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EVALUATION OF THE HISTAMINE LEVELS OF TUNA (EUTHYNNUS AFFINIS) BASED ON THE LENGTH OF STORAGE AND ITS IMPLICATIONS FOR HEALTH

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ABSTRACT
Increased histamine levels in tuna (Euthynnus affinis) during storage may pose health risks to consumers. However, the effects of storage duration on histamine levels are not yet fully understood. Objective: This study aimed to evaluate the increase in histamine levels in mackerel based on its duration of storage at 4°C and its implications for public health. Methods: The experimental research had a completely randomized design. This study consisted of 7 treatment groups: negative control, positive control, and storage days 2, 4, 6, 8, and 10. Tuna were collected from the Kedonganan Fish Market and were randomly selected for inclusion in each group. Histamine levels were measured using a spectrophotometric method at a wavelength of 496 nm and analyzed using SPSS (USA, Chicago, IL) version 25.0 at a 95% confidence level ($P < 0.05$). Results: There was a correlation between the length of storage and the increase in histamine levels in tuna ($p = 0.001$). The histamine levels in tuna on the second day of storage were 46.19 mg/kg, those on the fourth day were 46.57 mg/kg, those on the sixth day were 50.97 mg/kg, those on the eighth day were 54.76 mg/kg, and those on the tenth day were 58.52 mg/kg. The longer the tuna is stored, the higher the histamine levels are. The most striking increase occurred after the sixth day of storage. However, the histamine levels remained within the standard limits set by the government until the 10th day of storage. Conclusion: Storage for 4°C significantly increased histamine levels in tuna. The implications of this study include the need for fish storage management as well as the need for a consumer understanding of health risks due to prolonged fish storage and good fish selection.

Keywords: aquatic disease; food safety; histamine; storage duration; tongkol fish

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INTRODUCTION

Tuna (*Euthynnus affinis*) is a type of fish that has high nutritional value and is an important source of animal protein for people in various parts of the world, including Indonesia (Baliwati & Putri, 2012; Hansamali et al., 2020; Norita et al., 2019). According to information from the Ministry of Maritime Affairs and Fisheries Republic of Indonesia, there has been an increase in national fish consumption, reaching 46.49 kg/cap; the most consumed fish is tuna, which is widely exported to foreign countries (Ministry of Maritime Affairs and Fisheries Republic of Indonesia, 2017). Although tuna has high nutritional value, one of the problems that often arises is the increase in histamine [*2-(1 H-imidazol-4-yl)ethanamine*] levels during storage (Arulkumar et al., 2017; Norita et al., 2019). Increased histamine levels during storage can pose a serious problem for consumer health. High histamine levels in fish can cause various allergic reactions such as hives, skin rashes, and anaphylaxis in sensitive individuals. Thus, evaluations of food safety, especially the health of raw food materials, must be performed continuously to minimize the occurrence of extraordinary events (Adnyana, 2023;

Hafid et al., 2023).

According to the standards set by the National Standardization Agency (SNI 01-2729), fresh fish must meet the requirements of freshness, cleanliness, and health; namely, fish must not be obtained from polluted waters and must have an intact form. Fish have bright brilliant eyes, a fresh odor, and an organoleptically elastic, dense, and compact texture. If fish do not meet raw material requirements, this can lead to potential hazards, such as interference with food *safety*, and histamine levels in fish should not exceed 100 mg/kg (Badan Standarisasi Nasional, 2006). Several studies have reported that histamine is formed in the *postmortem* phase, steadily increases during the process of exogenous decarboxylation produced by fish microbes and the heating process, and is resistant to processing, such as canning(Prasetiawan et al., 2013). Aminah (2015) reported that endogenous histamine is necessary for normal physiological function, but large doses of histamine are toxic because they enter the circulatory system. WHO (2018) reported that the temperature and storage time of tuna strongly affect histamine formation. Fish that are not processed immediately should be stored in a refrigerator at a temperature close to 0°C or in a temperature range of 0-5°C to prevent the growth of pathogenic bacteria and histamine formation. *Fatty fish* can be stored at 4°C or in a temperature range of 5-10°C.

Given these issues, the evaluation of histamine levels in tuna based on storage duration is highly relevant in the context of public health, the food industry, fisheries, and marine environments. By understanding the changes in histamine levels in mackerel during storage, it is possible to identify the factors that influence increased histamine levels and to develop storage strategies that can reduce the risk of histamine poisoning in consumers. This study also has important implications for the development of guidelines for the safe and high-quality storage and distribution of tuna, which will increase consumer confidence in fishery products and maintain better public health. Therefore, this study aimed to evaluate the histamine levels in tuna based on the length of storage and its health implications. With a better understanding of the dynamics of changes in histamine levels in tuna, improvements can be made in fish storage practices and supply chain management, thereby reducing the risk of food poisoning and improving the quality of fishery products as well as efforts to ensure food safety and consumer welfare.

METHOD

This study used a completely randomized experimental research design (RAL) (Darwin et al., 2021). The research was conducted for two months, from May to June 2023. The tuna (Euthynnus affinis) was stored under different conditions to evaluate changes in histamine levels during storage. The tuna used in this study were obtained from the Kedongan Fish Market, Kuta, Bali. Tuna samples were randomly selected from the population for inclusion in the treatment groups (Adnyana, 2021). The tuna samples were determined based on inclusion and exclusion criteria, including tuna that did not come from polluted waters and had an intact shape. Organoleptically, fish have a fresh odor, bright brilliant eyes, and an elastic, dense, and compact texture. Sampling was performed immediately after the fishermen finished fishing and returned to land. This study was exempted from ethical feasibility because it did not use live animals during the study, but the implementation of the study was approved by the Bali Province Investment and One-Stop Integrated Service Office with protocol number 070/2953/IZIN-C/DISPMPT.

In this study, there were seven treatment groups (n = 28) that were grouped as follows: negative control (K-) or Blanko, positive control K(+) with 10 mg/kg histamine; treatment 1

(P1) tuna stored on day 2; treatment 2 (P2) tuna stored on day 4; treatment 3 (P3) tuna stored on day 6; treatment 4 (P4) tuna stored on day 8; and treatment 5 (P5) tuna stored on day 10. The variable observed in this study was the histamine level in tuna. The histamine levels were measured using a spectrophotometric method at a wavelength of 496 nm. This research procedure began with sample preparation; that is, tuna was purchased from market fishermen and divided into treatment groups according to the research design. Each treatment group was stored according to predetermined conditions. Tuna samples were collected at predetermined time intervals to analyze the histamine levels. The tuna samples were processed using a spectrophotometric method to measure the histamine levels. The histamine levels will be statistically analyzed to evaluate changes in the histamine levels under various storage conditions. Data on histamine levels will be analyzed using descriptive and inferential statistical analyses with Mann–Whitney and Kruskal–Wallis tests with a 95% confidence level ($p<0.05$). IBM SPSS software (Chicago, IL, USA), version 25.0. All the test results are presented in tables, graphs, and narratives.

RESULTS

Histamine levels

Examination of the histamine levels in tuna (*Euthynnus affinis*) obtained from the Kedonganan Fish Market, Kuta, Bali, revealed that the histamine levels in tuna increased after storage. The average histamine concentration on day 2 was 46.19 mg/kg, an increase of 0.38 mg/kg; thus, the histamine concentration on day 4 of storage was 46.75 mg/kg. On the 6th day of storage, the histamine concentration increased by 4.4 mg/kg, so the histamine concentration was 50.97 mg/kg. On the 8th day of storage, the histamine concentration increased by 3.79 mg/kg, so the histamine concentration decreased to 54.76 mg/kg. On the 10th day of storage, the histamine concentration increased by 3.76 mg/kg, and the histamine concentration increased by 58.52 mg/kg.Based on the test results, histamine levels increase starting on the 6th day of storage. The greatest increase in the histamine level was observed on day 6. Starting on the 8th day of storage, the increase in histamine stabilized at 3.7 mg/kg. The results revealed that there was an increase in the histamine level after 2, 4, 6, 8, or 10 days of storage. Although the tuna was stored in a refrigerator at 4°C, an increase in histamine levels still occurred. Histamine levels were still within government standards until the 10th day of storage. The average histamine level in each treatment group is shown in Table 1 and Figure 1.

Table 1.
Average Histamine Levels of Tuna Fish

Treatment Group	Histamine Level of Tuna (mg/kg)
Negative control (KN)	46,080±0,535
Positive control (KP)	56,080±1,194
2nd day of storage (P1)	46,192±0,696
4th day storage (P2)	46,570±1,660
6th day of storage (P3)	50,967±1,097
8th day of storage (P4)	54,757±1,166
10th day of storage (P5)	58,517±3,337

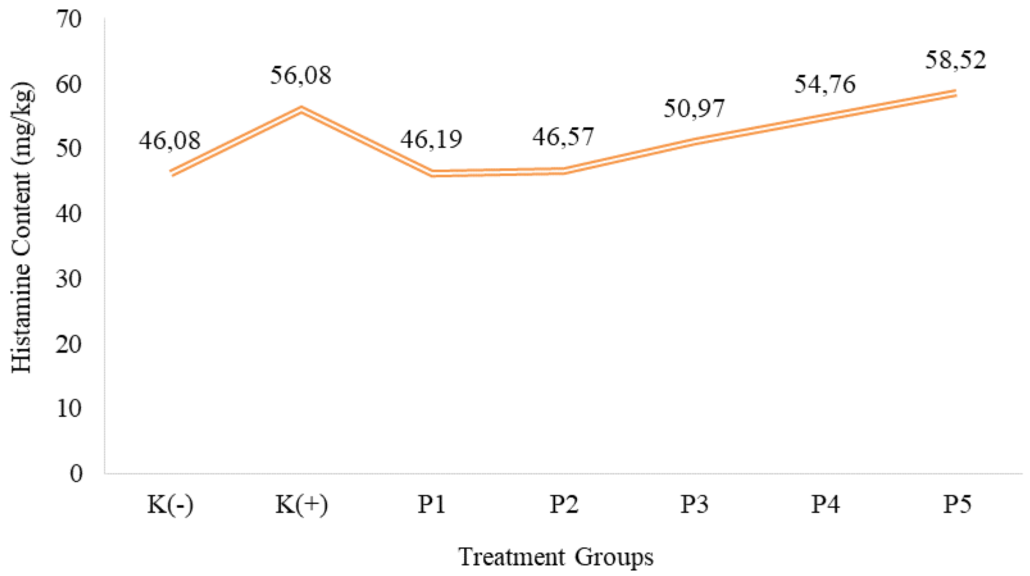

Figure 1. Increase in histamine levels in tuna based on length of storage. Notes: K(-)= Blank; K(+)= Histamine 10 mg/kg; P1= 2nd day storage; P2= 4th day storage; P3= 6th day storage; P4= 8th day storage; P5= 10th day storage.

Relationship between the storage time and the histamine content of Tuna

Based on the results of the normality test using *the Shapiro–Wilk test, the* normality test results were p>0.05. This indicates that the group was normally distributed. However, after the homogeneity test using the *Levene test, the* test result (p<0.05) indicated that the data were not homogeneous, so the comparative analysis was continued with the *Kruskal–Wallis* test. According to the results of the *Kruskal–Wallis* test, the probability value was 0.001 (p<0.05). Thus, there was a difference in the histamine levels between the treatment groups for the length of storage of the togkol fish at 4°C; thus, H1 was accepted, and H0 was rejected. The results of the normality, homogeneity, and *Kruskal–Wallis* tests are presented in Table 2.

Table 2.
Results of normality, homogeneity and *Kruskal–Wallis* tests (n=28)

Treatment Group	*df*	*Sig. Shapiro–Wilk*	*Kruskal–Wallis*
Negative control (KN)	4	0.338	0,001*
Positive control (KP)	4	0.294	
2nd day of storage (P1)	4	0.617	
4th day storage (P2)	4	0.140	
6th day of storage (P3)	4	0.834	
8th day of storage (P4)	4	0.192	
10^{th} day of storage (P5)	4	0.285	

Notes: * There was a significant difference at the 95% confidence level (p<0.05).

Furthermore, the Mann–Whitney analysis was used for post hoc tests. The P1 group had a dose of 46.080 ± 0.535 mg/kg, which was significantly different (p <0.05) from that of the KP, P3, P4, and P5 groups. However, the KN and P2 groups were not significantly different (p>0.05). In the P2 group, 46,570 ± 1,660 mg/kg was significantly different (p <0.05) among the KP, P3, P4, and P5 groups. The KN and P1 groups did not significantly differ (p>0.05). Treatment in the P3 group resulted in 50.967 ± 1.097 mg/kg, which was significantly different (p < 0.05) from that in the KN, KP, P1, P2, P4, and P5 groups. The P4 group showed 54,757 ± 1,166 mg/kg, which was significantly different (p <0.05) from those of the KN, P1, P2, and P3 groups. However, in the KP group, the P5 levels were not significantly different (P >0.05).

In the P5 group, the concentration was 58.517 ± 3.337 mg/kg, which was significantly different (p <0.05) from that in the KN, P1, P2, and P3 groups. The KP and P4 groups were not significantly different (P >0.05). The results of post hock testing are presented in Table 3.

Table 3.
Comparison of Histamine Levels between Groups According to the Mann–Whitney Test

	KN	KP	P1	P2	P3	P4	P5
KN	-	0,021	0,773*	1,000*	0,021	0,021	0,021
KP		-	0,021	0,021	0,021	0,149*	0,248*
P1			-	0,773*	0,021	0,021	0,021
P2				-	0,021	0,021	0,021
P3					-	0,021	0,021
P4						-	0,083*
P5							-

Notes: * There was a significant difference at the 95% confidence level (p<0.05).

DISCUSSION

Histamine levels in tuna (*Euthynnus affinis*) stored under various conditions significantly increased with increasing storage time. A significant increase in histamine levels occurred starting on the 6th day of storage, when the greatest increase was recorded. From the 8th to the 10th days of storage, the increase in histamine levels remained stable, with an increase of 3.7 mg/kg. This finding indicates that there is a correlation between the length of storage and the increase in histamine levels in tuna. Although the tuna was stored in a refrigerator at 4°C, an increase in histamine levels still occurred. This indicates that even when the storage temperature is controlled, other factors, such as storage time and environmental conditions, still affect the increase in histamine levels in tuna (Torre et al., 2020). However, the histamine levels measured in this study were still within the standard limits set by the government until the 10th day of storage. This indicates that tuna stored under regulated conditions is still safe for consumption for up to a certain period.

According to FAO & WHO (2013), storage in a refrigerator at 4°C can inhibit the growth of mesophilic histamine-producing bacteria and slow the growth of psychrotrophic histamine-producing bacteria (Wodi et al., 2019). The increase in histamine levels will stop when freezing and storing at freezing temperatures (-18°C or lower) due to the cessation of growth of all bacteria and prevention of the preformed histidine decarboxylation enzyme from producing histamine (Cicero et al., 2020; Firman et al., 2021). In the context of public health, these findings provide insights into the risk of histamine poisoning that can arise from the consumption of tuna stored under various conditions. Although histamine levels are still within standard limits, it should be kept in mind that a significant increase in histamine levels may increase the risk of allergic reactions or food poisoning in sensitive individuals (Kung et al., 2016; Sivamaruthi et al., 2021). Therefore, tuna storage management needs to be carefully considered to ensure optimal food safety and consumer health.

Furthermore, based on the analysis of treatment group differences using the Kruskal–Wallis test, there was a significant difference in histamine levels between treatment groups for tuna stored at 4°C. Thus, the research hypothesis (H1), which states that there is a difference in histamine levels between treatment groups, is accepted, whereas the null hypothesis (H0), which states that there is no difference, is rejected. A significant increase in histamine levels began to occur on the 6th day of storage, when the most striking increase occurred. This indicates that tuna are susceptible to increased histamine levels after a relatively short storage period. This result is in line with the findings of Norita et al. (2019), who reported that histamine levels increase with increasing length of storage. His findings showed that histamine concentrations increased from day 0 to day 18, and on day 18, histamine concentrations in longtail tuna reached 59.99 mg/kg and 77.98 mg/kg in frozen and chilled storage, respectively. These results exceeded the FDA criteria but met the FAO criteria.

Furthermore, Silva et al. (1998) reported that the number of histamine-forming bacteria increased during storage at 4°C and 10°C and that histamine formation was suppressed at 4°C. These findings have important implications for the management of tuna storage and food safety. Careful storage management is needed to minimize the increase in histamine levels and to maintain the quality and safety of tuna consumed by the public. In addition, information on the risk of histamine poisoning that can arise from the consumption of stored tuna needs to be socialized to the public, especially for individuals who are prone to allergic reactions or food poisoning.

Implications for Public Health and Food Safety

Increased histamine levels in tuna (*Euthynnus affinis*) based on the length of storage have significant implications for public health and food safety (Puttonen et al., 2017; Visciano et al., 2017). Histamine is a biogenic compound that can cause allergic reactions or food poisoning when consumed in high amounts (Heruwati et al., 2008; Suranaya Pandit, 2012). Allergic reactions, such as hives, skin rashes, and anaphylaxis, can occur if a person consumes tuna containing high levels of histamine. In addition, histamine poisoning can cause gastrointestinal symptoms such as nausea, vomiting, diarrhea, and abdominal pain. This can impair the health and quality of life of consumers, especially those with food allergies or a weak immune system. Although histamine levels are still within normal limits or meet quality standards, the increase in histamine levels in tuna raises concerns regarding food safety (Heruwati et al., 2008; Rachmawati & Triwibowo, 2022). The consumption of tuna with high histamine levels can cause serious health problems for consumers. Therefore, it is important for the fishing industry and the government to implement strict supervision and control measures in the management of tuna storage and distribution to ensure that fish products sold to consumers are safe for consumption.

When choosing fish for consumption, consumers must pay attention to several factors to avoid the health risks associated with high histamine levels. Some of the factors that need to be considered include the following: a) source, consumers should choose fish from trusted and safe sources, such as fish markets that have high hygiene and safety standards; b) storage, consumers should ensure that fish are stored in proper conditions, including appropriate storage temperatures and storage durations that are not too long; and c) periodic inspection, consumers should check the physical condition of the fish before buying, such as color, aroma, and texture, to determine whether the fish have been exposed to damage or not. Increased histamine levels in tuna can be recognized through several physical characteristics, including a) unusual aroma, generally fish containing high histamine levels can have an unusual or unfresh aroma, such as a fishy smell or a strong sour smell; b) changes in color that occur in fish meat, such as brownish or greenish, which can be an indicator of increased histamine levels; and c) unusual texture, such as being softer or harder than usual, can be a sign of increased histamine levels.

Research Limitations

Although this study provides valuable insights into the increase in histamine levels in tuna based on storage duration, it has some limitations that need to be considered. This study focused only on the effect of storage duration on histamine levels; other factors, such as temperature, humidity, and other storage methods, can also affect histamine levels in tuna. The findings of this study may not be directly generalizable to all types of fish and storage conditions. Further research is needed to validate these findings under different fish types and storage conditions. Finally, this study only evaluated the histamine levels in tuna after up to 10 days of storage. Further research with longer storage durations may provide a more comprehensive understanding of the changes in histamine levels over a longer period of time.

CONCLUSION

These findings show that there is a correlation between the length of storage and the increase in histamine levels in tuna. The histamine levels in tuna on the second day of storage were 46.19 mg/kg, those on the fourth day were 46.57 mg/kg, those on the sixth day were 50.97 mg/kg, those on the eighth day were 54.76 mg/kg, and those on the tenth day were 58.52 mg/kg. The longer the tuna is stored, the higher the histamine levels are. Further research should explore the factors that influence the increase in histamine levels in tuna during storage and identify more effective storage strategies to minimize health risks associated with tuna consumption. Thus, the results of this study can serve as a basis for the development of more appropriate storage guidelines and effective policies for maintaining food safety and consumer health.

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