Changes in microbial composition and quality characteristics of yellowfin tuna under different storage temperature

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Abstract

Yellowfin tuna is one of the commercially important fish varieties, and inappropriate storing may deteriorate its safety and quality. This study aimed to investigate the microbial composition and quality characteristics of yellowfin tuna stored at different temperatures for varying amounts of time. With an increase in the storage temperature and storage time, the biogenic amines, the total volatile basic nitrogen TVB-N, and the total viable cell count steadily increased, which influenced the quality of tuna. The most significant histamine concerning food safety reached levels of 21.25, 235.05, 1166.18, and 3799.29 mg/kg, respectively. The values of total viable cell counts were increased to 7.04, 7.97, 8.24, and 8.91 log CFU/g after storage at 0, 4, 10, and 20 °C for 12 days, 7 days, 7 days, 3 days, respectively. Additionally, changes in microbial composition were evaluated by high-throughput sequencing, and the results showed that *Pseudomonas* was the dominant spoilage bacteria in yellowfin tuna. The bacterial dynamics and their correlation with biogenic amines and TVB-N in yellowfin tuna were analyzed. A positive correlation between *Pseudomonas*, *Shewanella*, *Morganella*, *Acinetobacter*, and biogenic amines was found. *Pseudomonas* showed significant correlation with histamine, cadaverine, and putrescine. This study provides insights into yellowfin tuna quality and microbial composition, which provide theoretical guidance for maintaining seafood safety and quality during distribution and storage.

Keywords: biogenic amines; correlation; high-throughput sequencing; microbial composition; quality characteristics; yellowfin tuna

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Introduction

Yellowfin tuna (*Thunnus albacores*) is an economically important marine fish in the international market because of its excellent nutritional content and appealing flavors (Wang *et al.*, 2021). Yellowfin tuna is highly susceptible to spoilage during storage and transportation as it contains high protein and high moisture content, which provide favorable conditions for rapid microbial growth (Li *et al.*, 2020a). The microbiota of fisheries is influenced by the species, the area in which they were captured, and their feeding habits (Jaaskelainen *et al.*, 2019; Li *et al.*, 2021), and microorganisms have different metabolic activities under varied environmental conditions. Therefore, an in-depth understanding of the spoilage of seafood requires monitoring the bacterial changes during storage and subsequently developing strategies for protecting seafood safety and quality.

The traditional method of analyzing bacterial changes is the culture-dependent incubation method. However, this method can only determine the cultivable bacteria, and the results are highly affected by the culture and the researcher's experience. This approach cannot comprehensively reflect the microbial components in the background. Of late high-throughput sequencing technology is an effective method of analyzing microbiota in food and has been widely used in recent studies. It has been applied to investigate the microbiota of sausages (Zhang *et al.*, 2021), roast duck (Chen *et al.*, 2020), fermented fish (Shen *et al.*, 2021), fish surimi (Zhao *et al.*, 2021), and so on. High-throughput sequencing can detect species undetected by culture-dependent method and first-generation sequencing in microbial food composition.

Quality characteristics of seafood can be determined by biogenic amines and total volatile basic nitrogen (TVB-N). Previous studies have shown that the presence of biogenic amines in seafood is also associated with safety (Mohamed *et al.*, 2009; Ruiz-Capillas *et al.*, 2019). The accumulation of biogenic amines and TVB-N in fish during storage is related to the microbial flora, fish species, and environmental conditions (Takahashi *et al.*, 2015; Wang *et al.*, 2020b). Moreover, biogenic amines accumulation mainly occurs by bacterial decarboxylation of free amino acids in seafood (Moniente *et al.*, 2021).

Therefore, the objective of this study was to evaluate the bacterial composition and quality of yellowfin tuna changes because of varying storage temperatures and later reveal the relationships between them. This research contributes to a better understanding of yellowfin tuna shelf life, spoiling, and the control of bacterial growth in it. It will also help devise better measures to preserve the safety and quality of yellowfin tuna during distribution and storage.

Materials and Methods

Sampling and storage

Fresh yellowfin tuna (*T. albacores*) was purchased from Guangdong Shun Xin Sea Fisheries Group Co., Ltd. (Guangdong, China). The yellowfin tuna was captured within 10 hours before the study commencement, gutted, and transported to the laboratory in ice water. Later it was sliced to approximately 100 grams pieces and placed in stomacher bags (Seward Ltd., Worthing, U.K.) for storage at temperatures of 0, 4, 10, and 20 °C, respectively.

Culture-dependent microbial analysis

The culture-dependent microbial analysis was performed by the spread plate method (Wang *et al.*, 2020a). About 25 grams of yellowfin tuna were aseptically minced and transferred into 225 mL sterile bags with 0.85% saline and homogenized for 5 minutes. After serial dilution (1:10) with phosphate buffer saline, 100 μL of the solution was spread on tryptic soy agar (Huankai Microbial Sci. & Tech. Co., Ltd, Guangdong, China). The total viable cell count (TVC) was determined after the plate incubation at 25 °C for 48 hours.

Culture-independent microbial analysis

The culture-independent microbial analysis was performed using Illumina-MiSeq high-throughput sequencing (Illumina Corp., San Diego, CA, USA). Yellowfin tuna samples of 5 grams each and10 mL 0.85% sterile saline were mixed. Later the homogenized solutions were centrifuged at 150*g* for 7 minutes, and the supernatant was separated. The supernatant was recentrifuged at 15,000*g* for 10 minutes at 4 °C for the final supernatant separation. Bacterial precipitation was used to extract the DNA by MagPure stool DNA KF kit B (Magen, Shanghai, China) following the manufacturer's protocol. Invitrogen Qubit 4 (Invitrogen, CA, USA) was used to quantify the DNA, and the quality of DNA was analyzed using 1% agarose gel electrophoresis. The primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') were used to amplify the V4 regions of the bacterial 16S rRNA gene by polymerase chain reaction (PCR) in triplicate. The 20 μL reaction mixtures for PCR contained 20 ng template DNA, 0.8 μL of each primer (5 μM), 2

μL of dNTPs (2.5 mM), 0.4 μL of FastPfu Polymerase, 4 μL of 5X FastPfu Buffer. The program of PCR was as follows: denaturation (98 °C, 3 minutes), 30 cycles (45 seconds, 98 °C), annealing (45 seconds, 55 °C), elongation (45 seconds, 72 °C), and final extension for 7 min at 72 °C. The PCR products were purified with AMPure XP beads (Agencourt, Woerden, Netherlands). Purified amplicons were sequenced on the Illumina HiSeq 2500 platform (Illumina Corp.). Raw reads were demultiplexed and quality-filtered using Fastp (v0.19.6) and merged by FLASH (v1.2.11). Operational taxonomic units (OTUs) were clustered with 97% cutoff value by UPARSE (v7.0.1090) software, and the OUT-representative sequence was analyzed by Ribosomal Database Project Classifier (v2.11).

Determination of biogenic amines

Biogenic amines were determined in yellowfin tuna using high-performance liquid chromatography (HPLC). Briefly, 5 grams of tuna meat were mixed with 20 mL of 0.4 M cold perchloric acid, centrifuged at 10,000*g* for 10 min at 4 °C. The supernatant was collected and analyzed for biogenic amines. About 20 μL samples were injected into HPLC (LC-20AD; Shimadzu, Japan) equipped with a reversed-phase chromatographic column (ChromCoreTM C18, 250×4.6 mm; Nano Chrom, China) at 254 nm wavelength. The samples were separated at 30 °C with a flow rate of 0.8 mL/min, and the mobile phase contained 45% ultrapure water and 55% methanol. The gradient elution procedure was performed as described by Zhao *et al.* (2021).

Determination of TVB-N

TVB-N was determined as described by Li *et al.* (2020b). About 5 grams of minced yellowfin tuna meat was dissolved in deionized water in a ratio of 1:10. The homogenized mixture was stirred for 30 minutes, and the mixture was centrifuged at 2000*g* for 5 minutes at 4 °C. Subsequently, the supernatant was equally mixed with 1% magnesium oxide and transferred to the Kjeldahl tube, and distilled with Kjeldahl nitrogen apparatus (Kjeldahl™2300, Foss, Denmark). The TVB-N value was expressed in units of mg N/100 g of yellowfin tuna meat.

Statistical analysis

All the experiments were done in triplicate, and the data were analyzed by one-way analysis of variance with multiple comparison Tukey's honestly significant difference tests. The significance level was determined at $P < 0.05$. Correlations of microbial abundances, biogenic amines, and TVB-N were computed using Spearman correlation analysis in R (v4.1.0), and the heatmap was drawn using the pheatmap package in R.

Results and discussion

Changes in TVC

The safety and quality of seafood mainly depend on its bacterial counts (Li *et al.*, 2020). The initial TVC values of the storage at 0, 4, 10, and 20 °C were 3.23, 3.46, 3.47, 3.52 log CFU/g, respectively, which indicated that the yellowfin tuna was of good quality from microbial aspects (Figure 1; Jaaskelainen *et al.*, 2019). Storage temperature is widely known to be a pivotal factor affecting microbial growth. The lower storage conditions will cause the microbial growth rate to decrease. The TVC steadily increased at 0 °C groups, and the cell counts were higher than 7 log CFU/g at 12 days. Compared with storage at 0 °C, the bacterial populations reached 7.97 log CFU/g at 4 °C after 7 days storage. Moreover, the bacterial growth rate in 10 °C groups was faster than that in the 0 and 4 °C groups. The bacterial counts increased to 7.8 log CFU/g at 5 days and exceeded 8.2 log CFU/g at 7 days. During storage at 20 °C, the bacterial population reached 8.9 log CFU/mL at 3 days. The permissible level of total bacterial counts is 7 log CFU/g for fisheries and fisheries products (International Commission on Microbiological Specifications for Foods, 1986).

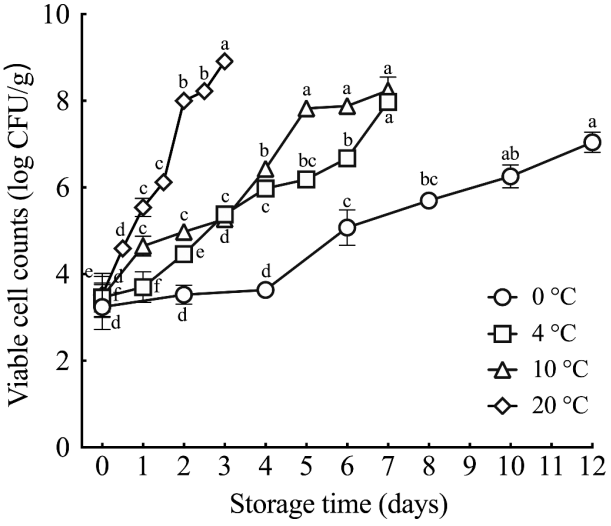

Figure 1. Changes in total viable cell count (TVC) in yellowfin tuna during storage at different temperatures. The lowercase letters in the same temperature indicate significant differences during storage ($P < 0.05$).

Microbial composition

To clarify the changes of microbial composition during yellowfin tuna storage at different temperature conditions. The relative proportions of the abundance microbial composition were analyzed at the genus level by high-throughput sequencing. As shown in Figure 2, the *Pseudomonas*, *Psychrobacter*, *Photobacterium*, *Acinetobacter*, and *Shewanella* constituted the top five microbial communities in fresh yellowfin tuna meat. In our study, the dominant microbe communities were *Pseudomonas* and *Shewanella*. Previous studies have shown that *Pseudomonas* and *Shewanella* are responsible for seafood spoilage (Bassey *et al.*, 2021; Sternisa *et al.*, 2020; Xie *et al.*, 2018). With increased storage time, *Pseudomonas* was the most abundant genus in all groups (85.30%, 70.58%, 59.16%, 59.89% after storage at 0, 4, 10, and 20 °C for 12 days, 7 days, 7 days, and 3 days, respectively. Similarly, Jaaskelainen *et al.* (2019) also identified *Pseudomonas* as the main OTUs in yellowfin tuna. Therefore, the results indicated that control of *Pseudomonas* and *Shewanella*, particularly the *Pseudomonas*, may play a significant role in extending the shelf-life of yellowfin tuna.

Biogenic amines

Biogenic amines are pivotal factors affecting seafood safety and quality (Visciano *et al.*, 2012). Histamine and tyramine are considered the most toxic biogenic amines associated with food poisoning (Santiyanont *et al.*, 2019). Table 1 shows the biogenic amine concentration changes during storage at different temperatures in yellowfin tuna. The histamine content increased with an increase in the storage duration. The concentration of histamines reached 51.00 and 322.20 mg/kg after storage for 5 days at 4 and 10 °C, respectively. At 20 °C, the histamine concentration significantly increased to 733.53 mg/kg after 2 days of storage which increased further to 3799.29 mg/kg after 3 days. Histamines cannot be destroyed by traditional cooking methods once formed by the histamine-producing bacteria (Chen *et al.*, 2016). The histamine toxicity limit for seafood is 50 mg/kg by Food and Drug Administration (2019). However, there is no evidence of tyramine contamination in seafood yet. The EFSA Panel on Biological Hazards (2011) reports 600 mg/kg as toxic levels for tyramine in food. In this study, the tyramine concentration ranged from 1.60 to 81.19 mg/kg, well within the acceptable range in tuna during the storage period. Putrescine and cadaverine are useful indicators for seafood freshness and quality, but a toxicity limit level in seafood for these two has not been established (Codex Alimentarius Commission, 2012). At end of the observation period, putrescine and cadaverine concentrations for storage at 0 °C for 12 days, 4 °C for 7 days, 10 °C for 7 days, and 20 °C for 3 days was 14.55, 40.04, 153.59, and 144.05 mg/kg and 5.60, 29.97, 113.72, and 100.53 mg/kg, respectively. Although toxicity levels for putrescine and cadaverine have not been set. They can enhance the histamine poisoning reaction (Xu *et al.*, 2010).

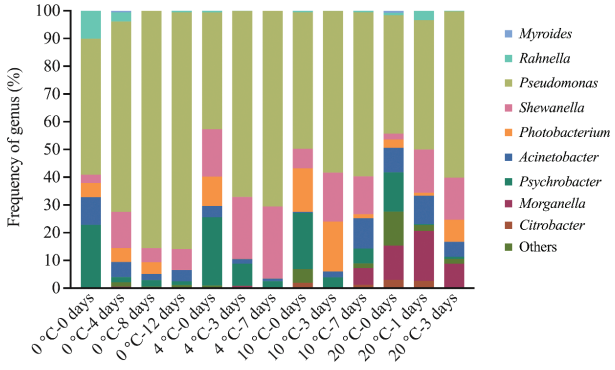

Figure 2. Changes in the relative abundance of microbial composition in yellowfin tuna during storage at genus levels.

TVB-N

The TVB-N values are related to bacterial activity and seafood spoilage (Huang *et al.*, 2018). As shown in Figure 3, the TVB-N values of four storage groups at different temperatures showed an uptrend with the extension of storage time. After 2 days, the TVB-N value was greater than 20 mg N/100 grams at 20 °C. Several studies have reported that the TVB-N value increases during cold storage (Kang *et al.*, 2020; Li *et al.*, 2020; Zhao *et al.*, 2019). The TVB-N values steadily increased at 10 °C and reached 21.21 mg N/100 grams after 4 days. For storage at 4 °C, 22.81 mg N/100 grams was recorded after 6 days. And the TVB-N value after storage at 0 °C increased slightly and reached 22.34 mg N/100 grams after 12 days. Sikorski *et al.* (1990) reported that the acceptable limit of TVB-N for fish products was 20 mg N/100 grams, and the increase of the TVB-N level was because of the protein degradation caused by decayed microorganism growth and endogenous enzymes activity (Qian *et al.*, 2018) , agreeing with our study outcomes. The fish decaying increased the TVC levels.

Correlation of microbial composition with biogenic amines and TVB-N

To further investigate the relationships between the bacterial changes, biogenic amines concentrations, and TVB-N during storage, Spearman's correlation tests were performed (Figure 4). Eight biogenic amines and TVB-N were positively correlated with *Pseudomonas*, *Shewanella*, *Morganella*, and *Acinetobacter*. Some previous studies

Table 1. Changes in biogenic amines in yellowfin tuna during storage at different temperatures.

Storage temperature	Days	Biogenic amines (mg/kg)							
		Tryptamine	Phenethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine
0 °C	0	ND	ND	ND	ND	ND	1.68±0.75^{c}	ND	ND
	2	ND	ND	ND	2.04±0.05^{a}	2.16±0.60^{c}	7.43±1.43^{bc}	ND	4.05±1.53^{b}
	4	ND	ND	ND	2.47±0.66^{a}	3.69±0.47^{c}	18.39±4.79^{abc}	ND	6.86±0.64^{a}
	6	4.76±2.03^{a}	5.51±2.81^{b}	4.54±2.88^{b}	3.85±1.52^{a}	10.85±1.66^{b}	28.47±4.98^{ab}	5.17±2.62^{ab}	7.17±0.81^{a}
	8	6.63±0.72^{a}	12.89±1.71^{a}	11.16±2.41^{a}	4.19±2.46^{a}	12.89±0.43^{b}	34.27±11.44^{a}	3.80±0.18^{b}	6.21±0.27^{ab}
	10	6.86±2.46^{a}	15.27±4.21^{a}	13.39±2.29^{a}	3.49±1.26^{a}	14.99±1.00^{b}	34.57±10.48^{a}	5.58±0.35^{ab}	7.94±1.02^{a}
	12	6.38±2.70^{a}	15.91±2.60^{a}	14.55±3.63^{a}	5.60±2.89^{a}	21.25±2.50^{a}	41.15±16.43^{a}	6.90±0.50^{a}	7.92±0.08^{a}
4 °C	0	ND	ND	ND	ND	ND	1.60±0.81^{d}	ND	0.59±1.03^{b}
	1	ND	4.98±2.35^{c}	4.16±0.34^{c}	2.17±1.17^{c}	4.53±4.18^{c}	8.03±3.66^{cd}	4.24±1.30^{ab}	7.04±1.75^{a}
	2	1.45±0.10^{b}	8.00±1.65^{bc}	6.75±2.94^{c}	1.47±1.44^{c}	4.25±2.02^{c}	17.56±3.79^{bc}	2.56±0.98^{b}	8.77±2.11^{a}
	3	4.63±1.29^{ab}	9.65±3.66^{bc}	10.96±1.95^{bc}	2.41±0.55^{c}	3.31±0.85^{c}	22.06±8.51^{b}	4.18±0.62^{ab}	8.83±1.38^{a}
	4	4.24±1.90^{ab}	13.48±2.21^{ab}	16.28±4.00^{b}	13.52±0.94^{b}	19.50±1.83^{c}	19.92±3.94^{bc}	4.41±1.17^{ab}	7.34±1.32^{a}
	5	4.42±1.11^{ab}	15.77±4.67^{ab}	15.56±2.58^{b}	26.56±2.73^{a}	51.00±11.39^{b}	53.85±3.22^{a}	4.88±0.84^{ab}	7.09±0.78^{a}
	6	5.15±2.41^{ab}	15.57±2.39^{ab}	15.31±2.05^{b}	24.75±3.91^{a}	73.99±3.08^{b}	57.45±4.30^{a}	5.23±0.61^{a}	7.38±0.31^{a}
	7	6.83±2.49^{a}	18.16±2.51^{a}	40.04±3.07^{a}	29.97±5.64^{a}	235.05±23.32^{a}	61.70±3.19^{a}	5.50±0.90^{a}	7.70±0.73^{a}
10 °C	0	ND	ND	ND	ND	ND	1.62±0.80^{c}	ND	0.93±1.61^{a}
	1	4.73±1.98^{c}	13.86±3.43^{a}	5.19±0.16^{c}	3.22±0.49^{c}	3.58±1.96^{c}	4.56±2.25^{c}	2.88±2.84^{a}	5.98±1.23^{a}
	2	6.02±1.95^{bc}	14.19±0.76^{a}	5.23±0.55^{c}	3.90±0.77^{c}	4.04±0.89^{c}	18.38±8.10^{c}	4.70±0.23^{a}	6.74±1.48^{a}
	3	8.25±2.44^{abc}	12.79±2.30^{a}	5.57±2.81^{c}	6.57±2.97^{bc}	6.54±1.84^{c}	48.76±8.05^{b}	6.06±4.20^{a}	7.08±1.47^{a}
	4	8.89±0.83^{abc}	14.62±2.38^{a}	7.37±2.53^{c}	37.62±10.19^{bc}	37.84±5.72^{c}	70.38±5.62^{a}	8.32±0.84^{a}	7.90±0.68^{a}
	5	12.63±3.21^{ab}	15.55±5.62^{a}	16.07±5.38^{c}	54.03±20.23^{abc}	322.20±177.42^{bc}	69.33±5.82^{a}	6.59±1.26^{a}	7.60±0.88^{a}
	6	13.07±1.89^{ab}	18.96±2.89^{a}	99.27±7.82^{b}	78.29±58.63^{ab}	820.20±372.89^{ab}	77.98±4.37^{a}	7.33±0.57^{a}	7.58±0.81^{a}
	7	14.92±4.91^{a}	18.80±0.93^{a}	153.59±11.7^{a}	113.72±39.23^{a}	1166.18±298.98^{a}	81.19±6.51^{a}	7.02±1.62^{a}	9.32±1.25^{a}
20 °C	0	ND	ND	ND	ND	ND	2.93±2.02^{d}	ND	0.93±1.61^{b}
	0.5	3.78±2.69^{b}	3.90±1.26^{b}	0.79±0.84^{d}	2.05±0.70^{b}	5.19±1.92^{d}	14.35±8.00^{cd}	6.83±4.01^{a}	6.77±1.52^{a}
	1	5.94±1.84^{b}	7.64±2.19^{ab}	1.27±0.51^{d}	2.34±0.41^{b}	7.97±2.72^{d}	18.56±7.99^{cd}	5.59±2.44^{a}	7.31±1.79^{a}
	1.5	7.51±1.20^{b}	5.01±0.90^{ab}	2.73±0.58^{d}	8.43±3.03^{b}	22.64±8.47^{d}	34.85±7.75^{bc}	5.63±1.74^{a}	8.05±1.29^{a}
	2	8.47±0.96^{b}	13.21±2.99^{ab}	32.71±5.05^{c}	27.41±11.48^{b}	773.53±192.01^{c}	51.73±8.48^{ab}	7.00±1.18^{a}	7.16±2.00^{a}
	2.5	18.08±4.43^{a}	18.99±12.91^{ab}	120.89±6.26^{b}	77.15±12.57^{a}	2666.26±433.67^{b}	56.63±10.94^{ab}	9.73±2.82^{a}	8.76±1.36^{a}
	3	25.01±4.30^{a}	23.77±11.75^{a}	144.05±11.05^{a}	100.53±34.58^{a}	3799.29±395.96^{a}	57.75±7.69^{a}	9.13±0.53^{a}	11.41±2.29^{a}

ND: not detected.

^{abc}Different lowercase superscripts letters in the same column within the same temperature indicated significant differences during storage ($P < 0.05$).

proved that *Morganella* is a potent histamine producer and can produce high histamine levels, and *Morganella psychrotolerans* has been shown to make toxic levels of histamine at 4 °C (Emborg *et al.*, 2006). The *Pseudomonas* showed significant positive correlation with histamine, cadaverine, putrescine, tyramine, and phenethylamine, and was reported as a histamine, cadaverine, and putrescine producer (Economou *et al.*, 2017; Fernández-No *et al.*, 2011; Xie *et al.*, 2018). *Shewanella* is also an important genus responsible for the accumulation of cadaverine and putrescine in fisheries and fisheries products (He *et al.*, 2017). The *Citrobacter*, *Rahnella*, *Myroides,* and *Psychrobacter* showed a negative correlation with histamine, cadaverine, and putrescine. The present study showed that the biogenic amines formation and increase of TVB-N values during the yellowfin tuna storage might be because of the metabolism of *Pseudomonas*, *Shewanella*, *Morganella*, and *Acinetobacter*.

Conclusion

As for improving the seafood safety and quality, we depth analyzed changes of bacterial and quality characteristics of yellowfin tuna during storage. The results showed *Pseudomonas* and *Shewanella* were the predominant microorganisms associated with the spoilage of yellowfin tuna. They exhibited positive correlations with the

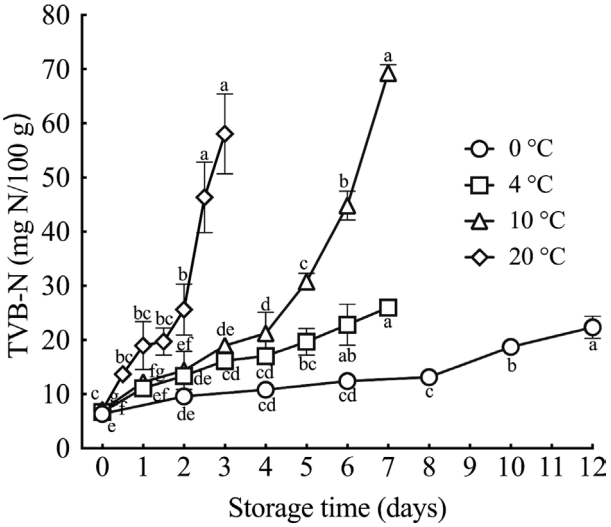

Figure 3. Changes in TVB-N values in yellowfin tuna during different storage temperatures. Different lowercase letters in the same temperature indicated significant differences during storage ($P < 0.05$).

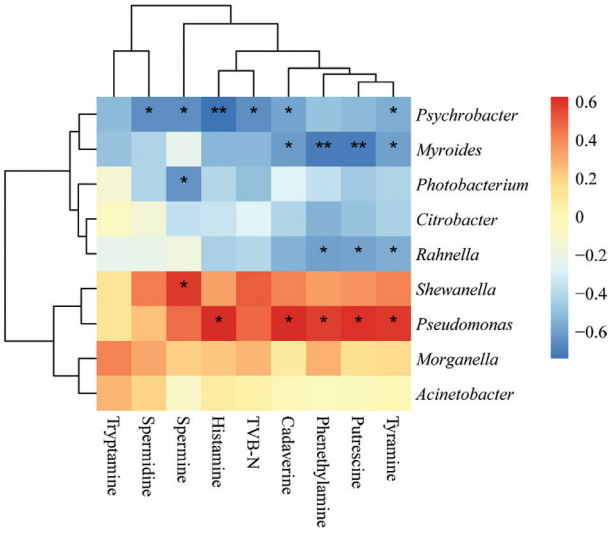

Figure 4. Heatmap visualization between microbial composition and quality characteristics of the yellowfin tuna during storage. * $P < 0.05$; ** $P < 0.01$.

accumulation of histamine, cadaverine, and putrescine in yellowfin tuna. The current study provides insights into understanding the shelf-life of yellowfin tuna and the relationships between the quality characteristics and microbial composition, which will help develop specific strategies to prevent yellowfin tuna spoilage and improve seafood safety and quality.

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Authorship Contributions

Di Wang: Methodology, validation, formal analysis, investigation, data curation, writing of the original draft, visualization, and funding acquisition.

Jianchao Deng: Software, methodology, and visualization.

Xupeng Li: Methodology and software.

Xianqing Yang: Supervision, draft reviewing and editing, and funding acquisition.

Shengjun Chen: Software and validation.

Yongqiang Zhao and Chunsheng Li: Draft reviewing and editing.

Yanyan Wu: Draft reviewing and editing, and funding acquisition.

Conflict of Interest

The authors declare no conflict of interests.

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