Reduction of histamine levels in tuna fish with micro nano bubble ozone venturi system treatment

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Abstract. Histamine content is a key indicator of marine fish safety. Histamine levels above 15 ppm may trigger allergic reactions, while concentrations exceeding 100 ppm can cause poisoning. This study aimed to reduce histamine levels in tuna using the Micro Nano Bubble Ozone (MNBO) venturi system. The research employed three treatments: untreated tuna (control) and washed tuna with MNBO for 15, 30, 45, and 60 minutes. Tuna samples weighed an average of 250 g. The MNBO system consisted of a 20 g/h ozone generator, a 900 L ozonized water tank equipped with a 150 W submersible pump, and a ¾-inch venturi injector, connected to a 1.0 × 1.5 × 0.7 m washing tank. During washing, dissolved ozone concentrations range from 0.09 to 0.19 ppm. Histamine was analyzed using a spectrofluorometric method following SNI 2354.10:2016. The 45-minute MNBO treatment produced the most significant reduction, achieving a decrease of 1.76 log cycles. These findings demonstrate that MNBO technology is effective in lowering histamine levels in tuna and has potential applications in enhancing seafood safety during post-harvest handling.

1 Introduction

Tuna (*Euthynnus affinis*) is a marine fish species with high nutritional value and is widely consumed as a source of animal protein [1]; [2]. However, during storage, tuna is susceptible to histamine formation due to the activity of histamine-producing bacteria, leading to the accumulation of 2-(1H-imidazol-4-yl) ethanamine (histamine) [3]. Elevated histamine levels pose serious food safety risks, as concentrations above 15 ppm may cause allergic reactions, while levels exceeding 100 ppm may lead to poisoning [4]. Therefore, continuous monitoring of raw fish quality is essential to ensure safety and prevent foodborne illness outbreaks [5]. Fresh fish standards set by the National Standardization Agency (SNI 01-2729) require products to meet criteria of freshness, cleanliness, and proper organoleptic characteristics. Tuna that does not meet these standards may pose hazards, including excessive histamine levels, which must remain below 100 mg/kg to ensure consumer safety [6].

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Several studies have explored the application of Micro Nano Bubble Ozone (MNBO) technology in various fields, such as improving water quality in aquaculture [7], enhancing oxygen transfer in catfish ponds [8], and reducing microbial loads in fish species such as tilapia and eel [9], [10]. MNBO technology offers advantages over conventional aeration or ozonation methods because nanobubbles are highly stable, have a long residence time, and possess a large surface area that enhances ozone dissolution and mass transfer efficiency [11]. Unlike larger bubbles that rise rapidly and escape from the system, nanobubbles remain suspended in water, enabling more effective interaction with microbial contaminants, including histamine-producing bacteria.

Although MNBO has been applied in aquaculture and water treatment, studies focusing specifically on histamine reduction in fishery products remain limited. Existing treatments such as conventional ozonation, cold storage, or washing with chlorinated water often show limited penetration efficiency or reduced microbial inactivation capabilities. MNBO is potentially superior because its nanobubble structure improves ozone stability, enhances oxidative reactions, and allows deeper penetration into fish tissues, which may reduce histamine accumulation more effectively than conventional methods.

Given this research gap, this study investigates the application of MNBO technology to reduce histamine levels in tuna (Euthynnus affinis) as an effort to enhance seafood safety and prevent histamine-related health hazards.

2 Materials and Methods

2.1 Sample Collection

Fresh tuna (*Euthynnus affinis*) were obtained from the Puger Fish Auction Place (TPI), Jember Regency, in August 2025. A total of 15 tuna samples were used, divided into five treatments, each consisting of three fish per treatment: 0 minutes (control), 15 minutes, 30 minutes, 45 minutes, and 60 minutes of MNBO washing. Each fish weighed approximately 250 g, and the spine temperature was checked to ensure it did not exceed 4°C.

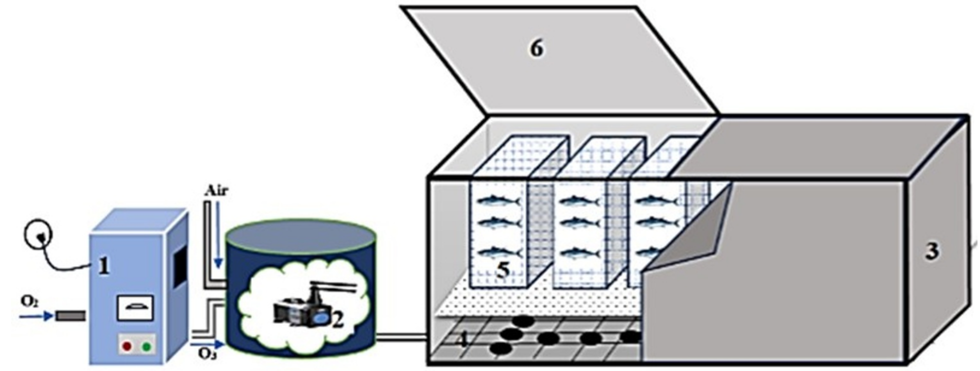
2.2 MNBO System Configuration

The MNBO system used in this study consisted of several components integrated to generate and dissolve ozone effectively. The system included:

- a 20 g/h ozone generator (Fig. 2),
- a 900 L ozonized water tank equipped with a 150 W submersible pump,
- a ¾-inch venturi injector for ozone dissolution (Fig. 3),
- a washing tank measuring 1.0 × 1.5 × 0.7 m,
- and an ozone analyzer DOZ-30 for monitoring dissolved ozone concentration (Fig. 4).

During the washing process, the dissolved ozone concentration remained in the range of 0.09–0.19 ppm. This relatively low concentration was intentionally maintained to prevent tissue oxidation in tuna while still achieving effective microbial inactivation. Nanobubbles produced by the MNBO system have a higher surface area and longer residence time, allowing ozone to remain active longer and increasing mass-transfer efficiency compared to conventional ozonation.

Figure 1 provides a schematic representation of the MNBO system and its operational configuration.



Caption:
1 Ozone generator with a capacity of 20 g/hour
2 50 liter capacity ozonized water tank equipped with submersible pump
3 Fish washing tank with MNBO venturi system technology
4 Nano Bubble Micro Aerator
5 Fish basket
6 Ozone wash tub door

Fig. 1. MNBO system components and operational layout

Fig. 2. Venturi injector used for ozone dissolution

Fig 3. Ozone generator unit (20 g/h)

Fig. 4. DOZ-30 ozone analyzer for dissolved ozone measurement

2.3 Experimental Procedure

The experimental procedure consisted of sequential stages beginning from sample selection to histamine quantification. A flowchart of the complete research workflow is presented in Figure 5, providing a visual overview of the MNBO-based histamine reduction process.

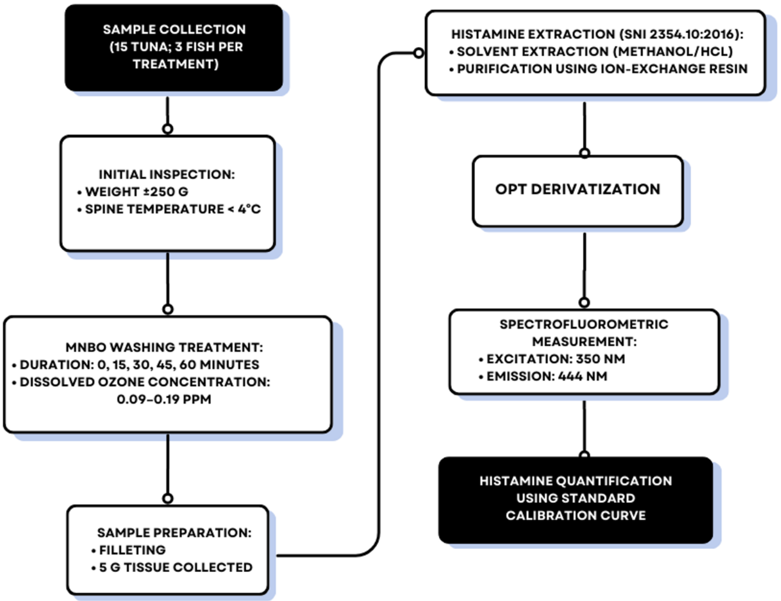


Fig. 5. Flowchart of the MNBO-based histamine reduction procedure

Each fish underwent MNBO washing according to its designated treatment. During this process, dissolved ozone concentration was monitored using a DOZ-30 analyzer and maintained between 0.09–0.19 ppm. This controlled low concentration was selected to prevent oxidative damage to tuna flesh while allowing nanobubbles to enhance ozone transfer efficiency, ensuring effective suppression of histamine-forming bacteria.

After treatment, tuna fillets were collected and a 5-gram tissue sample was prepared for histamine analysis. The extraction and purification steps followed the SNI 2354.10:2016 procedure, which included homogenization, solvent extraction, and purification using an ion-exchange resin to eliminate interfering compounds.

Purified extracts were derivatized with o-phthalaldehyde (OPT) to form a fluorescent complex. Fluorescence intensity was measured using a spectrofluorometer (excitation 350 nm; emission 444 nm). Histamine concentration was then calculated by comparing fluorescence values with a standard calibration curve prepared from histamine solutions of known concentrations, following the method specified in SNI 2354.10:2016 [12].

3 Results and Discussion

3.1 Technology MNBO

The MNBO (Micro–Nano Bubble Ozone) system is designed to enhance ozone dissolution and distribution in water through an integrated mechanism involving an ozone generator, a washing tank, a circulation pump, and a venturi injector. These components operate synergistically to ensure that ozone is absorbed into water efficiently and remains available for antimicrobial activity during the washing of tuna samples.

The venturi injector is the core component responsible for generating micro- and nanobubbles. When water flows through the constricted venturi section, the velocity increases, creating a pressure drop that produces a strong vacuum effect. This vacuum draws ozone gas from the generator into the water stream. As the mixture passes through the venturi throat, intense turbulence and shear forces fragment the ozone gas into extremely small bubbles, typically in the micro- and nanoscale range. Nanobubbles, which are less than 1 μm in diameter, exhibit unique physical characteristics, including high internal pressure, large interfacial surface area, and neutral buoyancy. These properties allow nanobubbles to remain suspended in water for long periods without rapidly rising to the surface, unlike conventional ozonated bubbles that escape almost immediately.

Once inside the washing tank, the circulating pump maintains continuous water flow, ensuring that micro- and nanobubbles are distributed homogeneously throughout the tank. The pump not only supports the formation of nanobubbles but also prevents bubble coalescence, which would otherwise reduce the effective surface area available for mass transfer. The ozone meter continuously monitors dissolved ozone concentration, ensuring that the system maintains the intended working range of 0.09–0.19 ppm. Although this concentration is relatively low compared to typical ozonation systems, MNBO technology compensates for the lower dosage through superior gas–liquid interaction efficiency. The configuration of the MNBO system can be seen in Fig. 6, whereas Fig. 7 illustrates the microbubble–ozone mixture generated during operation, showing the fine bubble dispersion characteristic of MNBO technology.

Fig. 6. MNBO Equipment

Fig. 7. A mixture of water and ozone is shown in a foam-like shape

Compared to conventional ozonation, MNBO significantly enhances ozone mass-transfer efficiency. In traditional systems, large ozone bubbles ascend rapidly and burst at the surface before substantial dissolution can occur, leading to poor ozone utilization. In contrast, the micro- and nanobubbles produced by MNBO minimize buoyancy-driven escape and maximize the amount of ozone that dissolves directly into the aqueous phase. Their exceptionally high surface area-to-volume ratio increases the interface where ozone can diffuse into water, while their long residence time prolongs ozone availability. This combination results in a more stable and sustained concentration of dissolved ozone, providing prolonged antimicrobial activity even at low applied doses.

The improved mass transfer and stability of ozone within the MNBO system make it particularly effective in reducing histamine-producing bacteria on tuna surfaces. These bacteria, such as *Morganella morganii*, *Proteus vulgaris*, and *Enterobacter aerogenes*, are highly sensitive to oxidative stress. The persistent micro- and nanobubbles facilitate continuous exposure of bacterial cells to dissolved ozone, increasing cell membrane damage, disrupting metabolic processes, and ultimately reducing bacterial populations more effectively than conventional ozonation. Therefore, MNBO not only improves ozone utilization efficiency but also enhances the disinfection potential of the system, enabling histamine control without the need for high ozone doses.

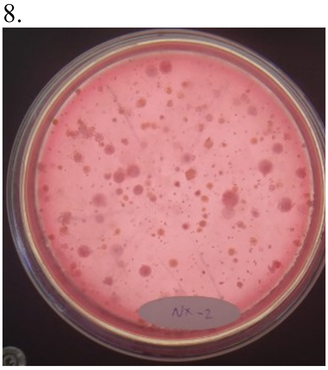
3.2 Reduction of Histamine-Producing Bacteria

The performance of the MNBO equipment on reducing bacteria in the control treatment, contact time of 15 minutes, 30 minutes, 45 minutes and 60 minutes is shown in Table 1.

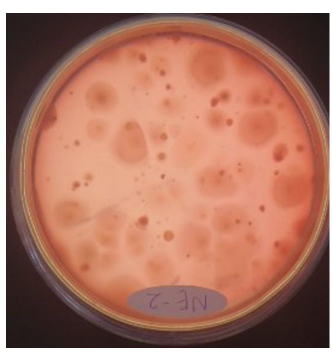
Table 1. Level of reduction in histamine-producing bacteria in various treatments

Treatment	Repeat (cycle log)			Observation value	Decline rate (log cycle)
	1	2	3		
Control	5,51	5,57	4,72	5,26 ± 0,47	0
Contact time 15 minutes	4,02	3,10	3,64	3,59 ± 0,46	1,67
Contact time 30 minutes	3,85	3,20	3,93	3,66 ± 0,40	1,60
Contact time 45 minutes	4,15	2,30	4,05	3,50 ± 1,04	1,76
Contact time 60 minutes	4,19	4,10	3,02	3,77 ± 0,65	1,49

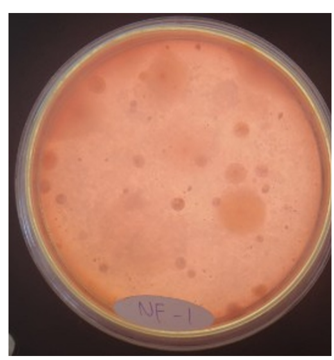
Table 1 shows the reduction of histamine-producing bacteria under each MNBO washing duration. The highest bacterial reduction was observed at 45 minutes, achieving a decrease of 1.76 log cycles, indicating that this duration is the most effective for suppressing histamine-forming microorganisms. Improvements were also seen at 15, 30, and 60 minutes, although the 45-minute exposure provided the greatest reduction under the ozone concentration used in this study. These findings align with previous ozonation studies, where increased exposure time contributed to greater microbial reduction efficiency [13]. Visual observations of bacterial colonies before and after treatment are shown in Fig. 8.



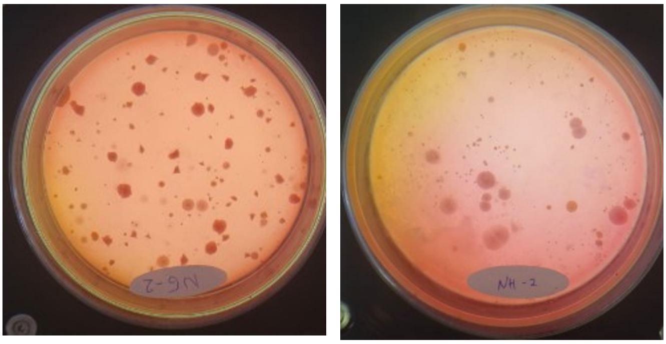
Control



Contact time 15 minutes



Contact time 30 minutes



Contact time 45 minutes Contact time 60 minutes

Fig. 8. Observation of histamine-producing bacteria

Research on reducing histamine levels and histamine-forming bacteria from pindang badeng tuna with 20% salt sprinkling treatment and boiling for 8 hours then stored at room temperature with sample storage at 0, 8, 16, 24 and 32 hours showed that storing pindang fish can reactivate the production of histamine levels which showed significantly different values during the 32-hour storage process [14].

Histamine can form during production, processing, and distribution. Bacterial growth is controlled by cold storage of tuna at temperatures below 4.4°C. Histamine formation can be stopped by freezing. However, once formed, histamine cannot be removed by conventional processing [15]. Freezing technology can maintain fish quality, but it cannot improve quality if the fish is no longer fresh or contaminated. Therefore, fish handling must be carried out to high standards to maintain quality and keep microorganism levels below the threshold of 5×10^5 CFU/gram [16].

Each country has different maximum histamine content standards. Indonesia, through the Indonesian National Standard (SNI), sets a maximum limit of 100 ppm [16]. Indonesian tuna exports have been rejected several times because histamine levels exceed the limit, indicating weaknesses in the handling and processing chain [17]. Based on 2013–2018 data from the FDA, CFIA Rejections of Products, and the EU RASFF portal, there have been a number of cases of tuna export rejections from Indonesia due to high histamine content [18]. This is an indicator that quality control needs to be improved throughout the process, from production to distribution.

Ozone can reduce histamine-producing bacteria by inhibiting the growth of microorganisms that cause histamine poisoning, such as Morganella morganii, Proteus vulgaris, and Enterobacter aerogenes, which convert histidine to histamine. Scientific research shows that ozone application can reduce the number of these bacteria and inhibit histamine formation, thereby improving the safety and quality of fishery products, especially fish that are susceptible to high histamine formation, such as tuna, as well as agricultural products such as edamame [19, 20].

Several studies have developed ozone-treated water as an alternative to chemicals to decontaminate microorganisms and reduce harmful chemical compounds. This technology is being applied in various fields such as wastewater treatment, healthcare, agriculture, and fisheries due to ozone's effective oxidizing properties. Several studies have shown that ozonated water can control microbes in strawberries [21], reduce pesticide residues in edamame [19], reduce total microbes; eliminate ammonia odor and improve the quality of shark [22]. Ozone water technology with a dose of 0.3 mg/L, with a contact time of 120 minutes, room temperature of 8oC, ozone was able to eliminate tuna fish bacteria by 91.2% and the protein content decreased to 0.22% when stored cold for 7 days [23]. There is no research data on the direct effect of Ozone technology on histamine reduction, but the indirect effect confirms that ozone is capable of reducing total bacteria, known as an effective agent in killing bacteria and other microorganisms. This means that ozone can help reduce the number of bacteria that break down histidine, which in turn can reduce the formation of histamine.

4 Conclusion

The MNBO system equipped with a 900-liter tank and a 20 g/h ozone generator was capable of producing dissolved ozone concentrations of 0.09–0.19 ppm, which effectively reduced histamine-producing bacteria in tuna by 1.76 log cycles at 45 minutes of treatment. This level of bacterial reduction indicates that MNBO technology has strong potential to support compliance with national and international histamine standards, thereby improving the safety and quality of tuna destined for export markets. The ability of MNBO to enhance microbial control at low ozone dosages also suggests promising applications in seafood processing operations, particularly as a non-thermal, residue-free sanitation method. However, this study was limited to a single fish species, a specific tank configuration, and relatively low ozone concentrations, without evaluating direct histamine levels or sensory impacts. Future research should explore the optimization of ozone dosage, treatment duration, and system scale-up, as well as assess MNBO performance across different fish species, processing conditions, and real industrial settings. Investigation into the correlation between bacterial reduction and actual histamine formation over storage time would also provide more comprehensive insight into the technology's effectiveness.

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