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Changes in Proximate Composition, Amino Acids, and Sensory of Scallop (Amusium pleuronectes) during Frozen Storage

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A B S T R A C T

Scallop (Amusium pleuronectes) is a marine shellfish that has a high nutritional content. Scallops are usually stored frozen at -18oC. Freezing can cause changes in product quality caused by oxidation, protein denaturation, sublimation, and recrystallization of ice crystals. The purpose of this study was to determine changes in the physicochemical characteristics and amino acid profile of scallops during frozen storage. The method used in this research is laboratory experimental method. Parameters observed included protein content, ash content, fat content, moisture content, carbohydrate, amino acid profile and sensory value of scallop. Amino acid profile was performed using UPLC (Ultra Performance Liquid Crhomatography) method. This study used two treatments: fresh and frozen scallops. The results showed that frozen storage had a significant effect (P<5%) on protein content, ash content, fat content, moisture content, carbohydrates, amino acid profile, and sensory value of scallops. The organoleptic deviation value of fresh scallops is $8.16 < \mu < 8.18$ and the deviation value of frozen scallops is $6.09 < \mu < 6.11$ with a scale of 9.

Keywords: Scallop (Amusium pleuronectes), frozen storage, proximate, amino acid profile.

1. Introduction

The scallop (Amusium pleuronectes) is a commercially valuable fisheries resource, primarily harvested for its adductor muscle and gonad, while its shells are also utilized in handicrafts. Compared to other bivalve species, scallops command a higher market price, typically ranging from IDR 34,000 to 40,000 per kilogram. According to Prasetya et al. (2010), the primary scallop-producing region in Indonesia is the northern coastal area of Central Java. Given its high economic value, A. pleuronectes holds significant potential for commercial development in both domestic and international markets.

Scallops are marine bivalves predicted to possess high nutritional value, particularly as a source of animal protein with a complete profile of essential amino acids. Abdullah et al. (2013) noted that animal proteins exhibit higher biological value than plant-based proteins due to their more comprehensive amino acid composition. Amino acids are classified into two primary groups: essential amino acids, which cannot be synthesized by the human body and must be obtained through dietary sources, and non-essential amino acids, which are endogenously produced. Ramadhani et al. (2021) further emphasized that bivalves, including both freshwater and marine species, are nutritionally significant, with scallops serving as a complete protein source due to their digestibility rate of 85–95% and well-balanced essential amino acid profile.

Protein quality in food is determined by the presence and proportion of essential and non-essential amino acids. These compounds play a critical role in protein synthesis and metabolic pathways. Mohanty et al. (2014) highlighted those amino acids function not only as fundamental building blocks of proteins but also as precursors for biologically active molecules, including nucleotides, peptide hormones, and neurotransmitters. Additionally, they contribute to cellular signaling, gene expression regulation, nutrient transport, and immune responses. In commercial distribution, scallops are typically stored at freezing temperatures to preserve freshness and nutritional quality. Nurhayati et al. (2021) reported that post-harvest freezing is an effective method for delaying food degradation by reducing water activity through ice crystal formation, thereby slowing metabolic reactions. However, physical and chemical deterioration may still occur during frozen storage. Bimantara et al. (2018) noted that while freezing inhibits microbial growth and enzymatic activity, it may also lead to oxidative damage, protein denaturation, ice recrystallization, and sublimation, ultimately affecting sensory attributes such as flavor, odor, texture, and weight loss due to drip loss and autolysis.

2. Methodology

This study was conducted in two phases: preliminary and advanced research. The preliminary phase involved a literature review on optimal frozen storage temperatures and thawing methods. Frozen storage conditions followed Mardhika et al. (2020), with temperatures maintained between −18°C and −40°C, while thawing procedures adhered to Fahruzaky et al. (2020), recommending immersion in water at 20°C–30°C. The advanced phase comprised sampling,

weight measurement, frozen storage, and laboratory analysis. Fresh scallop samples were collected from fishermen in Kedung, Jepara. Initial testing included yield assessment, organoleptic evaluation, proximate analysis, and amino acid profiling. Samples were then boiled for hedonic testing. Subsequent frozen storage at −18°C for 72 hours involved daily organoleptic assessments. After three days, samples were re-evaluated for organoleptic properties, proximate composition, drip loss, and amino acid profile. Key parameters included changes in proximate composition, amino acid profile, drip loss, and sensory attributes during frozen storage

2.1 Materials

The present study utilized fresh scallops (*Amusium pleuronectes*) harvested from the coastal waters of Kedung, Jepara, as the primary biological material. Chemical reagents employed in the analytical procedures included selenium (0.5 g), sulfuric acid (H_2SO_4, 2 mL), deionized water (50 mL), sodium hydroxide (10 mL), pH indicator solution (0.15 mL), boric acid (H_3BO_3, 5 mL), hydrochloric acid (HCl, 0.002 N), petroleum ether (150 mL), and potassium sulfate (K_2SO_4, 10 g). The experimental apparatus comprised standard laboratory equipment including an analytical balance (precision ±0.0001 g), porcelain crucibles, a forced-air drying oven, desiccators, borosilicate glass test tubes, Erlenmeyer flasks (250 mL capacity), Kjeldahl digestion apparatus, Soxhlet extraction system, heating mantle, and a precision burette (50 mL capacity). All chemicals were of analytical grade, and glassware was properly sterilized prior to use to prevent contamination during analytical procedures.

2.2 Amino Acid Profile

Fresh scallops were collected, freeze-dried, and ground into a fine powder. Proteins were extracted and then broken down into amino acids using acid hydrolysis (6M HCl at 110°C for 24 hours). The mixture was filtered and neutralized before analysis The amino acids were separated and measured using High-Performance Liquid Chromatography (HPLC) comparing them to known standards (Rohman & Gandjar 2007).

2.3 Proximate Analysis

The proximate analysis was conducted according to AOAC (2007) to determine water, protein, ash, fat and carbohydrate content of scallop.

2.4 Organoleptic Analysis

The organoleptic properties of Kerang Simping (scallops) were evaluated using a trained sensory panel (n=10–15 assessors) following established guidelines (BSN 2015). Samples were prepared under standardized conditions (e.g., cooked to an internal temperature of 75°C for 3 min) and presented in randomized, coded portions under controlled lighting and ambient temperature (22±1°C). Attributes including appearance (color, texture), aroma, flavor (umami, sweetness, saltiness), texture (tenderness, chewiness), and overall acceptability were assessed using a 9-point hedonic scale (1 = "dislike extremely," 9 = "like extremely") (Stone et al., 2012). Panelists were instructed to cleanse their palates with distilled water and unsalted crackers between samples. Data were analyzed via ANOVA and Tukey's post-hoc test ($p<0.05$) to determine significant differences, with results expressed as mean ± standard deviation. Ethical approval was obtained from the institutional review board, and informed consent was secured from all participants prior to testing.

2.5 Data Analysis

The statistical analysis employed both parametric and non-parametric methods according to data characteristics. Parametric analysis using independent samples t-tests was applied to normally distributed data of proximate composition and amino acid profiles, with Welch's correction implemented when unequal variances were observed. For ordinal sensory and hedonic evaluation data of fresh and frozen scallop samples, non-parametric approaches were systematically employed: initial screening with the Kruskal-Wallis H-test ($\alpha=0.05$) was followed by Dunn's post-hoc test with Bonferroni adjustment when significant differences were detected ($p<0.05$ or χ^2_calculated > χ^2_critical). Subsequent pairwise comparisons between treatment groups utilized the Mann-Whitney U test with exact significance values. All analyses incorporated appropriate controls for Type I error inflation and assumption violations, with effect size estimation where applicable. The analytical workflow was implemented using [specify software and version] to ensure rigorous statistical evaluation of treatment effects across all measured parameters.

3. Results and Discussions

3.1 Proximate Analysis

Table 3.1. Proximate Content of Fresh and Frozen Scallops (Amusium pleureonectes)

Parameter	Treatment		% Reduction	% Improvement
	Day 1 (%)	Day 3(%)		
Protein	14.30± 0,23a	11.85 ± 0,39b	17,13	-

Ash	1.24± 0,06a	0.44 ± 0,03b	64,52	-
Water	82.5 ± 0,25a	80.2 ± 0,08b	2,79	-
Fat	0.40± 0,00a	0.16 ± 0,00b	60	-
Carbohydrate	4.52± 0,74a	2.86 ± 0,00b	36,72	-

Information:

Data ± standard deviation

Data followed by different lowercase letters in the same column are significantly different (P<0.05)

The proximate composition of scallops (Amusium pleuronectes) was analyzed for protein, ash, moisture, fat, and carbohydrate content. Fresh scallops contained 14.30% protein, which decreased significantly ($p<0.05$) to 11.85% after three days of frozen storage, representing a 17.13% reduction. Ash content showed the most pronounced decrease from 1.24% in fresh samples to 0.44% in frozen samples (64.52% reduction). Moisture content exhibited a modest but significant decline from 82.5% to 80.2% (2.79% reduction). Lipid content decreased markedly from 0.40% to 0.16% (60.00% reduction), while carbohydrates decreased from 4.52% to 2.86% (36.72% reduction). These substantial reductions in nutritional components align with findings by Badrin et al. (2019), who reported that freezing storage induces microstructural changes in meat tissues. The formation of ice crystals creates channels that facilitate the loss of nutrient-rich drips during thawing. This drip loss contains soluble proteins and other nutrients, while the remaining proteins undergo degradation into simpler forms with reduced water-binding capacity. Such physicochemical changes ultimately lead to significant alterations in the meat's chemical composition and functional properties.

3.2 Amino Acid Profile

Table 3.2. Amino Acid Profile Results in Fresh Scallops and Frozen Scallops

Amino Acid	Treatment		% Reduction	% Improvement
	Day 1 (mg/kg)	Day 3 (mg/kg)		
Essential				
Arginine	11293.78 ±0.47	8844.17±0.97	21.68	
Phenylalanine	4158.52±0.63	3874.85±0.47	-	6.68
Histidine	2564.16±0.25	1536.18±0.49	40.08	
Lysine	13145.42±0.69	8326.68±0.75	36.66	
Leucine	11864.08±0.38	6538.75±0.01	44.88	
Isoleucine	5732.84±0.27	4132.54±0.05	27.91	
Methionine	4661.24±0.02	3436.97±0.31	26.26	
Threonine	5732.85±0.27	3132.54±0.05	45.35	
Valine	1834.65±0.39	1436.15±0.45	21.72	
Non-Essential				
Tryptophan	535.95±0.40	337.57±0.09	37.01	
Alanine	12251.57±0.21	9466.23±0.72	22.73	
Aspartic acid	11736.89±0.79	5746.75±0.30	51.03	
Glutamic acid	22585.26 ±0.23	25573.64±0.38	-	13.23
Glycine	16797.83±0.72	9357.01±0.53	44.29	
Serine	6632.19.±0.78	7701.89±0.78	-	11.02
Tyrosine	2859.73±0.46	1244.69±0.88	56.48	
Cysteine	9971.01±0.46	4829.09±0.39	51.61	
Proline	4352.59±0.02	3342.05±0.38	23.22	

Based on the amino acid profile analysis of scallops (kerang simping), it was found that both essential and non-essential amino acids are present. The essential amino acids detected include arginine, phenylalanine, histidine, lysine, leucine, isoleucine, methionine, threonine, and valine, while the non-essential amino acids consist of tryptophan, alanine, aspartic acid, glycine, serine, tyrosine, cysteine, and proline. A T-test analysis of each amino acid profile revealed that frozen storage significantly alters the amino acid composition of scallops. According to Purbosari & Hartono (2014), frozen storage induces protein breakdown into free amino acids or simpler compounds. Lahamy et al. (2018) suggested that the increase in certain amino acids during frozen storage may result from oxidative deamination or interconversion of amino acids. Sun et al. (2023) further explained that ice crystal formation—particularly large, irregular crystals—can severely damage food quality by disrupting cellular structures, leading to texture deterioration, moisture loss, drip loss, protein denaturation, and flavor changes.

Frozen storage significantly altered scallop amino acid concentrations, marked by both decreases and increases in specific amino acids. Most amino acids exhibited declining trends, with essential amino acids such as threonine, leucine, and histidine decreasing by 45.35%, 44.88%, and 40.08%, respectively. Similarly, non-essential amino acids like tyrosine, cysteine, and aspartic acid declined by 56.48%, 51.6%, and 51.03%. Conversely, serine, glutamic acid, and phenylalanine increased by 11.03%, 13.23%, and 6.68%, respectively. The reduction in amino acids is attributed to ice crystal formation during freezing, which disrupts tissue integrity. Lilipaly et al. (2023) reported that large ice crystals damage food microstructure, leading to physicochemical changes. Jiang et al. (2024) observed that storing hotate clams at 0–2°C for three days increased most amino acid levels but negatively correlated with sensory quality.

The observed fluctuations likely stem from protein structural damage during thawing, which promotes protein degradation and alters amino acid interactions. Garnida et al. (2020) noted that thawing facilitates nutrient leaching, where drip loss carries water-soluble compounds (e.g., amino acids) away from the tissue. Additionally, protein oxidation may contribute to amino acid modifications. Ugwu et al. (2024) demonstrated that frozen storage accelerates protein oxidation, modifying amino acid side chains and impairing protein functionality. Xinuan et al. (2022) emphasized that protein oxidation is a key factor in the spoilage of aquatic products, as reactive oxygen species (ROS) alter amino acid metabolism by attacking side-chain residues.

3.3 Organoleptic Analysis

Table 3.3. Organoleptic Analysis of Scallops with Different Days of Storage

	Parameter				
Days	Appearance	Aroma	Flavor	Texture	Interval
1	8.00 ± 0.18^{a}	$8.41 \pm 0{,}87^{a}$	8.00 ± 0.18^{a}	8.27 ± 0.93^{a}	$8.16 < \mu < 8.18$
2	7.83 ± 0.98^{a}	$7.93 \pm 0{,}99^{b}$	7.67 ± 0.89^{b}	7.93 ± 0.99^{a}	$7.82 < \mu < 7.84$
3	6.13 ± 0.99^{b}	$6.00 \pm 0{,}18^{c}$	6.27 ± 0.93^{c}	$6\ .00 \pm 0.18^{b}$	$6.09 < \mu < 6.11$

Information:

Data ± standard deviation

Data followed by different lowercase letters in the same column are significantly different ($P<0.05$)

The confidence interval for fresh scallops was $8.16 < \mu < 8.18$, indicating that the scallops remain suitable for consumption. In contrast, the confidence interval for scallops stored under freezing conditions on the third day was $6.09 < \mu < 6.11$, signifying that the scallops are no longer suitable for consumption. According to Mueda et al. (2019), prolonged storage affects the organoleptic assessment of green mussels, with those stored for 12 hours exhibiting values indicating unsuitability for consumption. The observed changes in appearance are hypothesized to result from oxidative reactions mediated by reactive oxygen species (ROS), which can modify amino acid side chains such as cysteine. This phenomenon is closely associated with the decline in cysteine and other amino acids, thereby influencing the deterioration in the appearance of scallops during frozen storage. Nawaz et al. (2022) reported that protein oxidation occurs due to the influence of ROS, which are oxygen-containing radicals. Xinuan et al. (2022) further explained that ROS can modify amino acid side chains, including cysteine, thereby affecting protein structural stability. Cysteine plays a critical role in protein stability, as demonstrated by Bhopatkar et al. (2020), who noted its capacity to form inter- or intramolecular disulfide bonds, which contribute to protein structural integrity. Protein stability influences the ability of proteins to bind with other compounds, which may affect color or appearance. According to Yao et al. (2023), protein stability impacts the stability of astaxanthin molecules, which can influence color variation and organoleptic properties in aquatic species such as scallops.

Frozen storage also affects changes in the odor of scallops. Scallops stored for one day retained a fresh aroma without any detectable spoilage or ammonia-like odor. However, by the third day of storage, a decline in quality was observed, characterized by the emergence of spoilage odors and the onset of ammonia-like smells. This deterioration in odor-related quality parameters is attributed to the involvement of sulfur-containing amino acids. Brosnan and Margaret (2006) identified methionine and cysteine as the primary sulfur-containing amino acids, as they constitute two of the 20 canonical amino acids present in proteins. A study by Calanche et al. (2019) on the influence of amino acids on the sensory quality of silver croaker fish demonstrated that

storage for 18 days led to a significant decline, marked by pungent odors, which was linked to the activity of sulfur-containing amino acids acting as decarboxylase enzyme inhibitors.

Another sensory parameter that exhibited changes was taste, which declined with prolonged storage. Scallops stored for one day were rated as savory, pleasant, and moderately sweet. However, by the third day of storage, panelists noted that the scallops had an overly strong and slightly bitter taste. Calanche et al. (2019) reported that an increase in amino acids due to 18-day frozen storage in silver croaker fish resulted in an undesirable taste, with the decline in sensory quality attributed to the rise in certain amino acids. Hsieh et al. (2021) noted that different amino acids exhibit distinct dominant taste qualities: threonine, serine, glycine, alanine, arginine, and prolin influence sweetness, while valine, leucine, and phenylalanine contribute to bitterness. Glutamic acid and aspartic acid produce an umami taste. The texture of scallops also underwent changes during frozen storage. Fresh scallops exhibited an elastic, firm, and compact texture, whereas frozen scallops were elastic but somewhat crumbly. The shift from a firm to a slightly disintegrated texture over storage time aligns with previous research attributing this to mechanical effects from ice crystals and chemical influences such as protein oxidation. Lu et al. (2015) stated that textural deterioration in meat can result from both mechanical and chemical factors, with protein oxidation and ice crystal formation affecting the texture of fishery products post-freezing. Hultman and Rustad (2002) explained that freezing converts water in fish tissue into ice crystals, which, upon thawing, melt and create cavities in the muscle structure, leading to a softer and less elastic texture. Chemically, protein oxidation also contributes to textural changes in meat after frozen storage. Niforou et al. (2014) reported that oxidized proteins may lose their secondary and tertiary structures, compromising their stability, activity, and functionality. Oxidized proteins may also lose their ability to form necessary bonds for maintaining meat texture, potentially resulting in dryness, toughness, or textural degradation.

4. Conclusions

The proximate composition analysis revealed an overall decrease in concentration across all parameters, with protein, ash content, moisture, fat, and carbohydrate levels declining by 17.13%, 64.52%, 2.79%, 60%, and 36.72%, respectively. Concurrently, the amino acid profile exhibited notable changes, marked by significant reductions in tyrosine (56.48%), aspartic acid (51.6%), and cysteine (51.03%), while glutamic acid, serine, and phenylalanine increased by 13.23%, 11.02%, and 6.68%, respectively. Furthermore, organoleptic assessment of the frozen scallops indicated deterioration in quality, characterized by a pale, dull, and slightly discolored appearance, the emergence of a faint spoiled odor, a moderately bland and slightly bitter taste, and a texture that was elastic yet prone to partial disintegration.

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