	5.93 ± 0.34[a]	5.78 ± 0.18[a]
Alanine	10.68 ± 0.76[a]	9.78 ± 0.82[a]	9.62 ± 0.88[a]	10.13 ± 0.61[a]
Tyrosine	2.38 ± 0.14[a]	2.71 ± 0.18[a]	2.39 ± 0.35[a]	2.58 ± 0.13[a]

Notes: Different letters in the same row indicate a significantly different at p<0.05.

they are involved in the reaction (Kchaou et al., 2019; Li et al., 2022). Non-essential amino acids showed that glutamic acid had the highest concentration, followed by aspartic acid and alanine. In this study, processing was found to have no significant effect on the concentration of non-essential amino acids, consistent with Islam et al. (2020) in their research on mud eel processing.

Conclusion

The results indicated that the processing affected the nutritional value, albumin, minerals, and amino acids. Fresh eel had the highest protein content, followed by smoked eel, while there was no significant difference between shirayaki and kabayaki. The protein digestibility was highest in smoked (67.59%), but there was no significant difference among the others (63.30-64.44%). Calcium (Ca), magnesium (Mg), and potassium (K) contents were highest in smoked eel compared to fresh eel and decreased significantly in shirayaki and kabayaki, but for zinc (Zn), shirayaki and kabayaki tend to be higher than smoked eel. An analysis of essential amino acids showed no significant difference between the processing methods. Leucine had the highest concentration, followed by valine, threonine, and isoleucine, with concentrations ranging from 4.02% to 7.83%. The research found that the best processing method was smoked, and there was no significant difference between kabayaki and shirayaki.

Acknowledgments

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