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Morganella morganii GROWTH IN SKIPJACK TUNA UNDER DIFFERENT STORAGE CONDITIONS AND HISTAMINE DETECTION USING TLC METHOD

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Abstract

Morganella morganii, a strong histamine-producing bacterium (HPB), has been frequently detected in seafood, such as skipjack tuna. Temperature fluctuations and improper packaging have resulted in bacterial proliferation and histamine production. This study aimed to determine the effects of different packaging and temperature conditions on *M. morganii* growth and to examine histamine formation in skipjack tuna. A factorial design with two factors, namely packaging type (vacuum and non-vacuum) and storage temperature (4, 15, 30, and 40°C), was used in this study. The bacterial growth model over time was analyzed using DMFit software. Histamine production was analyzed using thin-layer chromatography (TLC) combined with ImageJ program visualization. The results indicated that different temperatures significantly affected the bacterial growth rate ($p<0.05$). The application of vacuum packaging at 4 °C retarded histamine formation in skipjack tuna cubes. The highest growth rate (0.2652 log $CFU^{-1}h^{-1}$) was observed in samples under non-vacuum packaging stored at 40 °C. *M. morganii*. At 15 °C, a 3 to 4 log increase was observed, starting from 3.2 to 7.5 (vacuum packaging) and from 5.8 to 8.3 log CFU-*1* mL-*1* (non-vacuum packaging) at the end. Nevertheless, the production of histamine in vacuum-packed samples stored at 15°C after days 3 and 4 of incubation were 446 ppm and 443.5 ppm, respectively. These findings highlight the importance of proper packaging of skipjack tuna using a cold chain system during storage. This study also confirmed the potential application of TLC for the detection of histidine and histamine.

Keywords: growth rate, non-vacuum packaging, temperature, thin-layer chromatography, vacuum-packaging

Pertumbuhan *Morganella morganii* pada Cakalang dengan Kondisi Penyimpanan Berbeda dan Deteksi Histamin Metode TLC

Abstrak

Morganella morganii sebagai bakteri penghasil histamin yang kuat sering kali terdeteksi pada hasil perikanan, misalnya cakalang. Faktanya, fluktuasi suhu dan pengemasan yang buruk menyebabkan peningkatan pertumbuhan bakteri dan produksi histamin. Penelitian ini bertujuan mengetahui pengaruh kondisi pengemasan dan suhu terhadap pertumbuhan *M. morganii* dan menghitung pembentukan histamin pada daging cakalang. Penelitian ini menggunakan rancangan faktorial dengan dua faktor meliputi jenis kemasan (vakum dan nonvakum) dan suhu penyimpanan (4, 15, 30, dan 40°C). Model pertumbuhan

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bakteri dianalisis menggunakan program DMFit. Produksi histamin dianalisis menggunakan kromatografi lapis tipis (TLC) yang divisualisasikan dengan program ImageJ. Hasil penelitian menunjukkan bahwa suhu berbeda secara signifikan memengaruhi laju pertumbuhan bakteri (p<0,05). Pengemasan vakum dengan kombinasi penyimpanan suhu 4°C mampu menghambat pembentukan histamin pada daging cakalang. Laju pertumbuhan tertinggi (0,2652 log $CFU^{-1}h^{-1}$) diamati pada sampel dalam kemasan udara suhu penyimpanan 40°C. Pada suhu 15°C, peningkatan jumlah bakteri sebesar 3 hingga 4 log ditunjukkan pada sampel kemasan vakum (3,2–7,5 log CFU^{-1} mL^{-1}) dan sampel kemasan udara (5,8–8,3 log CFU^{-1} mL^{-1}). Namun, produksi histamin pada sampel vakum dengan penyimpanan suhu 15°C memiliki kadar histamin tinggi setelah 3 dan 4 hari penyimpanan dengan konsentrasi masing-masing sebesar 446 dan 443,5 ppm. Temuan ini menyoroti pentingnya kemasan yang sesuai pada cakalang perlu diaplikasikan bersama dengan sistem rantai dingin selama penyimpanan. Studi ini juga mengonfirmasi potensi aplikasi TLC untuk deteksi pembetukan histidina dan histamin.

Kata kunci: kemasan vakum, kemasan nonvakum, kromatografi lapis tipis, laju pertumbuhan, suhu

INTRODUCTION

As stated in the Regulation of the Ministry of Marine Affairs and Fisheries of the Republic of Indonesia number 35/PERMEN-KP/2014, skipjack tuna, an important fish commodity, is geographically well-distributed in Indonesia (Rachmawati *et al.,* 2024). Production increased from 432,845 tons in 2021 to 474,810 tons in 2022, with an export value of 960,266,000 USD (Setiawan *et al* 2024). Skipjack tuna is widely recognized for its high nutritional value, containing 73.8% moisture, 21.2% protein (of which 35.4–74.9% comprises essential amino acids), 2.2% fat (with a total of 37.7% polyunsaturated fatty acids (PUFAs)), 1.6% ash, and 1.2% carbohydrates (Chakma *et al.,*2022). In terms of amino acid profiles, skipjack tuna is notable for its relatively high histidine content, ranging from 1.9 to 8.9 mg/100 g (Chakma *et al.,*2022; Chakraborty *et al.,*2017) which plays a vital role in the biosynthesis of proteins and enzymes, such as serine proteases (Brosnan & Brosnan, 2020). Histidine, while essential for protein and enzyme synthesis, serves as a precursor for histamine formation through the catalytic action of histamine decarboxylase (HDC*)*.

Histamine is a primary mediator of allergic reactions and is responsible for a wide range of poisoning symptoms, including headache, nasal secretion, hypotension, urticaria, abdominal cramps, gastric acid, inflammation, burning sensation, and central nervous system symptoms. In severe cases, histamine exposure can lead to fatal outcome (Rachmawati & Triwibowo, 2022; Rahmani *et al.,*2018; Schirone *et al.,*2017; Yoshikawa *et al.,*2014). Several cases of histamine poisoning have been reported globally. For instance, in the United States, a case involving an adult showed three phases of symptoms: first, prodromal or gastrointestinal; then, acute or dermal; and finally, subacute or respiratory (Tamasi *et al.,*2022). In 2017, an outbreak of histamine poisoning occurred in France, where 40 adults were reported to have been affected after consuming tuna (40 cases) (Velut *et al.,*2019). When compared to tuna and bullet tuna, skipjack tuna has contained the highest histamine levels (36–174.2 mg/kg) exceeding maximum limit set by FDA (50 ppm). A risk analysis study reported that Indonesian communities might be exposed to 4.6–10.38 mg of histamine/day/individual by consuming skipjack tuna based on the estimated daily intake (26.42–59.57 g/day) (Rachmawati *et al.,*2024).

HDC enzyme is produced by *M. morganii, Klebsiella pneumoniae, Raoultella* sp.*, Photobacterium* sp.*, Enterobacter aerogenes, Vibrio harveyi, Pseudomonas fluoroscens, Streptomyces griseus, Fusobacterium varium, Clostridum perfringens,* and *Limosilactobacillus reuteri* are responsible to HDC production (*Alya'ainun et al.,* 2021*; Dityanawarman et al.,* 2020*; Schirone et al.,* 2017). *M. morganii* is considered one of the most potent histamine-producing bacteria (HPB), capable of producing histamine concentrations exceeding 2,000 ppm (Dityanawarman *et al.,* 2020), which is higher than those produced by *Citrobacter freundii* (<100 ppm) (Margareta *et al.,* 2020), *V. alginolyticus* (200-500 ppm) (Tao

et al., 2022a), *P. khistanii* (1,038 ppm), and *P. phosphoreum* (<1,188 ppm) (Bjornsdottir-Butler *et al.,* 2018).

M. morganii has been reported tolerant to wide ranges of temperatures, pressures, pH, and oxygen. Although *M. morganii* has demonstrated tolerance to low temperatures, it did not produce histamine during storage at 4°C (Dityanawarman *et al.,* 2020). However, the long distribution of skipjack tuna has faced temperature variations, which contribute to histamine formation (Dityanawarman *et al.,* 2020, Rachmawati *et al.,* 2024). The growth and histamine production of *M. morganii* increased with increasing temperatures (4, 15, 30, and 40 °C). *M. morganii* grown in seafood matrices has been characterized as resistant to thermal (50 – 58 °C) and high-pressure (200 – 600 MPa) conditions (Enache *et al.,* 2013; Lee *et al.,* 2020). Moreover, *hdc* genes have been reported to be highly induced under low pH (4.5 to 6.7) and histidine-rich substrates (Yang *et al.,* 2020). Histamine formation typically affects the increase in pH values (Octariani *et al.,* 2020). At low pH values, this bacterium can produce > 1, 000 ppm of histamine in salted-pickled and fermented fish products (Tao *et al.,* 2022b; Yang *et al.,* 2020). Since this bacterium thrives under the aforementioned extreme conditions and poses a high potential hazard, its growth and histamine formation should be monitored, particularly in skipjack tuna.

In addition to temperature control, vacuum packaging serves as an alternative method for inhibiting the formation of histamine in fish products. Vacuum packaging is typically applied to chilled and smoked skipjack tuna to maintain quality during storage by minimizing oxygen exposure (Mentang *et al.,* 2022; Patil *et al.,* 2020; Zhang *et al.,* 2015). Using High-performance liquid chromatography (HPLC), a previous study detected 50 ppm histamine in vacuum-packed fish stored at 25 °C for 48 h, which was not significantly different from that in non-vacuum packaging (Lee *et al.,* 2019). Whereas the Ultra Performance Liquid Chromatography (UPLC) has successfully analyzed 133 ppm of histamine from trout samples stored at 12 °C for seven days, higher than those stored at 3.5°C for 14 days (1.1 ppm) (Matějková *et al.,* 2013). Vacuum packaging inhibited the growth and activity of HPB.

Thin-layer chromatography (TLC) is an alternative, simple, rapid, and practical technique for assessing histamine levels when high-technology elucidation techniques such as HPLC, UPLC, gas chromatography (GC), fluorometric assay, or Enzyme-Linked Immunosorbent Assay (ELISA) are not available. This technique has the potential for practical use in on-the-spot histamine monitoring in skipjack tuna during distribution. A similar method has been previously developed using Pauly's or diazonium reagents, as well as lane and spot analyzers or ImageJ for image visualization (Tao *et al.,* 2011; Yu *et al.,* 2018). However, there are several limitations, such as the requirement for freshly prepared Pauly's reagent, which must be stored at 4°C and protected from light (Ma *et al.,* 2023). Furthermore, diazonium is a reactive compound with potential explosive hazards (Sheng *et al.,* 2015). Thus, we alternatively offered the application of ninhydrin reagent combined with ImageJ visualization for rapid and simple semi-quantitative TLC analysis. This study aimed to elucidate the effect of temperature on the growth of *M. morganii* and to compare histamine production in vacuum- and non-vacuum-packed skipjack tuna using the TLC method. It is expected that the outcome can provide essential insights for establishing best practices in skipjack tuna handling and safety management systems.

MATERIALS AND METHODS

Sample Preparation

Fresh skipjack tuna weighing approximately 6 kg was obtained from Sadeng Port, Gunung Kidul, Yogyakarta, during a one-week fishing trip. The sample was immediately transported to the Laboratory of Fish Product Quality and Safety, Fisheries Department, Gadjah Mada University, using a cold box to maintain the cold chain. The dorsal part of the fish (± 5 cm from the back of the head and from the tip of the tail) was filleted. Skipjack tuna was prepared as a growth medium according to a previous study (Guizani *et al.,*

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2005), in which the fillets were cut into cubes measuring approximately 1.5 cm × 1.5 cm and weighing 5 g each. The skipjack tuna cubes were then stored at -20 °C before use. When needed, the cubes were thawed at 4 °C for 24 h.

Histamine Producing Bacteria Cultivation

M. morganii TK07 isolated from spoiled tuna and *M. morganii* ATCC 25830 (Thermo Scientific, Germany) isolates were initially grown in tryptone soy agar (TSA; Oxoid) and incubated at 37°C for 24 hours. Single colonies were subsequently transferred into tryptone soy broth (TSB; Oxoid) and incubated under the same conditions for 24 h. The resulting cultures were streaked onto slanted TSA medium and incubated at 37°C for 24 h to establish working stocks. Inoculum preparation followed the method described by Nei (2014) with modifications. A single colony from the working culture was inoculated into TSB under the same incubation conditions. The resulting broth culture was serially diluted four times with 0.85% NaCl until the bacterial count reached 4 log CFU^{-1} mL^{-1}. A final volume of 45 mL of bacterial inoculum was prepared for subsequent experiments.

Bacterial Inoculation to Skipjack Tuna Cubes

M. morganii inoculation was performed according to a previous study (Lee *et al.,* 2019). Prior to inoculation, skipjack tuna cubes were surface-sterilized by immersion in a 70% alcohol solution for 2 min, followed by draining under aseptic conditions for 2 min. Skipjack tuna cubes were then immersed in the bacterial inoculum solution using the dipping method for an additional 2 min. The sample was aseptically redrained for 2 min. Skipjack tuna cubes inoculated with *M. morganii* TK07 were individually packed under vacuum for 1.5 min. Meanwhile, those inoculated with *M. morganii* ATCC 25830 were packed using a Ziplock film.

The inoculated samples were incubated at four different temperatures: 4, 15, 30, and 40°C. The 4˚C condition was selected based on the critical limit temperature for histamine formation set by the FDA at 4.4˚C. The 15 °C incubation temperature was related to the body temperature of the fish during the landing process at the Fishing Port. The temperature of 30 °C used in this study was assumed to be room temperature or the temperature of the fish storage facility, while 40 °C simulated the unusually elevated daytime ambient temperature at the port. Incubation at 4°C and 15°C was carried out for 7 days with daily sampling to monitor pH values and bacterial growth dynamics. Incubation at 30°C and 40°C was carried out for 24 h, with observations made at 3-hours interval. The parameters observed for each analysis were total plate count, pH, and histamine levels. The study was conducted in two repetitions.

Histamine and Histidine Levels using Thin Layer Chromatography (TLC) Method

A 1 mL of the homogenized sample, prepared during the total plate count (TPC) stage, was centrifuged at 12,000 rpm for 5 min. The supernatant was collected and used for thin-layer chromatography (TLC) analysis. TLC silica gel plates (60F254, Merck, Germany) measuring 10 × 20 cm were prepared using silica as the stationary phase. The mobile phase consisted of an ammonia-methanol mixture at a 1:20 (v/v) ratio. The mobile phase was then purified into the chamber and slowly homogenized. The TLC plate was marked with a pencil at 1.5 cm from the bottom edge (application line) and 1 cm from the top edge (solvent limit).

A 0.5 µL aliquot of the sample supernatant was carefully spotted onto the designed application point on the air-dried TLC plate. The plate is then inserted into a chamber filled with the mobile phase. The chromatography development proceeded until the solvent front reached the top edge. The plate was then removed and dried with a hairdryer. The plate was sprayed with a ninhydrin solution and re-dried using a hairdryer until dark red spots, indicating the presence of histamine, appeared. Ninhydrin solution was prepared by mixing 300 mg of ninhydrin in 100 mL of n-butanol with 2 mL of glacial acetic acid. The plate was then

scanned immediately after the appearance of colored spots for documentation and further analysis.

Histamine and histidine levels were then quantified based on the dark-red spot-area observed on the TLC plate, using the ImageJ software. The concentration of histidine was analyzed using ImageJ software with a threshold of 225 ppm at 4, 15, and 30 °C and a threshold of 215 ppm at 40 °C. For standard calibration, histamine (TCI, Germany) was analyzed in the range of 300–1,500 ppm using the developed sensor. Histidine (TCI, Germany) was analyzed in the standard range of 2,000–10,000 ppm. The corresponding spot areas for both the histamine and histidine standards were measured using the ImageJ software.

pH Values

Samples were homogenized with Butterfield's phosphate buffer (BPB) at a ratio of 1:9 (w/v) of skipjack tuna cubes to BPB. The pH meter was then immersed in the homogenized sample, and the pH value was recorded once a stable reading was achieved (Ratnawati *et al.,* 2023).

Data Analysis

Statistical analyses were performed using SPSS version 26.0. Analysis of Variance (ANOVA) was conducted to evaluate the effect of different temperatures, packaging conditions, and storage time, with these variables treated as independent factors ($p<0.05$). The Tukey test was used to assess significant differences among the factors. Additionally, total plate count (TPC) data were plotted to analyze bacterial growth rates as a function of temperature and storage time using the dynamic modeling software DMFit (www.combase.cc). DMFit is a tool designed to illustrate the effects of various temperatures on the cell concentration of bacteria (Bancalari *et al.,* 2016).

A primary growth model was developed by plotting bacterial growth against storage time, allowing the determination of key kinetic parameters: lag time, growth rate (μ_{max}), and maximum population density (N_{max}). The values of μmax represent the growth rate, $N_{initial}$ indicates the initial bacterial population, and N_{final} indicates the final bacterial population. The secondary square root model was used to predict μ_{max} as a function of temperature (Powell *et al.,*2015).

RESULTS AND DISCUSSION

The Growth of *M. morganii* TK07

The growth of *M. morganii* TK07 in skipjack tuna cubes packed under different packaging conditions and stored at 4, 15, 30, and 40 °C is shown in Figure 1. Overall, bacterial growth showed different trends throughout storage ($p<0.05$).

The initial population of vacuum-packed skipjack tuna cubes inoculated with *M. morganii* ranged from 2.85 to 3.52 log CFU^{-1} mL^{-1} (Figure 1). In contrast, under non-vacuum packaging conditions, the initial bacterial load was higher, ranging from 4.7 to 6.2 log CFU^{-1} mL^{-1}. A similar phenomenon has occurred in *C. freundii* and *K. aerogenes* grown in fish matrices, resulting in high variability of the initial bacterial population accounting for 3.8–4.8 log CFU^{-1} mL^{-1} and 3.0–4.2 log CFU^{-1} mL^{-1}, respectively (Margareta *et al.,* 2020; Rachmawati *et al.,* 2022). Additionally, the samples used in our study were specifically collected from the dorsal part of two individual fish. Therefore, biological variation may have resulted in the distinction of the initial bacterial populations. A previous study reported high variability in the microbiome among fish individuals and different body parts (Berggren *et al.,* 2022).

After four to seven days of storage at 4 °C, no significant growth was observed in the inoculated samples. With TPC values ranging from 2.85 to 3.03 log CFU^{-1} mL^{-1}, the bacterial loads of vacuum-packed skipjack tuna cubes were approximately half of those observed in non-vacuum samples. The findings showed that low-temperature control is crucial for inhibiting bacterial growth and histamine formation. Our study showed a decrease and stagnant trend in bacterial population, ranging from 5.7 to 4.4 log CFU^{-1} mL^{-1} in non-vacuum samples and from 2.8 to 3.0 log CFU^{-1} mL^{-1} in vacuum-packaged samples during storage. This result was consistent with a previous study that observed a decline in the growth of

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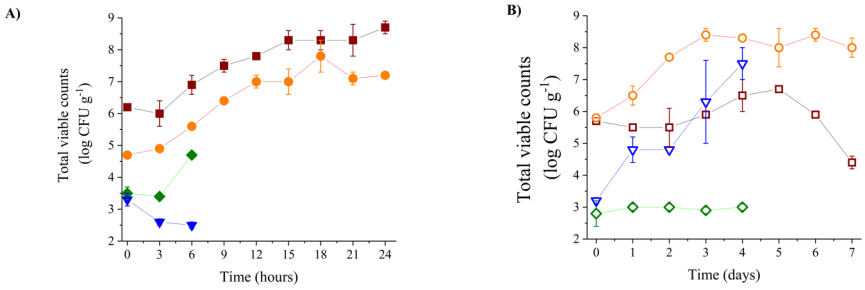

Figure 1 The growth of *Morganella morganii* in skipjack tuna cubes under (◇) vacuum packed and stored at 4˚C; (▽) vacuum packed and stored at 15˚C; (◆) vacuum packed and stored at 30˚C; (▼) vacuum packed and stored at 40˚C; (○) non-vacuum packed and stored at 4˚C; (□) non-vacuum packed and stored at 15˚C; (●) non-vacuum packed and stored at 30˚C; (■) and non-vacuum and stored at 40˚C . Note: A= time (hours) and B= time (days).

M. psychrotolerans and *M. morganii* at 4 – 5 °C (< 4 log CFU/g) (Nei, 2014; Wang *et al.,* 2020).

At low temperatures, the retardation growth of bacteria is confirmed as the acclimation or lag phase, signaled by the halt of protein production, resulting in a slower growth rate than those incubated at optimal temperatures (Barria *et al.,* 2013). In contrast, at 15 °C, the increase in the number of bacteria was pronounced in the inoculated samples ($p< 0.05$). Within 4–7 days, 3 to 4 log increases of bacterial counted from 3.2 to 7.5 log CFU^{-1} mL^{-1} in vacuum-packed samples, and from 5.8 to 8.3 log CFU^{-1} mL^{-1} in non-vacuum-packed samples. Bacterial loads in non-vacuum packed and vacuum-packed samples stored at 30 °C increased by 16% and 42% after six hours, respectively. This trend was also noted in non-vacuum-packed skipjack tuna cubes stored at 40 °C. Conversely, the latter temperature resulted in stagnant growth of bacteria in vacuum-packed samples.

To further investigate the effects of packaging and temperature on the growth rate of *M. morganii*, a growth kinetic analysis was performed to enumerate the growth rate (μ_{max}), initial bacterial population ($N_{initial}$), and final bacterial population (N_{final}) (Table 1).

The results demonstrated the sensitivity of the bacteria to temperature changes. As shown in Table 1, the maximum growth rate in vacuum-packaged samples stored at 4 °C was higher (μmax = 0.03 log $CFU^{-1}h^{-1}$) than that in non-vacuum-packaged samples (μmax = 0.01 log $CFU^{-1}h^{-1}$). When stored at 15 ˚C, no significant difference in bacterial growth rates was found between non-vacuum (μmax = 0.06 log $CFU^{-1}h^{-1}$) and vacuum-packed samples (μmax = 0.05 log $CFU^{-1}h^{-1}$). Vacuum-packed samples stored at 30 °C exhibited a higher maximum growth rate (μmax = 0.19 log $CFU^{-1}h^{-1}$). Notably, the lowest growth rate was observed in vacuum-packed samples stored at 40 °C, with a value of -0.11 log CFU^{-1} mL^{-1}. In contrast, non-vacuum samples stored under the same conditions revealed the highest growth rate of 0.26 log CFU^{-1} mL^{-1}. These findings are consistent with the bacterial growth shown in Figure 2. Although the initial number of bacteria in non-vacuum samples at 40 ˚C was lower (4.42 log CFU^{-1} mL^{-1}) than that at 30˚C (7.16 log CFU^{-1} mL^{-1}), the maximum growth was achieved in just 18 h.

Under vacuum packaging, however, a different pattern was observed, with a decline in bacterial population from 3.23 to 2.54 log CFU^{-1} mL^{-1}, resulting in a negative maximum growth rate (μmax = -0.11 log CFU^{-1} mL^{-1}). A negative μmax was in contrast to that inoculated in tuna fish infusion broth (TFIB), which led to the highest value (μmax = 0.578 log CFU^{-1} mL^{-1}) (Dityanawarman *et al.,*2020). In our study, at higher temperatures, vacuum

Table 1 Growth rate of *Morganella morganii* TK07* and ATCC 25830** on skipjack tuna flesh with different incubation temperatures

Packaging condition	Temperature (˚C)	μmax (log CFU/h)	$N_{initial}$ (log CFU^{-1} mL^{-1})	N_{final} (log CFU^{-1} mL^{-1})	Incubation time (h)
Vacuum*	4	0.03[d]	2.85	3.00	96
	15	0.05[d]	2.95	7.54	96
	30	0.19[b]	3.52	4.67	6
	40	-0.11[f]	3.23	2.54	6
Non-vacuum**	4	0.01[e]	6.94	4.10	168
	15	0.06[d]	7.89	7.74	168
	30	0.10[c]	7.16	9.98	24
	40	0.26[a]	4.42	7.15	24

Different supercripts on the same column indicate significant different of maximum growth rates (p<0.05)

packaging may have retarded the growth of *M. morganii*. Prior studies have reported stagnant growth of vacuum-packed tuna inoculated with *M. morganii*. and stored at 25 °C (Lee *et al.,* 2020b).

In non-vacuum samples, the growth rate of *M. morganii* on skipjack tuna cubes increased with increasing temperature, from 0.01 log $CFU^{-1}h^{-1}$ at 4 °C to 0.26 log $CFU^{-1}h^{-1}$ at 40 °C. In addition, our findings revealed the application of low temperature at 4 °C to inhibit bacterial growth (μmax = 0.01– 0.03 log $CFU^{-1}h^{-1}$). Similarly, the growth rate of *M. morganii* at a temperature of 4 ˚C has been reported to be relatively low (μmax = 0.01 log $CFU^{-1}h^{-1}$) and did not experience a significant increase in their population (4.2–6.0 log CFU^{-1} mL^{-1}) (Dityanawarman *et al.,* 2020). The growth of *M. morganii* in non-vacuum packaging tended to increase with higher incubation temperatures.

pH Values

Acidity (pH) is an indicator of fish freshness. The pH values of skipjack tuna cubes packed in vacuum and non-vacuum packaging at 4, 15, 30, and 40 °C are presented in Figure 2.

Figure 2 shows the pH changes in skipjack tuna cubes during storage at different temperatures. Overall, the pH values (5.1–6.9) of skipjack tuna cubes in our study were within the pH range (5.2–7.0) of fresh fish (European Food Safety Authority, 2015). The differences in pH values in the early days of storage can be attributed to biological variations in samples, microbial loads, handling or storage procedures, and stress response (Barbosa *et al.,* 2018). Our study revealed that the pH values of non-vacuum samples stored at 4, 15, and 30 °C increased to 5.1–5.3, 5.9–6.9, and 5.6–6.7, respectively. The increase in pH values may be attributed to the accumulation of biogenic amines and volatile compounds during storage (Ratnawati *et al.,*2023). At a lower pH value of 5.6 at 37 ˚C, denaturation rapidly occurred in tuna meat, resulting in auto-oxidation. Denaturation transforms myoglobin to metmyoglobin, leading to browning and the release of histidine (Nurilmala *et al.,* 2018; Nurilmala & Ochiai, 2016), which is a precursor of histamine formation. The activity of the hdc enzyme occurred at pH 4–8 and temperature ranges of 10–50 °C. The *hdc* enzyme increased and reached an optimum point at pH 7 and 30 °C before decreasing with increasing temperature (Wang *et al.,* 2020).

In contrast, the pH values of vacuum-packed samples in this study decreased slightly, ranging from 6.8 to 6.7 (30 °C) and from 6.3 to 6.2 (40 °C). A weak negative correlation with storage time has previously been observed in canned sardines stored at 4 ˚C (Cruz *et al.,* 2022). The decrease in pH values is possibly due to autolysis, protein

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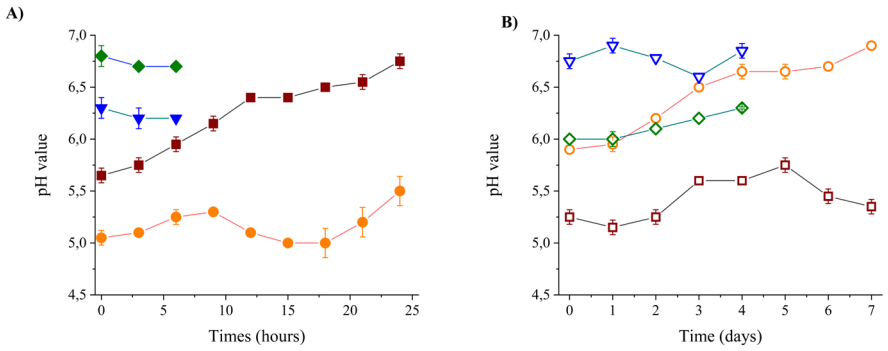

Figure 2 pH values of *Morganella morganii* in skipjack tuna cubes under (◇) vacuum packed and stored at 4˚C; (▽) vacuum packed and stored at 15˚C; (◆) vacuum packed and stored at 30˚C; (▼) vacuum packed and stored at 40˚C; (○) non-vacuum packed and stored at 4˚C; (□) non-vacuum packed and stored at 15˚C; (●) non-vacuum packed and stored at 30˚C; (■) and non-vacuum and stored at 40˚C. Note: A= time (hours) and B= time (days).

denaturation, and bacterial activity, which produces acidic compounds. During the autolysis stage, autolytic enzymes degrade adenosine triphosphate (ATP) and related compounds, resulting in rigor mortis and thus structural decay, myofibril change, lactic acid production, a decrease in pH values, discoloration, and quality loss (Barbosa *et al.,* 2018; Huang *et al.,* 2023). Moreover, the multiplication of lactic acid bacteria in vacuum-packed samples might lead to a decrease in pH due to the production of acid compounds during storage (Lee *et al.,* 2019; Yang *et al.,* 2020).

Histamine and Histidine Productions

In this study, the effects of temperature and packaging conditions on the growth of *M. morganii* and histamine production in skipjack tuna were elucidated. Owing to the hazardous risk of histamine, regulatory authorities have established the maximum allowable concentration in food products. A histamine concentration of 50 ppm is considered indicative of food deterioration, whereas a concentration of 200 ppm is associated with the potential to cause human illness (Food and Drug Administration, 2024). This concentration limit is also relevant for EU countries, Gulf state countries, Australia, New Zealand, China and Korea. In other words, a histamine level of 50 ppm indicates rejection of food, including seafood products (Debeer *et al.,* 2021; Schirone *et al.,* 2017).

Histamine and histidine contents (Merck, Germany) in vacuum and non-vacuum packed skipjack tuna cubes was monitored at different storage temperatures. Histidine levels were determined because this amino acid is a precursor of histamine formation in the presence of *hdc* (Wang *et al.,*2020). To enumerate histidine and histamine levels, a TLC method was performed, and quantification was performed using ImageJ software. The limit of histamine detection (LOD) using the TLC method is 20-23 ppm lower than the standard limit established by the FDA (Tao *et al.,* 2011; Yu *et al.,* 2018). By utilizing a standard curve with different ranges of histamine and histidine concentrations, the technique could detect the increase in histidine and histamine levels as a function of the spotted areas. The standard curves for histamine and histidine were generated from the data obtained from the standards (Figure 3), and the following equations were derived from the respective standard curves: for histamine, y = 0.0002x-0.0402 with an R^2 value = 0.9263, and for histidine, y = 0.3457x-0.0018 with an R^2 value = 0.9291.

The formation of histamine and histidine is presented in Table 2. The results indicated that histamine and histidine were well separated on the TLC plates. Histidine

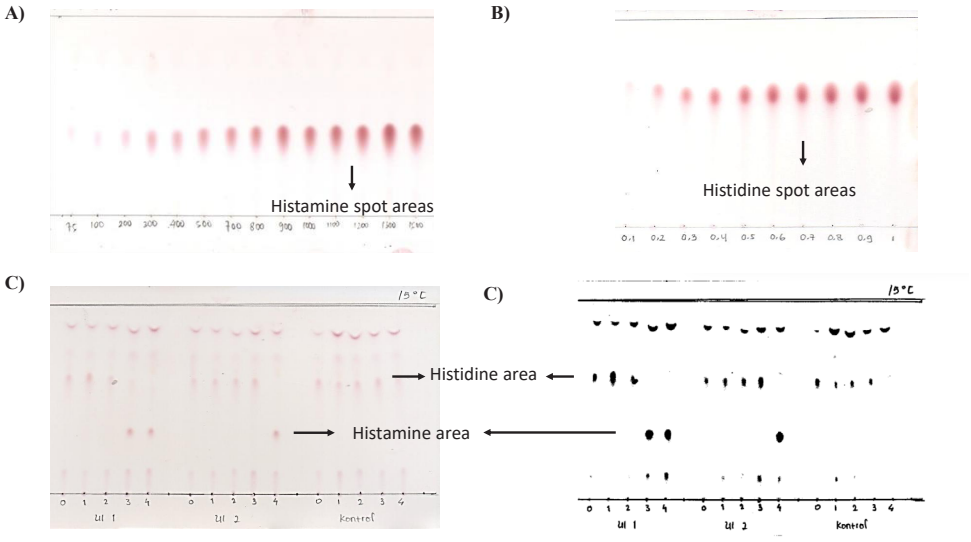

Figure 3 A) Histamine and B) histidine standards, C) histamine production in vacuum-packed skipjack tuna inoculated with *Morganella morganii* TK07 on thin layer chromatography (TLC) plates, D) ImageJ visualisation

Table 2 Histamine formation by *Morganella morganii* TK07* and ATCC 25830* in fish meat with different incubation temperatures

Time (day)	4˚C			15˚C		
	Histidine levels (ppm)	Histamine levels (ppm)		Histidine levels (ppm)	Histamine levels (ppm)	
		Vacuum-packed*	Non-vacuum packed**		Vacuum-packed*	Non-vacuum packed**
0	1,421±5.2	n.d.	n.d.	2,014±9.5	n.d.	n.d.
1	1,075±3.3	n.d.	n.d.	3,105±16.0	n.d.	n.d.
2	1,305±7.0	n.d.	n.d.	3,527±5.5	n.d.	35.08
3	1,720±12.8	n.d.	5.1	1,503±14.0	446	75.38
4	2,306±12.3	n.d.	43.8	1,358±8.4	443.5	114.56
Time (h)	30˚C			40˚C		
	Histidine levels (ppm)	Histamine levels (ppm)		Histidine levels (ppm)	Histamine levels (ppm)	
		Vacuum-packed*	Non-vacuum packed**		Vacuum-packed*	Non-vacuum packed**
0	3,201±21.2	n.d.	n.d.	2,450±20.8	n.d.	n.d.
3	3,512±23.4	n.d.	n.d.	3,072±14	n.d.	n.d.
6	3,342±11.9	n.d.	n.d.	3,930±15	n.d.	128.73
9	3,300±9.8	n.d.	1,186.40	3,214±22	n.d.	n.d.

n.d.: not detected

http://dx.doi.org/10.17844/4mms8195

content analysis revealed high variability in histidine levels across the samples. For instance, the initial histidine concentration in samples stored at 4˚C was 1,421±5.2 ppm, while samples stored at 15, 30, and 40 ˚C exhibited higher histidine levels of 3,201±21.2 ppm. There was also a fluctuation in histidine concentrations during storage, from 1,358±8.4 to 3,300±9.8 ppm at the end of storage. This variation might be attributed to the use of several fish as the medium for skipjack tuna cubes. In this study, skipjack tuna cubes were prepared from the dorsal parts of the fish (± 5 cm from the back of the head and the tip of the tail). Although histidine is abundant in the muscle tissues of tuna, including those in the dorsal parts, the denaturation process releases free histidine that is transported to the cytoplasm of bacterial cells, where histamine production occurs in the presence of *hdc* (Nurilmala *et al.,* 2018; Oktariani *et al.,* 2022). Indeed, the TLC method detected histidine levels. Unfortunately, this technique cannot distinguish between total and free histidine. Thus, further analysis is required to calculate the free histidine levels that affect hdc expression and histamine formation.

As indicated in Table 2, no histamine formation was observed in vacuum-packed samples stored at 4, 30, and 40 °C, presumably because the number of bacteria was still relatively low, approximately 2.8–3.5 log CFU^{-1} mL^{-1}. Under aerobic conditions at 4 °C, however, our study uncovered that bacterial populations of at least 5.9±0.1 log CFU^{-1} mL^{-1} were capable of producing 5.1 ppm histamine. This observation is in contrast to previous studies, which have reported a higher minimum bacterial threshold of 6.3 log CFU^{-1} mL^{-1} as a benchmark for the formation of histamine by *M. morganii* at 5 °C of storage (Emborg & Dalgaard, 2008).

Notably, at the same temperature, vacuum packaging effectively retarded the formation of histamine in skipjack tuna cubes, as evidenced by the absence of histamine in samples stored under these conditions. Supporting this, no histamine formation was detected in *M. morganii* culture incubated at 4 °C for 10 days (Wang *et al.,* 2020). In contrast, it has been reported that at this temperature, histamine production of 30-50 ppm in samples commenced after nine days (Lee *et al.,* 2019). At lower temperatures, bacterial enzyme activity is reduced, and cytoplasmic membrane fluidity decreases, thereby impairing cellular transport mechanisms. A prior study stated that at 10 °C, the activity of the HDC enzyme was limited to 40% and possibly decreased at lower temperatures (Wang *et al.,*2020). This is consistent with previous findings that demonstrated low levels of histamine (<1 ppm) in tuna flesh stored at 4 °C for 5 days, while at the same period, samples spiked with histamine demonstrated an increase from 12.8 to 68.2 ppm as the temperature and number of histamine-forming bacteria increased (Altafini *et al.,* 2022; Dityanawarman *et al.,* 2020).

In contrast, *M. morganii* in vacuum-packed samples at 15 °C produced histamine production was evident on the third and fourth days, with values of 466 ppm and 443.5 ppm, respectively, exceeding the FDA maximum standard (50 ppm). In non-vacuum samples, faster but lower histamine levels were observed on the second day (35.08 ppm). Interestingly, despite the higher number of bacteria (8.3 log CFU^{-1} mL^{-1}), histamine levels on the fourth day were significantly lower (114.56 ppm) than those observed under vacuum conditions. With a bacterial count of 7.5 log CFU^{-1} mL^{-1}, higher histamine production (443.5 ppm) was observed in vacuum-packed samples stored at 15 °C for four days. Our study revealed that at a higher temperature of 15 °C, 4.8±0.5 log CFU^{-1} $mL^{-1}$of bacteria in vacuum-packed skipjack tuna cubes were able to produce five-fold higher histamine levels (446 ppm) than those in non-vacuum samples. This phenomenon contrasts with prior studies on mackerel stored at 15 °C, which demonstrated lower histamine formation in vacuum-packed samples than in non-vacuum-packed samples. When the TPC values were > 7 log CFU^{-1} mL^{-1}, the histamine levels started to increase to > 10 ppm (vacuum-packed samples) and > 40 ppm (non-vacuum samples) (Lee *et al.,*2019). M. morganii is a facultative anaerobic bacterium, which explains its ability to grow under

vacuum-packed conditions. Thus, bacteria survived under anoxic conditions in vacuum-packaged samples.

Within 9 h of incubation at 30 and 40 °C, only the non-vacuum packaged fish exhibited histamine production of 1,186.40 ppm (30 °C) and 128.73 ppm (40 °C). Differences in bacterial strain types might affect histamine production, and *M. morganii* ATCC 25830 exhibited weaker histamine-forming activity. At higher temperatures (30 and 40 °C), histamine production was detected only under aerobic conditions. Within 9 h of incubation and total bacteria > 7 log CFU/g, high levels of histamine were detected at 30 °C, resulting in 1,186.40 ppm of histamine or 10-fold higher than those at 40 °C. This finding was consistent with the study of *Citrobacter freundii* CK01 in bullet tuna, which reported the maximum histamine levels of approximately 100 ppm in samples stored at 15 and 30 °C within 48 and 12 h, respectively (Margareta *et al.,* 2020). Pronouncing at higher levels, 412–450 ppm of histamines has been detected from body parts of sardines, when the bacteria population counted > 5 log CFU/g at 21 °C of incubation (Mohamed *et al.*,2022). Another study demonstrated that the production of histamine at 37 °C was higher (350–600 ppm) than that at 25 °C during 24 h of incubation (Lee *et al.,* 2016). Previous studies have demonstrated that the relative activity of *HDC* peaked at 30 °C, reaching 100%, but declined at elevated temperatures above 30 °C, falling below 40% (Wang *et al.,* 2020).

Overall, this study revealed that vacuum packaging in combination with low temperature at 4 °C was effective in inhibiting the growth of *M. morganii* and histamine formation for 4 days. However, the use of vacuum packaging was not effective at 15 °C. This is because *M. morganii* is a facultative anaerobic bacterium that can grow with or without oxygen. There are two subspecies, morganii and siboni, and different strains that have different decarboxylase abilities (Ryser *et al.,* 2021). Two isolates of histamine-producing bacteria (HPB), *M. morganii* ATCC 25830 and TK07, were used in our study. The findings revealed that histamine content was predominantly detected in *M. morganii* ATCC 25830 than in TK07. However, histamine levels were detected in samples inoculated with *M. morganii* TK07 when the populations were > 6 log CFU.mL^{-1}. It should be noted that *M. morganii* ATCC 25830 was isolated from patients with summer diarrhea, whereas *M. morganii* TK07 was isolated from spoiled tuna.

Our study revealed that a total of 35.08 ppm of histamine was produced by inoculated non-vacuum skipjack tuna cubes with ATCC 25830 strain when TPC exceeded 7 log CFU. mL^{-1}. *M. morganii* ATCC 25830 has been tested positive for *hdc* (Dityanawarman *et al.,* 2020). Although *hdc* has been reported to be sensitive to at least 4 log CFU.mL^{-1} of *M. morganii* ATCC 25830 in standard medium, a higher population at 8 log CFU.mL^{-1} in TSB had the capability to produce 26.71 ppm of histamine (Wongsariya *et al.,* 2016). Different *hdc* activities might occur, resulting in different histamine formation. Regarding histamine formation, it is suggested to analyze the phenotype and genotype characterization of these two isolates to understand their metabolic activities and how environmental factors influence their expression.

CONCLUSION

This study demonstrated that the differences in incubation temperatures and types of packaging significantly affected the growth of *M. morganii* in skipjack tuna cubes. At the same temperature, vacuum-packed samples exhibited the lowest growth rate of *M. morganii*, which was -0.114 log CFU^{-1} mL^{-1}. This study also confirmed the potential application of the TLC method to detect histamine formation in skipjack tuna under different packaging and storage conditions, with maximum histamine levels of up to 1,186.40 ppm. The implementation of a low temperature of 4 °C in combination with vacuum and non-vacuum packaging is required to retard the growth of *M. morganii* and histamine production in skipjack tuna.

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REFERENCES

Altafini, A., Roncada, P., Guerrini, A., Sonfack, G. M., Accurso, D., & Caprai, E. (2022). Development of histamine in fresh and canned tuna steaks stored under different experimental temperature conditions. *Foods*, 11(24), 4034 https://doi.org/10.3390/foods11244034

Alya'ainun, R., Fathoni, E. Y., & Puspita, I. D. (2021, July 28-29). The effect of pH on bacterial growth and histamine formation by *Klebsiella pneumoniae* CK02 and *Raoultella ornithinolytica* TN01 [conference session]. *The 4th International Symposium on Marine and Fisheries Research, Yogyakarta, Indonesia. IOP Conference Series: Earth and Environmental Science*, 919(1), 012039 https://doi.org/10.1088/1755-1315/919/1/012039

Bancalari, E., Bernini, V., Bottari, B., Neviani, E., & Gatti, M. (2016). Application of impedance microbiology for evaluating potential acidifying performances of starter lactic acid bacteria to employ in milk transformation. *Frontiers in Microbiology*, 7(1628), 1–11. https://doi.org/10.3389/fmicb.2016.01628

Barbosa, R. G., Gonzaga, L. V., Lodetti, E., Olivo, G., Costa, A. C. O., Aubourg, S. P., & Fett, R. (2018). Biogenic amines assessment during different stages of the canning process of skipjack tuna (*Katsuwonus pelamis*). *International Journal of Food Science and Technology*, 53(5), 1236–1245. https://doi.org/10.1111/ijfs.13703

Barria, C., Malecki, M., & Arraiano, C. M. (2013). Bacterial adaptation to cold. *Microbiology*, 159 (12), 2437–2443. https://doi.org/10.1099/mic.0.052209-0

Berggren, H., Tibblin, P., Yıldırım, Y., Broman, E., Larsson, P., Lundin, D., & Forsman, A. (2022). Fish skin microbiomes are highly variable among individuals and populations but not within individuals. *Frontiers in Microbiology*, 12, 1-13 https://doi.org/10.3389/fmicb.2021.767770

Bjornsdottir-Butler, K., Abraham, A., Harper, A., Dunlap, P. V., & Benner, R. A. (2018). Biogenic amine production by and phylogenetic analysis of 23 *Photobacterium* species. *Journal of Food Protection*, 81(8), 1264–1274. https://doi.org/10.4315/0362-028X.JFP-18-022

Brosnan, M. E., & Brosnan, J. T. (2020). Histidine metabolism and function. *Journal of Nutrition*, 150, 2570S-2575S. https://doi.org/10.1093/jn/nxaa079

Chakma, S., Rahman, M. A., Mali, S. K., Debnath, S., Hoque, M. S., & Siddik, M. A. B. (2022). Influence of frozen storage period on the biochemical, nutritional, and microbial quality of Skipjack tuna (*Katsuwonus pelamis)* collected from the Bay of Bengal coast of Bangladesh. *Food Chemistry Advances*, 1(100139), 1–7. https://doi.org/10.1016/j.focha.2022.100139

Chakraborty, K., Krishna Raola, V., Joy, M., & Makkar, F. (2017). Nutritional attributes in the fillet of skipjack tuna (*Katsuwonus pelamis*) from the Arabian Sea near the south-west coast of India. *Journal of the Marine Biological Association of the United Kingdom*, 97(2), 419–432. https://doi.org/10.1017/S0025315416000527

Cruz, R., Pereira, V., Pinho, T., Ferreira, I. M. P. L. V. O., Novais, C., & Casal, S. (2022). Safety and quality of canned sardines after opening: A shelf-stability study. *Foods*, 11(991), 1-16 https://doi.org/10.3390/foods11070991

Debeer, J., Bell, J. W., Nolte, F., Arcieri, J., & Correa, G. (2021). Histamine limits by country: A survey and review. *Journal of Food Protection*, 84(9), 1610–1628. https://doi.org/10.4315/JFP-21-129

Dityanawarman, A., Puspita, I. D., Ratnawati, S. E., Ekantari, N., & Tamplin, M. (2020). Growth rate and histamine production of *Klebsiella* sp. Ck02 isolated from skipjack tuna compared with *Morganella morganii* ATCC 25830 at various incubation temperatures. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology*, 15(1), 1–9. https://doi.org/10.15578/squalen.

v15i1.441

Emborg, J., & Dalgaard, P. (2008). Growth, inactivation and histamine formation of *Morganella psychrotolerans* and *Morganella morganii* — development and evaluation of predictive models. *International Journal of Food Microbiology*, 128, 234–243. https://doi.org/10.1016/j.ijfoodmi

Enache, E., Kataoka, A., Glenn Black, D., Weddig, L., Hayman, M., & Bjornsdottir-Butler, K. (2013). Heat resistance of histamine-producing bacteria in irradiated tuna loins. *Journal of Food Protection*, 76(9), 1608–1614. https://doi.org/10.4315/0362-028X.JFP-12-467

European Food Safety Authority. (2015). Scientifica and technical assistance on the evaluation of the temperature to be applied to pre-packed fishery products at retail level. *Scientific Report of EFSA*, 13(7), 1–48.

Food and Drug Administration. (2024). *Sec. 540.525 Scombrotoxin (histamine)-forming fish and fishery Products-decomposition and histamine (CPG 7108.24) compliance policy guide: guidance for FDA Staff*. https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/compliance-

Guizani, N., Al-Busaidy, M. A., Al-Belushi, I. M., Mothershaw, A., & Rahman, M. S. (2005). The effect of storage temperature on histamine production and the freshness of yellowfin tuna (*Thunnus albacares*). *Food Research International*, 38(2), 215–222. https://doi.org/10.1016/j.foodres.2004.09.011

Huang, X., Wang, H., & Tu, Z. (2023). A comprehensive review of the control and utilization of aquatic animal products by autolysis-based processes: Mechanism, process, factors, and application. *Food Research International*, 164, 112325 https://doi.org/10.1016/j.foodres.2022.112325

Lee, Y. C., Chen, Y. F., Huang, Y. L., Kung, H. F., Chen, T. Y., & Tsai, Y. H. (2016). Hygienic quality, adulteration of pork and histamine production by *Raoultella ornithinolytica* in milkfish dumpling. *Journal of Food and Drug Analysis*, 24(4), 762–770. https://doi.org/10.1016/j.jfda.2016.04.005

Lee, Y. C., Hsieh, C. Y., Chen, M. L., Wang, C. Y., Lin, C. Saint, & Tsai, Y. H. (2020). High-pressure inactivation of histamine-forming bacteria *Morganella morganii* and *Photobacterium phosphoreum*. *Journal of Food Protection*, 83(4), 621–627. https://doi.org/10.4315/0362-028X.JFP-19-267

Lee, Y. C., Tseng, P. H., Hwang, C. C., Kung, H. F., Huang, Y. L., Lin, C. Saint, Wei, C. I., & Tsai, Y. H. (2019). Effect of vacuum packaging on histamine production in Japanese Spanish mackerel (*Scomberomorus niphonius*) stored at various temperatures. *Journal of Food Protection*, 82(11), 1931–1937. https://doi.org/10.4315/0362-028X.JFP-19-143

Ma, J. X., Liu, P., Wang, Y. X., Ren, X., Zhang, R., & Li, L. W. (2023). A histidine-rich fusion tag enables real-time monitoring of recombinant protein expression by Pauly reaction-based colorimetric assay. *Biochemical and Biophysical Research Communications*, 666, 128–136. https://doi.org/10.1016/j.bbrc.2023.05.004

Margareta, G., Ratnawati, S. E., & Puspita, I. D. (2020, February 2020). Growth Rate and Histamine Production of *Citrobacter freundii* CK01 in Various Incubation Temperatures. *The 3rd International Symposium on Marine and Fisheries Research, Yogyakarta, Indonesia. E3S Web of Conferences*, 147, 03018 https://doi.org/10.1051/e3sconf/202014703018

Matějková, K., Křížek, M., Vácha, F., & Dadáková, E. (2013). Effect of high-pressure treatment on biogenic amines formation in vacuum-packed trout flesh (*Oncorhynchus mykiss*). *Food Chemistry*, 137(1–4), 31–36. https://doi.org/10.1016/j.foodchem.2012.10.011

Mentang, F., Montolalu, R. I., Dien, H. A., Meko, A., & Berhimpon, S. (2022). Shelf life and presence of pathogens in liquid-smoked Skipjack pampis packed in vacuum packaging (VP), modified atmosphere packaging (MAP), and stored at

ambient temperature. *Nutricion Clinica y Dietetica Hospitalaria*, 42(4), 73–78. https://doi.org/10.12873/424mentang

Mohamed, W. A., Hassanen, E. I., Mansour, H. A., & Mahmoud, M. A. (2022). Bacteria forming histamine and shelf life of sardine (*Sardina pilchardus*) at different temperatures and storage times with an emphasis on histopathological changes in the skeletal musculature. *Egyptian Journal of Aquatic Biology & Fisheries,* 26(5), 345–360. www.ejabf.journals.ekb.eg

Nei, D. (2014). Evaluation of non-bacterial factors contributing to histamine accumulation in fish fillets. *Food Control*, 35(1), 142–145. https://doi.org/10.1016/j.foodcont.2013.06.037

Nurilmala, M., & Ochiai, Y. (2016). Molecular characterization of southern bluefin tuna myoglobin (*Thunnus maccoyii*). *Fish Physiology and Biochemistry*, 42(5), 1407–1416. https://doi.org/10.1007/s10695-016-0228-0

Nurilmala, M., Ushio, H., & Ochiai, Y. (2018). pH- and temperature-dependent denaturation profiles of tuna myoglobin. *Fisheries Science*, 84(3), 579–587. https://doi.org/10.1007/s12562-018-1198-1

Oktariani, A. F., Ramona, Y., Sudaryatma, P. E., Dewi, I. A. M. M., & Shetty, K. (2022). Role of marine bacterial contaminants in histamine formation in seafood products: A Review. *In Microorganisms*, 10 (6), 11197 https://doi.org/10.3390/microorganisms10061197

Patil, A. R., Chogale, N. D., Pagarkar, A. U., Koli, J. M., Bhosale, B. P., Sharangdhar, S. T., Gaikwad, B. V, & Kulkarni, G. N. (2020). Vacuum packaging is a tool for shelf life extension of fish product : a review. *Article in Journal of Experimental Zoology*, 23, 807–810. https://www.researchgate.net/publication/339842514

Powell, S. M., Ratkowsky, D. A., & Tamplin, M. L. (2015). Predictive model for the growth of spoilage bacteria on modified atmosphere packaged Atlantic salmon produced in Australia. *Food Microbiology*, 47, 111–115. https://doi.org/10.1016/j.fm.2014.12.001

Rachmawati, N., Ariyani, F., Triwibowo, R., Januar, H. I., Dwiyitno, D., Yennie, Y., Kusmarwati, A., & Poernomo, A. (2024). Exposure assessment and semi-quantitative risk analysis of histamine in tuna and tuna-like fish from Indonesia. *Food Additives and Contaminants - Part A*, 41(11), 1498–1508. https://doi.org/10.1080/19440049.2024.2396971

Rachmawati, N., Powell, S. M., Triwibowo, R., Nichols, D. S., Ross, T., & Tamplin, M. L. (2022). Modelling growth and histamine formation of *Klebsiella aerogenes* TI24 isolated from Indonesian pindang. *International Journal of Food Microbiology*, 362(109459), 1–9. https://doi.org/10.1016/j.ijfoodmicro.2021.109459

Rachmawati, N., & Triwibowo, R. (2022, 25 March 2022). Histamine Fish Poisoning (HFP) in Indonesia: Current status and challenges. *International Food Conference, Surabaya, Indonesia. E3S Web of Conferences*, 344, 05001 https://doi.org/10.1051/e3sconf/202234405001

Rahmani, J., Miri, A. L. I., Mohseni-Bandpei, A., Fakhri, Y., Bjørklund, G., Keramati, H., Moradi, B., Amanidaz, N., Shariatifar, N., & Khaneghah, A. M. (2018). Contamination and prevalence of histamine in canned tuna from Iran: A systematic review, meta-analysis, and health risk assessment. *Journal of Food Protection*, 81(12), 2019–2027. https://doi.org/10.4315/0362-028X.JFP-18-301

Ratnawati, S. E., Kuuliala, L., Walgraeve, C., Demeestere, K., Ragaert, P., & Devlieghere, F. (2023). The effect of high oxygen modified atmospheres on the quality degradation of packed live blue mussels (*Mytilus edulis*). *LWT*, 177, 104047 https://doi.org/10.1016/j.lwt.2023.114537

Ryser, L. T., Arias-Roth, E., Perreten, V., Irmler, S., & Bruggmann, R. (2021). Genetic and phenotypic diversity of *Morganella morganii* isolated from cheese. *Frontiers in Microbiology*, 12, 1-11 https://doi.org/10.3389/fmicb.2021.738492

Schirone, M., Visciano, P., Tofalo, R., & Suzzi, G. (2017). Histamine food poisoning.

In Handbook of Experimental Pharmacology 241, 217–235. Springer New York LLC. https://doi.org/10.1007/164_2016_54

Setiawan, A., Wahyuni, T., Asianto, A.D., Malika, R., Wulansari, R.E., Retno, R.A., Listyowati, T., Rakhman, F.A., Indria, P.D., MAritong, M.L., Arifah,F.A., Putra, H.I.K., Narentar, J.E.S. (2024). Kelautan dan perikanan dalam angka tahun 2024. Pusat Data, Statistik dan Informasi. 504 hlm.

Sheng, M., Frurip, D., & Gorman, D. (2015). Reactive chemical hazards of diazonium salts. *Journal of Loss Prevention in the Process Industries*, 38, 114–118. https://doi.org/10.1016/j.jlp.2015.09.004

Tamasi, J., Balla, Z., Csuka, D., Kalabay, L., & Farkas, H. (2022). The missing link: A case of severe adverse reaction to histamine in food and beverages. *American Journal of Case Reports*, 23(1), 1-7 https://doi.org/10.12659/AJCR.934212

Tao, Z., Sato, M., Han, Y., Tan, Z., Yamaguchi, T., & Nakano, T. (2011). A simple and rapid method for histamine analysis in fish and fishery products by TLC determination. *Food Control*, 22(8), 1154–1157. https://doi.org/10.1016/j.foodcont.2010.12.014

Tao, Z., Wu, X., Liu, W., Takahashi, H., Xie, S., Ohshima, C., & He, Q. (2022a). Prevalence of histamine-forming vacteria in two kinds of salted fish at town markets of Guangdong Province of South China. *Journal of Food Protection*, 85(6), 956–960. https://doi.org/10.4315/JFP-21-215

Velut, G., Delon, F., Mérigaud, J. P., Tong, C., Duflos, G., Boissan, F., Watier-Grillot, S., Boni, M., Derkenne, C., Dia, A., Texier, G., Vest, P., Meynard, J. B., Fournier, P. E., Chesnay, A., & De Santi, V. P. (2019). Histamine food poisoning: A sudden, large outbreak linked to fresh yellowfin tuna from Reunion Island, France, April 2017. *Eurosurveillance*, 24(22), 1-8 https://doi.org/10.2807/1560-7917.ES.2019.24.22.1800405

Wang, D., Yamaki, S., Kawai, Y., & Yamazaki, K. (2020). Histamine production behaviors of a psychrotolerant histamine-producer, *Morganella psychrotolerans*, in various environmental conditions. *Current Microbiology*, 77(3), 460–467. https://doi.org/10.1007/s00284-019-01853-y

Wongsariya, K., Bunyapraphatsara, N., Yasawong, M., & Chomnawang, M. T. (2016). Development of molecular approach based on PCR assay for detection of histamine producing bacteria. *Journal of Food Science and Technology*, 53(1), 640–648. https://doi.org/10.1007/s13197-015-1982-1

Yang, Q., Meng, J., Zhang, W., Liu, L., He, L., Deng, L., Zeng, X., Ye, C. (2020). Effect of amino acid decarboxylase genes and pH on the amine formation of enteric bacteria from Chinese traditional fermented fish (Suan Yu). *Frontier in Microbiology*, 11, 1130: 1–14. https://doi.org/10.3389/fmicb.2020.01130

Yoshikawa, T., Nakamura, T., Shibakusa, T., Sugita, M., Naganuma, F., Iida, T., Miura, Y., Mohsen, A., Harada, R., & Yanai, K. (2014). Insufficient intake of L-histidine reduces brain histamine and causes anxiety-like behaviors in male mice. *Journal of Nutrition*, 144(10), 1637–1641. https://doi.org/10.3945/jn.114.196105

Yu, H., Zhuang, D., Hu, X., Zhang, S., He, Z., Zeng, M., Fang, X., Chen, J., & Chen, X. (2018). Rapid determination of histamine in fish by thin-layer chromatography-image analysis method using diazotized visualization reagent prepared with: P-nitroaniline. *Analytical Methods*, 10(27), 3386–3392. https://doi.org/10.1039/c8ay00336j

Zhang, B., Deng, S. gui, Gao, M., & Chen, J. (2015). Effect of slurry ice on the functional properties of proteins related to quality loss during skipjack tuna (*Katsuwonus pelamis*) chilled storage. *Journal of Food Science*, 80(4), C695–C702. https://doi.org/10.1111/1750-3841.12812