Benha Veterinary Medical Journal 48 (2025) 98-102

Official Journal Issued by Faculty of Veterinary Medicine

**Benha Veterinary Medical Journal**

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

*Original Paper*

# Experimental studies for controlling histamine produced by some bacteria in tuna fish using some essential oils

**Wesam S. Elshafey , Shimaa A. Abd El-Wahaab, Rabab Rawash**

*Department of Food Hygiene, Animal Health Research Institute, Agriculture Research Center (ARC), Egypt.*

**ARTICLE INFO**

**Keywords**

*Tuna fish*

*Histamine level*

*Thyme oil*

*Blackberry oil.*

***Received*** 02/02/2025
***Accepted*** 22/03/2025
***Available On-Line*** 01/04/2025

**ABSTRACT**

This study investigates the bacteriological quality and histamine levels in tuna fish, highlighting significant health risks associated with bacterial contamination throughout the fish's lifecycle. Bacteriological analysis of three tuna samples revealed the presence of pathogenic bacteria, including *Escherichia coli* (2/3), *Salmonella* (1/3), *Staphylococcus aureus* (2/3), and *Proteus* species (1/3). Histamine levels were assessed using ELISA, with concentrations recorded as 18.05 mg/100g, 27.4 mg/100g, and 20.1 mg/100g, indicating that one sample exceeded the permissible limit of 20 mg/100g as per the Egyptian standards guidelines. The study further explored the efficacy of essential oils, specifically thyme and blackberry oils, in reducing histamine levels in contaminated fish. Results demonstrated that thyme oil at 1% concentration significantly reduced histamine levels by 58.6% over nine days, while blackberry oil at 1% achieved a reduction of 46.1%. These findings suggest that essential oils can effectively mitigate histamine accumulation and microbial growth in tuna fish, presenting a promising approach to enhance food safety and extend shelf life. The study underscores the importance of monitoring bacterial contamination and histamine levels in tuna to ensure consumer safety and improve product quality through natural preservation methods.

## 1. INTRODUCTION

Food is a basic necessity in human life, so the quality of food represents a basic necessity when choosing the food consumed. Increasing human knowledge about food quality plays a great role in changing the human habitat. Fish represent one of the nutritional foods that supply the human body with several vital elements (Kawarazuka and Béné, 2011). Despite its beneficial value, it may represent a primary source of infection of humans by many hazardous agents, either biological as bacteria and parasites, or through chemical as toxins, allergens, and histamine (Yemmen and Gargouri, 2022). Tuna fish represent one of Scombridae fish that is characterized by their superior flesh type that popular by consumers (Maiz et al., 2019), despite that it ranked as the highest cause of food poisoning to human as it contains higher amount of amino acid known as histidine in their flesh that may decarboxylated by bacterial proliferation to histamine and so cause histamine poisoning (Hungerford 2010 & 2021; Bjornsdottir-Butler et al. 2015).

Most of the bacteria identified as histamine producers are gram-negative, accounting for 87% of the isolates. Furthermore, a significant portion of these isolates (80%) belong to the *Enterobacteriaceae* family (Traylor and Mathew, 2021). So, the level of histamine in fish may serve as an indicator for its freshness (Yemmen and Gargouri, 2022). Histamine is one of the biogenic amines that can tolerate the cooking process and freezing temperature. It is level must not exceed 20 mg/100 gm as reported by the Egyptian standards (E.S, 2005). Generally, the symptoms of histamine poisoning appear after ingestion of a dose ranging from 70-100 mg in a single dose. The poisoning symptoms vary from skin rashes, facial swelling, headache, and elevated heartbeats. Others may show gastrointestinal disturbance like stomach discomfort, nausea, vomiting, and diarrhea (Harmelin et al., 2018).

The tolerance nature of histamine to different processes with their severity attracts attention to try to reduce its production through controlling the proliferation of the histamine producing bacteria. Preventing histamine producers acts as an indirect way to prevent biogenic amines formation. The direction to use natural antibacterial products like plant extracts increased greatly due to their acceptability by consumers. Thymol represents one of the most common plants that is used as an antibacterial due to its potent inhibitory effects against both Gram- negative and Gram-positive bacteria (Kowalczyk et al., 2020). Thymol essential oil exhibits the strongest antibacterial agents that can penetrate the lipid layer of the cell wall of the microbe and affect it due to its small, lipophobic nature (Perez et al., 2019; Kowalczyk et al., 2020). Also, several authors demonstrate its effect in reducing the formation of biogenic amines in different food types (Özogul et al., 2015; Křížek et al., 2018).

In addition, fruits may act as another enhancing and antibacterial agent that may be used in food processing, such as blackberries. Blackberry is characterized by the presence of various essential components as phenolic compounds, mainly anthocyanins (Dai et al., 2007), flavonols, ellagitannins (Oszmia´nski et al., 2015), and phenolic acids (Zia-Ul-Haq et al., 2014). Their various chemical structures enable them to interact with biological molecules while providing antioxidant functions and metal-chelating abilities, leading to the influence of various physiological processes (Ropiak et al., 2016).

* Correspondence to:: wesamshaban080@gmail.com

The extant study aimed to examine the occurrence of bacteria producing histamine in tuna fish, together with detecting the level of histamine in the examined fish by ELISA, and examining the capability of thymol oil and blackberry oil in reducing the histamine level in the examined fish.

## 2. MATERIAL AND METHODS

*2.1. Sampling*

Three tuna fish (each one was 1 Kg) were collected from fisheries in Benha, Kalyobiya government. The collected fish were transferred to the lab in an insulated ice box for bacteriological examination.

*2.2. Bacteriological examination*

The tested samples were prepared following ISO 6887-1 (2017). Take 1 ml from prepared samples and inoculate it into nutrient broth and incubate at 37 ºC for 37 hrs, then streak it over lysine iron agar to determine the bacteria producing biogenic amines. The suspected colonies were streaked over MacConkey agar, XLD agar, and Baird-Parker agar. The produced colonies were determined based on their morphology and biochemical examination.

*2.3. Measurement of histamine level by using ELISA (Leszczynska et al., 2004).*

It is a quantitative test used to detect histamine levels in different tissues. By using HistaSure™ kite. The test is based on the measurement of the absorbance of the unknown sample and compares it with the absorbance of a reference curve prepared with known standard concentrations. The reaction is monitored at 450 nm.

*2.3.1. Preparation of reagents*

*Wash Buffer:* 20 mL of concentrated wash buffer diluted with D.W. to reach a final concentration of 1000 ml. The buffer is preserved at 4-8 ºC for 6 months.

*Acylation Diluent:* must ensure that the acylation diluent is homogeneous and free from any crystals before use, "take care that such diluent has reached a freezing point at 18.5°C, so it is preserved at room temperature 25°C.

*2.3.2. Sample preparation:*

Weigh approximately 10 grams of each fish flesh and add 240 mL of deionized or distilled water to it, then homogenize the mixture for 1-2 minutes until a uniform consistency is achieved. Filtrate the homogenate through folded filter paper to remove solid residues. Alternatively, centrifuge the homogenate at maximum speed (approx. 600 rpm) for about 5 minutes, and collect the supernatant. Use 50 µl of the sample extract for the acylation.

*2.3.3. Acylation*

This occurred based on the instructions of the kits as follows: on the Master Block pipette 50 µl of control(s) and sample extracts into the respective wells. Then add 1.5 ml of acylation buffer into all wells. And then add 50 µl of acylation reagent to all wells. Incubate the mixture at room temperature for 5 min in the shaker (approx. 600 rpm). Finally, take 50 µl for the ELISA.

*2.3.4. Histamine detection by ELISA.*

Pipette 50 µl of the acylated control(s) and samples into the wells of the Histamine Microtiter Strips. Then Pipette 100 µl of the Histamine Antiserum Conjugate into all wells. Incubate them for 10 min at room temperature (20 - 25 °C) on a shaker (approx. 600 rpm). Then discard or aspirate the contents of the wells and wash the plate 3 x by adding 300 µl of Wash Buffer, discarding the contents and blotting dry each time by tapping the inverted plate on absorbent material. After that, pipette 100 µl of the Substrate into all wells and incubate it for 10 min at RT (20 - 25 °C) on a shaker (approx. 600 rpm). Then stop the reaction by adding 100 µl of the Stop Solution to each well and shaking the microtiter plate to ensure a homogeneous distribution of the solution. Finally, read the absorbencies of the solutions in the wells within 10 minutes using a microplate reader set to 450 nm.

*2.3.5. Calculation of the results.*

The absorbance readings of the 6 controls (0, 3, 10, 30, 100, and 300 ppm) are used to establish a standard curve. Plot the absorbance readings of the controls (y-axis, linear) against the corresponding control concentrations (x-axis, log) using a concentration of 0.001 ppm for the 0-control. For the curve fitting, a non-linear regression has to be applied. The concentrations of the samples can be read directly from this standard curve.

If a sample is off curve it has to be diluted with water 1:10 and re-assayed. The result obtained has to be multiplied by the dilution factor of 10.

*2.4. Experimental trial to control histamine level by using essential oils*

The fish sample showed histamine levels above the permissible limit detected by E.S. (2005). "20mg/100g" was used in the experiment. Thyme and blackberry essential oils used were obtained from the El-Badawya company.

The experiment was carried out according to the method described by Barbosa et al. (2009). Five experimental groups of contaminated fish fillets were prepared, each group contained 100 gm of fish fillet. The control group dipped in sterile distilled water, while other groups dipped in thyme and blackberry essential oils with concentrations of 0.5% and 1%. The dipping period extended for 15 min, then allowed to dry for 5 min in a sterile stainless-steel wire. After drying, each group was kept separately in sterile polyethylene bags in a chilling storage at 2°C. The groups were subjected to the assessment at time zero (within 2 hours after treatment), and every 3 days (zero, 3, 6, and 9 days).

*2.5. Statistical analyses*

The data statistical analysis was conducted using Analysis of Variance (ANOVA) in SPSS software (version 20.0), following the methodology outlined by Feldman et al. (2003). The level of significance was set at $P \leq 0.05$.

## 3. RESULTS

3.1. Bacteriological analysis

The bacteriological examination of the fish samples reveals isolation of *Escherichia coli* (2/3), *Proteus species* (1/3), *Salmonella* (1/3), and *Staphylococcus aureus* (2/3) from the examined fish.

3.2. Level of Histamine in the examined fish

Tuna fish 2 sample showed an elevated level of histamine used for the experimental trial (Table 1).

Table 1. Concentration of histamine residue in the examined tuna fish (n=3).

| Samples | Concentration of histamine (mg/100g) |
|---|---|
| Tuna Fish 1 | 18.05 mg/100g |
| Tuna Fish 2 | 27.4 mg/100g |
| Tuna Fish 3 | 20.1 mg/100g |

* Histamine should not exceed 20mg/100g fish (E.S., 2005)

3.3. Effect of using essential oils on the level of histamine

The results presented in the table (2) regarding a steady increase in histamine levels in the control group from 27.4 mg% % at zero day to 34.3 mg% by day 9, indicating that histamine accumulation occurs naturally over time due to bacterial activity. While other groups treated with thyme oil and blackberry oil can effectively reduce histamine levels in fish, particularly at higher concentrations. In case of group treated with thyme oil 0.5%, it showed reduction in histamine level by 18.5%,34%, and 47.8% respectively over the extended period 3,6, and 9 days. While that treated with thyme oil 1% showed reduction in histamine level by 31.3%,45.1%, and 58.6% over the same period. In addition, the group treated with blackberry oil 0.5% detected a reduction in histamine level by 11.4%,24.8%, and 39.9% within 3,6, and 9 days. And the last group treated with blackberry oil 1% showed reduction levels of 17.1%, 31%, and 46.1%, respectively, over the period 3,6, and 9 days.

Table 2. Effects of the addition of essential oils on the level of histamine in the contaminated fish.

| Storage period | Control histamine level (mg%) | Thyme oil (0.5%) | | Thyme oil (1%) | | Blackberry oil (0.5%) | | Blackberry oil (1%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Level | R % | Level | R % | Level | R % | Level | R %* |
| Zero day | 27.4 | 27.4 | ------ | 27.4 | ------ | 27.4 | ------ | 27.4 | ------ |
| 3 days | $28.1^{A}$ | $22.9^{E,C}$ | 18.5 | $19.3^{D}$ | 31.3 | $24.9^{B}$ | 11.4 | $23.3^{C}$ | 17.1 |
| 6 days | $30.6^{A}$ | $20.2^{E,C}$ | 34 | $16.8^{D}$ | 45.1 | $23.0^{B}$ | 24.8 | $21.1^{C}$ | 31 |
| 9 days | $34.3^{A}$ | $17.9^{E,C}$ | 47.8 | $14.2^{D}$ | 58.6 | $20.6^{B}$ | 39.9 | $18.5^{C}$ | 46.1 |

Mean values with different superscripts in the same rows are significantly different at P<0.05

## 4. DISCUSSION

Fish flesh is typically free from bacteria when initially caught. However, the extent of bacterial contamination can differ based on environmental factors and the microbiological quality of the water. These infections may arise from direct contamination with polluted water or from secondary contamination that occurs during various stages, including capture, handling, processing, storage, distribution, or preparation for consumption (Yemmen and Gargouri, 2022). Fish may represent a source of infection to humans through transmission of various food poisoning bacteria like *Staphylococcus aureus, Salmonella* species, *Shigella* species, *Escherichia coli*, *Vibrio* species, *Aeromonas* species, *Clostridium botulinum, and Listeria monocytogenes* (Bensley et al., 2011; Eze and Echezona, 2011).

Tuna fish are among fish that may be contaminated by several pathogenic bacteria during their life and their consumption represents a potential health hazard for humans. As mentioned in this study, different bacteria have been isolated from examined fish as *Escherichia coli*, *Proteus* species, *Salmonella,* and *Staphylococcus aureus.* These findings slightly agree with the previous study reported by Hassan et al. (2019), who found that the consumption of tuna fish causes outbreaks by *Salmonella* in the USA with a total of 136 infections. Additionally, Liu et al. (2016) isolated *Salmonella* and *L. monocytogenes* from raw yellowfin tuna. Similarly, Pattipeilohy et al. (2017) reported the presence of *Staphylococcus saprophyticus, Planococcus citreus, and Micrococcus varians* in the examined fresh tuna and demonstrated that such infection may occur during post-harvesting. Furthermore, Mutmainnah et al. (2024) were able to isolate *Escherichia coli, Proteus* spp., and other pathogenic bacteria from fresh tuna fish.

Most of enteric bacteria were histamine producing bacteria such as *E. coli, Vibrio, Proteus, Klebsiella, Clostridium, Salmonella* and *Shigella* as they contain histidine decarboxylases enzyme that flourishes when the temperature elevated above chilling point led to decarboxylation of histidine and increase the level of histamine in contaminated fish (Maijala et al., 1993; Traylor and Mathew, 2021), their effects continue even if the bacterial growth ceases by refrigeration (Lehane and Olley, 2000).

Freshly caught tuna fish mainly contains histamine levels less than 2 mg/kg (FAO/WHO, 2013). That may not affect human health due to the presence of the amine oxidase in the human intestine that can detoxify these amines (Biji et al., 2016). When the fish die, the histamine-producing bacteria located in the gills and surface of the fish invade the muscle and decarboxylate histidine to histamine (Sabry et al., 2019). Also, exposure of tuna to warm water or air generates heat through their tissue that leads to the continuous release of histidine decarboxylase enzyme by microorganisms continuously and raises the histamine level in their flesh (FDA, 2011). This process could stop if the fish is refrigerated immediately after catching (Sabry et al., 2019). So, the level of histamine in fish represents an indicator for fish freshness (Yemmen and Gargouri, 2022).

The examination of the histamine level in the recent study by using ELISA revealed the presence of histamine with levels 18.05, 27.4, and 20.1 mg/100 gm in the examined fish, which indicated that only one fish exceeded the permissible limit "20 mg/100 g" detected by E.S. (2005). This disagrees with the result detected by Elbarbary and Abdelmotilib, (2023), who found the mean value of histamine in solid tuna samples was 48.33±1.12 ppm. Numerous studies have documented outbreaks of histamine poisoning linked to tuna consumption, attributed to increasing histamine levels in the fish. Colombo et al. (2018) conducted a review of 55 reports from 1959 to 2013 and found that 32.9% of the cases of histamine poisoning were associated with tuna consumption. Similarly, Velut et al. (2019) reported an outbreak in April 2017, specifically connected to tuna. Furthermore, Pereira et al. (2021) identified 51 cases of Scombrotoxin fish poisoning resulting from the consumption of tuna with elevated histamine levels.

The results of the histamine level-controlling trial using essential oils (thyme oil and blackberry oil with two concentrations, 0.5% and 1%) over 9 days indicated the ability of thyme oil and blackberry oil to effectively reduce histamine levels in fish, particularly at higher concentrations. This mainly refers to its higher concentration of phenolic compounds that act as potent antibacterial agents, which affect most Gram-positive and Gram-negative bacteria. Their actions mainly targeted the cell membrane, leading to leakage of its contents, inhibiting nucleic acid synthesis, and altering membrane permeability (Xie et al., 2015). The results of the present study are coordinated with those reported by Nader et al. (2016), who recorded a reduction in histamine level in frozen fish fillets by about 11.2% and 19.8% after addition of thyme oil (1%) for 3 days and 7 days. This came in alignment with the previous studies done by Özogul et al. (2015), who reported the ability of thyme essential oil in decreasing the level of histamine and other biogenic amines in Cyprinus carpio fillets. While Křížek et al. (2018) found that thyme essential oils could control the formation of eight kinds of biogenic amines in vacuum-packed fillets of Cyprinus carpio. Moreover, the addition of blackberry oil showed marked reduction in histamine level by increasing the concentration of oil, which

is attributed to the presence of phenolic compounds and also higher content of phytochemicals that affect microbes (Puupponen-Pimiä et al., 2001). The antibacterial activity of blackberry extracts is not always proportional to the total phenolic content (Jazi´c et al., 2018) but also may be indicative to other non-phenolic molecules such as terpenoids and organic acids that might exert selective antimicrobial activity (González et al., 2013).

The application of essential oil to fish fillets effectively inhibited microbiological growth throughout the storage period. These findings suggest that essential oil could be a promising method for extending the shelf life and preserving the quality of fish fillets (Cai et al., 2015).

## 5. CONCLUSIONS

This study underscores the critical risks associated with bacterial contamination in tuna fish, which can arise at multiple stages from capture to consumption. The detection of pathogenic bacteria such as *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus* highlights the health hazards linked to tuna consumption, particularly concerning the production of histamine and other biogenic amines. Elevated histamine levels in certain samples indicate that inadequate handling and storage practices pose significant food safety risks. The investigation into the application of essential oils, particularly thyme and blackberry oil, reveals their potential in effectively reducing histamine levels in fish fillets

## 6. REFERENCES


1. Barbosa, L., Rall, V., Fernades, A., Ushimaru, P., De Silva, I., Fernandes, T., 2009. Essential oils against food borne pathogens and spoilage bacteria in minced meat. Foodborne Pathogens and Disease, 6, 725-728.
2. Bensley, M., Dick, T.A., Hudson, C., Lumsden, J.S., Peters, K.K., Souter, W., Vannest, L., Donald, D.B., Nelson, R., 2011. Devils lake – red river basin fish parasite and pathogen project qualitative risk assessment. Prepared for the International Red River Board and International Joint Commission. 47.
3. Biji, K.B., Ravishankar, C.N., Venkateswarlu, R., Mohan, C.O., Srinivasa Gopal, T.K., 2016. Biogenic amines in seafood: a review. J Food Sci Technol., 53,5, 2210 – 2218.
4. Bjornsdottir-Butler, K. and Bowers, J.C., Benner, R.A., 2015. Prevalence and characterization of high histamine–producing bacteria in Gulf of Mexico fish species. J. Food Prot., 78 ,7, 1335–1342.
5. Cai, L., Cao, A., Li, Y., Song, Z., Leng, L., Li, J., 2015. The effects of essential oil treatment on the biogenic amines inhibition and quality preservation of red drum ,Sciaenops ocellatus) fillets. Food Control., 56, 1–8,
6. Colombo, F.M., Cattaneo, P., Confalonieri, E., Bernardi, C., 2018. Histamine food poisonings: A systematic review and meta-analysis. Critical Reviews in Food Science and Nutrition, 58,7, 1131–1151.
7. Dai, J., Patel, J.D., Mumper, R.J., 2007. Characterization of Blackberry Extract and Its Antiproliferative and Anti-Inflammatory Properties. J. Med. Food,10, 258–265.
8. Egyptian Standards "E.S", 2005. Reports related to ES: 3494/2005 for chilled fish. Egyptian Standards, Ministry of Industry, Egypt.
9. Elbarbary, N.K. and Abdelmotilib, N.M., 2023. Monitoring the Bacteriological Contamination and Histamine Formation in Canned Tuna. Egyptian Journal of Aquatic Biology & Fisheries Zoology, 27,4, 421 – 438.
10. Eze, E. and Echezona, B. 2011. Isolation and identification of pathogenic bacteria associated with frozen mackerel fish ,Scomber scombrus) in a humid tropical environment. African Journal of Agricultural Research, 6,7, 1918– 1922.
11. Feldman, D., Ganon, J., Haffman, R., Simpson, J., 2003. The solution for data analysis and presentation graphics. 2nd Ed., Abacus Lancripts, Inc., Berkeley, USA.
12. Food and Agriculture Organization of the United Nations/World Health Organization ,FAO/WHO). 2013. Public Health Risks of Histamine and other Biogenic Amines from Fish and Fishery Products. Meeting report.
13. Food and Drug Administration ,FDA). ,2011). Fish and Fishery Products Hazards and Controls Guidance, 4th edn. Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington DC.
14. González, O.A., Escamilla, C., Danaher, R.J., Dai, J., Ebersole, J.L., Mumper, R.J., Miller, C.S., 2013. Antibacterial Effects of Blackberry Extract Target Periodontopathogens. J. Periodontal Res., 48, 80–86.
15. Harmelin, Y., Hubiche, T., Pharaon, M., Del Giudice, P. 2018. Three cases of scombroid poisoning. Annales de Dermatologie et de Vénéréologie, 145,1, 29– 32.
16. Hassan, R., Whitney, B., Williams, D., Holloman, K., Grady, D., Thomas, D. et al., 2019. Multistate outbreaks of Salmonella infections linked to imported Maradol papayas – United States, December 2016– September 2017. Epidemiology and Infection, 147, e265.
17. Hungerford, J.M., 2010. Scombroid poisoning: a review. Toxicon 56, 231-43.
18. Hungerford, J.M., 2021. Histamine and Scombrotoxins. Toxicon 201, 115-126.
19. ISO ,International Organization for Standardization, 2017. International Organization for Standardization. No.6887-1. Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions.
20. Jazi´c, M.R., Vuli´c, J.J., Kukri´c, Z.Z., Topali´c-Trivunovi´c, L.N., Savi´c, A.V., 2018. Chemical Composition, Biological Potentials and Antimicrobial Activity of Wild and Cultivated Blackberries. Acta Period. Technol., 49, 65–79.
21. Kawarazuka, N. and Béné, C., 2011. The potential role of small fish species in improving micronutrient deficiencies in developing countries: building evidence. Public Health Nutrition, 14, 1927–1938.
22. Kowalczyk, A., Przychodna, M., Sopata, S., Bodalska, A., Fecka, I., 2020. Thymol and Thyme Essential Oil-New Insights into Selected Therapeutic Applications. Molecules., 25,18, 4125. doi: 10.3390/molecules25184125.
23. Křížek, M., Dadáková, E., Vácha, F., Pelikánová, T., Matějková, K., 2018. The effects of two essential oil and UV-light irradiation treatments on the formation of biogenic amines in vacuum-packed fillets of carp (Cyprinus carpio). LWT - Food Science and Technology, 95, 268– 273.
24. Lehane, L. and Olley, J., 2000. Histamine fish poisoning revisited. Int. J. Food Microbiol., 58, 1 – 37.
25. Leszczynska, J., Wiedlocha, M., Pytasz, U., 2004. The histamine content in some samples of food products. Czech J. Food Sci., 22, 81–86.
26. Liu, C., Mou, J., Su, Y.C. 2016. Behavior of *Salmonella* and *Listeria monocytogenes* in Raw Yellowfin Tuna during Cold Storage. Foods, 5(1), 16.
27. Maijala, R.L., Eerola, S.H., Aho, M.A., Hirn, J.A., 1993. The effect of GDL induced pH decrease on the formation of biogenic amines in meat. J. Food Protect., 56, 125 – 129.
28. Maiz, H.D.B., Guadix, E.M., Guadix, A., Gargouri, M., Espejo-Carpio, F.J., 2019. Valorisation of tuna viscera by endogenous enzymatic treatment. International Journal of Food Science, 54 ,4, 1100–1108.
29. Mutmainnah, Juharni, Nurmalasari, D., Yuliana, Khamsiah, A., Surahman, Edwarsyah, Vincentius, A., 2024. Identification of Pathogenic Bacteria in Fresh and Smoked Fish of the Tuna ,Thunnus sp.) with SNI 01-2332.2-2006. Egyptian Journal of Aquatic Biology & Fisheries, 28,4, 1495 – 1513.
30. Nader, Y.M., Ibrahim, I.A., Mohammed, Y.M., 2016. Reduction of Histamine in Frozen Basa Fish Fillets by Using Essential Oils. Global Veterinaria, 17 ,5, 420-423.
31. Oszmia´nski, J., Nowicka, P., Teleszko, M., Wojdyło, A., Cebulak, T., Oklejewicz, K., 2015. Analysis of Phenolic Compounds and Antioxidant Activity in Wild Blackberry Fruits. Int. J. Mol. Sci.,16, 14540–14553.
32. Özogul, F., Kacar, Ç., Hamed, I., 2015. Inhibition effects of carvacrol on biogenic amines formation by common food-borne

pathogens in histidine decarboxylase broth. LWT - Food Science and Technology, 64,1, 50– 55.
33. Pattipeilohy, M., Amin, M., Lestari, U., Suarsini, E. 2017. Isolation and identification of pathogenic bacteria in fresh tuna ,Katsuwonus pelamis) collected from fishermen in Ambon, Indonesia. Asian Journal of Microbiology, Biotechnology and Environmental Sciences, 19,4, 878– 883.
34. Pereira, E., Elliot, E.L., Singleton, L.S., Otto, M., Tesfai, A., Doyle, M., Hawk, H., Bloodgood, S., Benner, R.A., Ross, M.P., et al., 2021. An outbreak investigation of Scombrotoxin fish poisoning illnesses in the United States linked to yellowfin tuna imported from Vietnam- 2019. Journal of Food Protection, 84, 962–972.
35. Perez, A.P., Perez, N., Lozano, C.M.S., Altube, M.J., de Farias, M.A., Portugal, R.V., Buzzola, F., Morilla, M.J., Romero, E.L., 2019. The anti MRSA biofilm activity of Thymus vulgaris essential oil in nanovesicle. Phytomedicine, 57, 339–351.
36. Puupponen-Pimiä, R., Nohynek, L., Meier, C., Kähkönen, M., Heinonen, M., Hopia, A., Oksman-Caldentey, K.M. 2001. Antimicrobial properties of phenolic compounds from Finnish berries. J Appl Microbiol., 90,4, 494-507.
37. Ropiak, H.M., Ramsay, A., Mueller-Harvey, I., 2016. Condensed Tannins in Extracts from European Medicinal Plants and Herbal Products. J. Pharm. Biomed. Anal., 121, 225–231.
38. Sabry, M.A., Hayam, A.A.M., Ashour, R.M., Hamza, E., 2019. Histamine- Producing Bacteria and Histamine Induction in Retail Sardine and Mackerel from Fish Markets in Egypt. Food borne Pathog. Dis., 16, 597 - 603.
39. Traylor, J. and Mathew, D. 2021. Histamine Toxicity. In: Stat Pearls [Internet]. Treasure Island, FL: StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK29763046/
40. Velut, G., Delon, F., Mérigaud, J.P., Tong, C., Duflos, G., Boissan, F., Watier-Grillot, S., Boni, M., Derkenne, C., Dia, A., et al., 2019. Histamine food poisoning: a sudden, large outbreak linked to fresh yellowfin tuna from Reunion Island, France, April 2017. Eurosurveillance, 24, 1800405.
41. Xie, Y., Yang, W., Tang, F., Chen, X., Ren, L. 2015. Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. Curr. Med. Chem. 22, 132–149.
42. Yemmen, C. and Gargouri, M., 2022. Potential hazards associated with the consumption of Scombridae fish: Infection and toxicity from raw material and processing. Journal of Applied Microbiology, 132, 4077-4096.
43. Zia-Ul-Haq, M., Riaz, M., De Feo, V., Jaafar, H.Z.E., Moga, M., 2014. Rubus fruticosus L.: Constituents, Biological Activities and Health Related Uses. Molecules 19, 10998–11029.