MINISTRY OF EDUCATION AND TRAINING NHA TRANG UNIVERSITY

NGUYEN THI KIEU DIEM

STUDY ON CHANGES IN THE QUALITY OF CHILLED/FROZEN NILE TILAPIA (*Oreochromis niloticus*) AT THE FINAL STAGE OF SUPPLY CHAINS

SUMMARY OF THE DOCTORAL DISSERTATION

KHANH HOA - 2022

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Speciality:Aquatic Products ProcessingCode:9540105

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KHANH HOA – 2022

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LIST OF SIGNS AND ABBREVIATIONS

Signs and	Extension				
abbreviations	Extension				
AOAC	Association of Official Analyical Chemists				
FDA	Food and Drug Administration				
FFA	Free Fatty Acid				
GAA	Global Aquaculture Alliance				
IQF	Individual Quickly Freezer				
ISO	International Organization for Standardization				
NMKL	Nordic Committee on Food Analysis				
NCBI	National Center for Biotechnology Information				
PCA:	Principal Compoment Analysis				
PUFA	Poly-unsaturated fatty acid				
MUFA	Mono-unsaturated fatty acid				
PV	Peroxide Value				
QDA:	Quantitative Descriptive Analysis				
QI:	Quality Index				
QIM:	Quality Index Method				
SSO	Specific Spoilage Organism				
TBARs	Thiobarbituric Acid Reactive Substances				
TMA	Trimethylamine				
TMAO	Trimethylamine Oxide				
TVB-N	Total Volatile Basic Nitrogen				
TPC	Total Plate Counts				

KEY FINDINGS

Title: Study on changes in the quality of chilled/frozen Nile tilapia fillets (*Oreochromis niloticus*) at the final stage of supply chains.

Major: Aquatic Products Technology. Code: 9540105.

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Ph.D candidate: Nguyen Thi Kieu Diem

Supervisors: 1. Assoc. Prof. Dr. Mai Thi Tuyet Nga

2. Assoc. Prof. Dr. Ly Nguyen Binh

Institution: Nha Trang University

Key findings:

1. The study identified the initial chemical compositions and the presence of some initial microbial groups of Nile tilapia fillets.

2. The study demonstrated that cold air thawing $(7 \pm 1^{\circ}C)$ was the most appropriate approach for defrosting frozen fillets of Nile tilapia.

3. The study identified the changes in the sensory quality, the Total Volatile Basic Nitrogen (TVB-N), the Total Viable Count (TPC) of cold-tolerant arerobic bacteria, and the number of common spoilage bacteria (*Pseudomonas* spp.) and hygiene indicator microorganisms (coliforms and *E.coli*) of Nile tilapia fillets stored at different stable temperatures. In addition, the modification of Quality Index Method (QIM) criteria was made for the sensory assessment of fillets of Nile tilapia raised in Vietnam.

4. The study developed the growth models of TPC of cold-tolerant arerobic bacteria and *Pseudomonas* spp., with a relatively high value of R^2 . After being tested with fluctuating temperatures simulating real-world conditions, these models revealed a extremely high reliability. The models were also examined in real-world conditions at the end of supply chains, and the results showed that it is possible to apply these models in the prediction of bacterial counts based on the history of storage temperature and time, and the product shelf life during cold supply chains.

Supervisor

PhD Candidate

Assoc.Prof.Dr. Mai Thi Tuyet Nga

Nguyen Thi Kieu Diem

INTRODUCTION

Farmed Nile tilapia (Oreochromis niloticus) is known as one of the most popular aquaculture fish. It has become an important protein source in many countries around the world. In recent years, Vietnam has paid much attention on developing farmed Nile tilapia. Tilapia fish available at different retail businesses from supermarkets, grocery stores to traditional open markets is usually stored by ice or put in chill compartments of refrigerators. However, fish products found at these areas might get contaminated with various human activities from fish transportation, retail display or when customers choose their fish products. The growth of microorganisms in fish negatively affects the chemical composition and sensory quality of aquaculture products as well as consumers' health. It can be said that microorganisms are the primary reason for the quality damage of aquaculure products. More specifically, temperature plays a vital role in the microbial growth. An increase in temperature will promote the microbial counts, accelerating the rate of fish spoilage. During supply chains, if the quality of Nile tilapia (Oreochromis niloticus) fillets is properly controlled by appropriately recording temperatures in each fish batch, the recorded values can be used to predict the microbial numbers which then serves as reference for a quicker prediction of the remaining storage time of fish fillets. For that reason, the thesis named "Study on changes in the quality of chilled/frozen Nile Tilapia fillets (Oreochromis niloticus) at the final stage of supply chains" was carried out to establish kinetic models for the growth of some bacteria based on storage temperature and time, contributing to an improved monitoring and management of tilapia quality at the end of supply chains.

The research objectives

1. To monitor the quality of Nile tilapia fillets during cold storage through changes in microbial counts, chemical composition and sensory quality of products.

2. To establish kinetic models for changes in the Total Viable Count of cold-tolerant arerobic bacteria and common spoilage bacteria (*Pseudomonas* spp.) available on Nile tilapia fillets based on time and temperature of cold storage.

Research contents

1. The identification of the initial presence of some microbial groups and changes in the basic chemical composition of Nile tilapia fillets.

2. The investigation of the effects of frozen storage time and thawing methods on the quality of defrosted Nile tilapia fillets in order to determine the appropriate thawing practice.

3. The investigation of changes in some chemical composition and sensory quality of Nile tilapia during chilled storage.

4. The identification of the growth of hygiene indicator microorganisms (Coliforms and *E.coli*), TPC of cold-tolerant bacteria, and common food spoilage bacteria (*Pseudomonas* spp.) available in Nile tilapia fillets during chilled storage.

5. The establishment of kinetic models for changes in the TPC of coldtolerant bacteria and common food spoilage bacteria (*Pseudomonas* spp.) available in Nile tilapia based on time and temperature of chilled storage.

6. Testing the application of established models in quality management of Nile tilapia during storage, displaying and selling processes at retail stores at the end of supply chains.

Scientific and practical significance

In Vietnam, the thesis is recognized as the first study carried out systematically to evaluate changes in the quality of Nile tilapia in terms of bacterial counts, chemical composition and sensory properties at different temperatures simulating real-world conditions at the end of frozen/chilled product supply chains. The bacterial counts in this study refer to the Total Viable Counts (TPC) of cold-tolerant aerobes, and Pseudomonas spp., common spoilage bacteria. The bacterial counts were identified at constant and fluctuating temperatures simulating real-world storage conditions of the end of supply chains. That is database for the author to design mathetical models of the bacterial growth during product storage, displaying and selling processes. The results demonstrated that the designed models can help identify the bacteral counts based on records of storage temperature and time during supply chains, and this achievement can be used to predict the remaining strorage time of fish fillets, contributing to a better control of fish products to the end of supply chains.

CHAPTER 1. LITERATURE REVIEW

1.1. Raw materials

Name in Vietnamese: "Cá Rô Phi Vằn"

Name in English: Nile Tilapia

Scientific name: Oreochromis niloticus

Other names in Vietnamese: "Cá rô phi vằn", "cá phi", "cá rô phi sông Nin".

Nile tilapia is a tropical species that finds it favorable to live at a temperature of $24\div32^{\circ}$ C and pH of $6,5\div8,5$. This type of fish is able to survive in fresh, brackish and salt water, and has a fast growth rate, with its weight reaching up to 300 to 500 grams just in 8 months.

1.2. An overview of supply chain

A supply chain is a system within organizations, people, activities, information, and resources involved in supplying a product or service to a consumer. Supply chain activities involve the transformation of natural resources, raw materials, and components into a finished product and delivering the same to the end customer

1.3. An overview of freezing and chilled/frozen storage of aquaculture products

1.3.1. Freezing

Freezing is a well-known method for a long-term preservation of food. This process involves reducing food temperatures to below the freezing point at which liquid water inside food is transformed into ice crystals. This helps inhibit physical, chemical and biological changes and activities of enzymes and microorganisms. At -8°C, the majority of bacteria are inactivated, while most cold-loving bacteria cannot grow to spoil food at -12°C.

1.3.2. Thawing

Thawing, also known as ice melting, is a process during which water in a solid state (ice crystals) inside flesh is turned into liquid. In other words, thawing is a way of converting frozen food back to its original unfrozen form.

Thawing methods can be devided into two groups as follows:

The first one involves the use of heat emited from flesh. Thawing methods in this group use heat from various sources such as electric field, resistance and microwave.

The second one is related to the use of heat transferred from the outside to the center of frozen products. This defrosting approach can be subdivided into smaller groups such as defrosting by water, by saturated stream and by cold air.

1.3.3. Chilled storage of aquaculture products

1.3.3.1. Storage with ice

It is common to see that fish and other aquaculture products sold at supermarkets and retail stores are chilled by ice. One of the reasons for using ice in food storage is that it has a higher heat transfer coefficient and faster cooling rate compared to other materials like air. Besides, once ice melts, it will create a layer of water which prevents water inside food from evaporating into the atmosphere, helping to avoid weight loss of thawed products.

1.3.3.2. Storage with cold air

During the storage, cold air slows down changes as well as bacterial growth in aquaculture products. The effectiveness of the storage depends on how temperature is maintained by chilling equipment and devices. Normally, equipment and devices are chilled by the circulation of cold air generated from cooling systems. Therefore, the air convention, temperature and humidity in storage facilities and equipment such as warehousing and refrigerators need to be properly controlled to maintain appropriate storage temperature.

1.4. Changes in the quality of aquatic products during the chilled/frozen storage

1.4.1. Chemical changes

1.4.1.1. Changes in the Total Volatile Basic Nitrogen (TVB-N)

As a result of the activities of enzymes and bacteria, organic compounds with nitrogen content in aquaculture products are decomposed during storage. The decomposition generates low-grade products, increasing the content of volatile basic nitrogen compounds such as ammonia, dimethylamine, trimethylamine (TMA), creatine, free amino acids, nucleotides and urea, ect. This is why the TVB-N value is commonly used to evaluate the quality/freshness and the suitability of fresh and chilled/frozen products. With the bacterial growth, non-protein nitrogen compounds are easily converted into compounds giving bad smell.

1.4.1.2. Changes in lipids

After fish dies, fish lipid deterioration often occurs because of hydrolysis and oxidation, so fish containing high levels of unsaturated fatty acids is more easily oxidized than that of lower levels of unsaturated fatty acids. The oxidation of unsaturated fatty acids is affected by many factors including temperature, light and enzymes, and the process generates peroxide, aldehyde, ketones and free radicals. A common way to investigate lipid oxidation in food is the determination of oxidation products, with peroxide value (PV) becoming as the most popular indicator for measuring the amount of hydroperoxide or peroxide, the products of autoxidation. In addition, due to the unstability of peroxide, the level of a secondary oxidation product, namely thiobarbituric acids (TBARs), is also measured. The purpose is to measure the level of aldehydes formed during the hydrolysis of hydroperoxide at the next stage of oil rancidity. These reactions are very common in fatty fish and considered one of the reasons for reducing shelf life of frozen fish.

1.4.2. Changes in the product quality caused by microbial activities during the chilled/frozen storage

Food spoilage caused by microorganisms attracts a great concerns of people all over the world, with 25% of post-harvest food being deteriorated due to microbial activities. Food spoilage may result from various chemical and physical factors; however, microbial activities are major reasons. Substances such as sulfur, alcohols, aldehydes and organic compounds produced due to these microbial activities are responsible for bad odor in food spoilage. On fish surface, the majority of microbes are found on fish scales, skin (10^2-10^7 CFU/cm²), gills ($10^3 \div 10^9$ CFU/g) and internal organs (10^3-10^9 CFU/g). Meanwhile, with fresh fish, just a few microorganisms are available in the flesh. This is because immediately after harvest or when fish is alive, the immune system of fish functions to prevent the growth of bacteria in the flesh. In contrast, after death, the immune system is weaken, allowing bacteria to grow and invade fish.

Microorganisms invade fish flesh through fish muscle fibers. Once the number of bacteria available on fish skin goes up to 10^6 CFU/cm², bacteria in fish flesh can be detected. The microbial flora is the main cause of spoilage in aquaculture products in general, and in fish in particular.

1.4.3. Changes in the sensory quality of aquatic products and common methods of sensory evaluation

1.4.3.1. Changes in the sensory quality of aquatic products during chilled/frozen storage

Sensory changes refer to changes which can be recognized by five sensory organs such as appearance, odor, texture, and taste, while sensory evaluation is defined as a scientific method of staing, measuring, analyzing and explaining people's feeling about products through sensory systems including sense of vision, sense of smell, sense of touch, sense of taste and sense of hearing. Along with chemical and microbial criteria, sensory evaluation is a useful tool to quickly identify the freshness and the remaining storage time of products during storage. Today, sensory evaluation is regarded as one of the primary and most important approaches to assess the freshness of aquatic products.

1.4.3.2. Various methods of sensory evaluation in aquaculture

a. Quality Index Method (QIM)

b. Quantitative Descriptive Analysis (QDA)

c. Torry-score method

1.5. An overview of microbial models in food

1.5.1. The Baranyi-Roberts primary model for microorganisms in food

The Baranyi & Roberts model assumes specific conditions to identify the potential growth rate of microorganisms, and this rate will be higher the actual growth rate if time is close to the time of bacterial culture. Over time, the actual rate will be as fast as that of the potential growth. The ratio of the actual growth to the potential is defined as the adjustment function.

The potential growth can be described by the logistic equation:

 $\frac{dN(t)}{dt} = \mu(N). N(t); N_0 \le N \le N_{max}; N(0) = N_0 \qquad (1)$ In which:

N (cfu/ml or cfu/g) is the microbial number;

N₀ is the initial bacterial number;

Nmax is the maximum bacterial number;

 μ (1/hour) is the individual growth rate.

The above-mentioned correlation assumes that the individual growth rates gradually decrease to 0 when the microbial counts reach a peak of N_{max} , or $\mu(N_{max})$.

On the above equation, the lag time is essential for microbial cells to be familiar with new environments, and the purpose is to build a model which can describe the adjustment. Accordingly, factors affecting the actual growth of microorganisms can be modelled as the following model.

 $\frac{dN(t)}{dt} = \alpha(t).\,\mu(N).\,N;\ 0 \le \alpha(t) \le 1;\ N_0 \le N \le N_{max};\mu(N_{max}) = 0$ (2)

In which: $\alpha(t)$ is a adjustment function. The ratio of the actual growth rate to that of the potential growth is considered as the adjustment fuction with an increase of 0 to 1.

1.5.2. The secondary model for microorganisms in food

The secondary model is able to estimate the microbial growth under unstable environmental conditions (changing) which include pre-culture and culture conditions.

If temperature is the only changing parameter while other environmental factors can be regarded as constant/stable, then the maximum growth rate may be modelled by the square root model of Ratkowsky as follows:

$$\sqrt{\mu_0} = b(T - T_{min})$$

In which: Tmin is the minimum temperature for the microbial growth, and b is a parameter of the tested model. These models do not include the thermal inactivation, and the Ratkowsky model can be extended as follows:

$$\sqrt{\mu_0} = b_2(T - T_{min}) \big(1 - \exp(c_2(T - T_{max}))\big)$$

In which: T_{max} is the maximum temperature for the microbial growth, and b is a parameter of the tested model.

Chapter 2. MATERIALS AND METHODS

2.1. Materials

Nile tilapia fish was cut into fillets, and then treated by the process of Individual Quick Freezing (IQF) at a seafood processing company in the Mekong Delta, Vietnam. The weight of each fillet was about 120-170 grams (g).

2.2. Methods



Figure 2.2. The flow chart of the thesis

2.2.1. Investigation of the initial chemical composition and the initial presence of bacteria in Nile tilapia

2.2.2. Investigation of the effects of thawing processes on the quality of thawed tilapia

2.2.3. Investigation of changes in the quality of tilapia fillets during the storage at stable positive temperatures

- Investigation of changes in chemical quality, sensory quality and investigation of the growth of hygiene indicator bacteria (coliformss and *E.coli*) and the TPC of cold-tolerant bacteria, common spoilage microorganisms (*Pseudomonas* spp.) in tilapia fillets stored at stable positive temperatures.

2.2.4. Establishment of growth kinetic models for TPC and Pseudomonas spp. in tilapia fillets based on storage time and temperature during supply chains

2.2.4.1. Establishment of growth kinetic models for TPC and Pseudomonas spp.

- The building of the primary model: identified parameters of kinetic models for changes in the number of microorganisms over time at different stable temperatures following the model of Baranyi and Roberts.

- The building of the secondary model: investigated the correlation of the bacterial growth rate with temperatures based on the model of Ratkowski.

2.2.4.2. Accreditation of established models

The dynamic temperature regines 1: these temperatures were storage conditions for fish fillets that can be encountered in refrigerators $(1\pm1^{\circ}C)$ and open display areas $(19\pm1^{\circ}C)$ at supermarkets.

The dynamic temperature regines 2: this simulated temperatures during the transporting process from manufacturers to distributors $(4\pm1^{\circ}C)$, at retail stores $(9\pm1^{\circ}C)$, and temperature variation when customers take products out of display cabinets $(15\pm1^{\circ}C)$.

2.3.4.3. Testing the application of kinetic models for the growth of TPC in quality management of tilapia fillets during cold supply chains

Tilapia fillets were monitored in terms of variation in termpature when they were displayed for selling at retail stores. Self-recording thermometer sensors were used to continuously monitor the storage temperature, and recorded data were automatically forwarded to central devices through wireless sensor networks.

Chapter 3. RESULTS AND DISCUSSIONS

3.1. Investigation of the initial chemical composition and the presence of bacteria in Nile tilapia

3.1.1. Investigation of the initial chemical compositions in tilapia fillets after filleting and IQF

			-
Compositions	Unit	After filleting	After IQF
Lipid	%	1.19 ± 0.03	0.68 ± 0.04
Protein	%	17.7 ± 0.6	19.6 ± 0.2
Mosture	%	79.7 ± 1.1	78.0 ± 0.9
Ash	%	0.81 ± 0.04	0.78 ± 0.02

 Table 3.1. Chemical compositions of tilapia fillets after filleting and IQF

After filleting, the content of free fatty acids was 2.86g per 100g of lipids, and this figure increased to 4.87g per 100g of lipids after IQF (Table 3.2). There was no PV detected in fish fillets after processing. Meanwhile, the values of TBARs after filleting and freezing were 0.06 MDA/kg and 0.11mg MDA/kg, respectively.

Components	Unit	After filleting	After IQF
TVB-N	mgN/100g	11.13 ± 0.74	12.82 ± 1.22
FFA	(g/100g lipids)	2.86 ± 0.05	4.87 ± 0.12
PV	(meq/kg lipids)	0	0
TBARs	(mg MDA/kg)	0.06 ± 0.004	0.11 ± 0.012

Table 3.2. Quality index of tilapia fillets after filleting and IQF

The results in table 3.2 showed that after filleting and IQF, Nile tilapia fillets in the study met requirements in terms of TVB-N.

3.1.2. Investigation of the presence of microorganisms in tilapia fillets after filleting and IQF

The number of TPC was about $3.4.10^3$ to $3.9.10^3$ CFU/g, while the figures for the hygiene indicator microorganisms including coliforms and *E.coli* were less than 10 CFU/g. They are all within the permit level of 10^2 CFU/g. In addition, microorganisms including *Clostridium, Samonella, Staphylococcus, Vibrio*, and *Listeria* were not identified in Nile tilapia fillets after processing.

3.2. Investigation of the effects of thawing methods on the quality of frozen tilapia fillets

3.2.1. Investigation of the effects of thawing processes on the content of TVB-N in frozen tilapia fillets

The analytical results illustrated that all samples stored at various points of time and defrosted by 4 different methods had similar content of TVB-N which was less than <30 mgN/100g.



Figure 3.1. Content of TVB-N in tilapia fillets stored at a temperature of $-20 \pm 2^{\circ}C$ from from month #0 to month #8, thawed by 4 different methods

Note: data of different letters show statistically significant differences in the average content of TVB-N at p < 0.05 among various thawing approaches of the same storage month.

3.2.2. Investigation of the effects of thawing processes on the sensory quality of frozen tilapia fillets



Figure 3.2. QI scores of tilapia fillets stored at -20 ± 2°C from month #0 to month #8 and thawed by 4 different approached: I – by cold air (7 ± 1°C); II – by ambient air; III – by cold water (8 ± 1°C); và IV – by normal water

Note: data of different letters show statistically significant differences in in the average QI score at p < 0.05 among various thawing approaches of the same storage month.

The QI score of samples defrosted by ambient air was higher than that of samples thawed by other approaches (Figure 3.2). Cold air thawing showed the lowest score compared to researched methods.

3.2.3. Investigation of the effects of thawing processes on the bacterial growth in frozen tilapia fillets



Figure 3.3. Bacter counts in tilapia fillets stored at -20 ± 2°C from month #0 to month #8, and thawed by 4 different methods: I – by cold air (7 ± 1°C); II – by ambient air; III – by cold water (8 ± 1°C) và IV – by normal water

Note: data of different letters show statistically significant differences in the average number of bacteria at p < 0.05 among various thawing methods of the same storage month

The presence of *E.coli* bacteria was not detected in defrosted samples, indicating good hygienic conditions during processing and thawing. In terms of coliform counts in thawed tilapia fillets, there was no significant difference among 4 approaches (p > 0.05). However, the counts of the TPC and Pseudomonas spp. in fillets thawed by ambient air were higher than that of other thawing methods when data were collected at month #4, 5, 6 and 7.

Slow thawing by cold air $(7\pm1^{\circ}C)$ for 8 hours was demonstrated as the most appropriate way for tilapia fillets. Based on the advantages mentioned, this thawing method was chosen for futher studies in this thesis. 3.3. Investigation of changes in the quality of tilapia fillets during cold storage at stable temperatures

3.3.1. Investigation of chemical changes in tilapia fillets during cold storage at stable temperatures

3.3.1.1. Investigation of changes in the content of TVB-N in Nile tilapia fillets during cold storage at stable temperatures



Figure 3.4. Changes in the average content of TVB-N in tilapia fillets at stable cold temperatures of 1, 4, 9, 15, 19, 25, 29±1°C and ambient temperature of about 30-33.5°C

Note: data of different letters show statistically significant differences in the average content of TVB-N among storage durations of the same temperature

Stored at different cold temperatures, tilapia fillets had the content of TVB-N exceeding a threshold of 30 mgN/100g after 240 hours of storage at $4 \pm 1^{\circ}$ C, 192 hours at $9\pm1^{\circ}$ C, 87 hours at $15\pm1^{\circ}$ C, 44 hours at $19\pm1^{\circ}$ C, 14 hours at $25\pm1^{\circ}$ C, and 19 hours at $29\pm1^{\circ}$ C. However, after 312 hours of storage at $1\pm1^{\circ}$ C and 16 hours at ambient temperatures of 30-33.5°C, the contents of TVB-N were not higher than the regulated level.

3.3.1.2. Investigation of changes in lipid composition in Nile tilapia fillets during cold storage at stable temperatures.

Tables 3.4 and 3.5 illustrate the analytical results of lipid composition. Table 3.4. Changes in lipids of tilapia fillets over storage time at temperatures of $1 \pm 1^{\circ}$ C and $4 \pm 1^{\circ}$ C

T	Storage	Lipid	FFA	PV	TBARs	
Temperature	time	(%)	(%)	(meq/kg)	(mg MDA/kg)	
	Day 0	0.68 ± 0.04	4.87 ± 0.11	-	0.11 ± 0.01	
	Day 6	2.51 ± 0.09	$\boldsymbol{6.89 \pm 0.11}$	-	0.91 ± 0.05	
1 ± 1°C	Day 9	1.66 ± 0.02	3.39 ± 0.05	-	0.85 ± 0.06	
	Day 13	1.81 ± 0.05	1.93 ± 0.05	2	0.75 ± 0.05	
	Day 4	3.35 ± 0.06	4.67 ± 0.08	0	0.49 ± 0.27	
4 ± 1°C	Day 7	2.90 ± 0.10	6.54 ± 0.24	0	1.02 ± 0.13	
	Day 10	3.36 ± 0.13	6.30 ± 0.05	1.82	1.23 ± 0.16	

The PV of fillets stored on day 10 at $1 \pm 1^{\circ}$ C and $4 \pm 1^{\circ}$ C were 2 meq/kg and 1.82 meq/kg, respectively. At the end of cold storage at these 2 temperature conditions, tilapia fillets had TBARs of only 0.75 mgMDA/kg at $1 \pm 1^{\circ}$ C and 1.23 mgMDA/kg at $4 \pm 1^{\circ}$ C.

3.3.2. Investigation of changes in the sensory quality of tilapia fillets during cold storage at stable temperatures

3.3.2.1. Investigation of changes in the sensory quality of tilapia fillets during cold storage at stable positive temperatures by using QIM





Figure 3.5. QI scores of tilapia fillets during storage at temperatures of 1, 4, 9, 15, 19, 25, 29 ± 1°C and ambitent temperatures of about 30-33.5°C

Note: different letters show statistically significant differences in the average QI scores at p < 0.05 among different storage durations of the same temperature.

At all stable temperatures, the QI scores at the end of storage period increased by 75% compared to the maximum QI level, exceeding the acceptable limit for sensory quality.

Table 3.6	6. Regression	equation	and	correlation	coefficient	of	QI	scores	(y)	over
storage ti	me (x, hour)	at stable te	empe	eratures						

Temperature	Regression equation	R ²
$1 \pm 1^{\circ}C$	y = 0.02x + 0.6514	$R^2 = 0.8669$
$4 \pm 1^{\circ}C$	y = 0.0408x + 1.1526	$R^2 = 0.9858$
$9\pm1^\circ C$	y = 0.0572x + 0.6065	$R^2 = 0.9805$
$15 \pm 1^{\circ}C$	y = 0.1139x + 0.434	$R^2 = 0.9588$
$19 \pm 1^{\circ}C$	y = 0.1503x + 0.8612	$R^2 = 0.9759$
$25\pm1^{\circ}C$	y = 0.1519x + 2.2549	$R^2 = 0.9082$
$29 \pm 1^{\circ}C$	y = 0.3984x + 0.0276	$R^2 = 0.8360$
30-33.5°C	y = 0.5673x + 0.0886	$R^2 = 0.8626$

The QI increased linearly with storage durations according to the regression equations described in Table 3.6, with a high value of $R^2 > 0.8$.

3.3.2.2. Investigation of changes in the sensory quality of tilapia fillets during chilled storage at $1 \pm 1^{\circ}C$ and $4 \pm 1^{\circ}C$ by QDA

	0				~	-		
		0	72	144	192	216	240	264
	Boiled potatoes	45.73ª	44.60 ^a	51.07ª	56.40ª	54.07ª	45.07ª	59.67ª
	Fishy	20.40 ^a	24.53ª	25.33ª	17.33ª	24.53ª	23.40 ^a	17.47ª
100	Mud	15.87ª	14.20 ^a	10.20ª	11.67ª	11.67ª	11.33ª	12.33ª
ĨĊ	Ammonia	7.73 ^a	5.27ª	4.87 ^a	9.93ª	7.93ª	10.73ª	14.33ª
	Mouldy	1.73 ^a	3.60 ^a	3.07 ^a	2.53ª	4.07 ^a	3.53ª	3.60 ^a
	Rancid	0.60 ^a	1.27 ^a	0.73 ^a	1.20 ^a	1.73 ^a	1.33 ^a	2.07 ^a
	Rotten	0.93ª	1.33 ^a	1.40 ^a	3.93ª	3.20 ^a	2.80 ^a	6.13 ^a
		0	48	96	120	144	168	192
	Boiled potatoes	46.67 ^a	51.53ª	49.93ª	47.40 ^a	44.53 ^a	49.00 ^a	43.73ª
- - 4ºC -	Fishy	19.53ª	18.27ª	19.53ª	15.87ª	15.93ª	22.40 ^a	14.53ª
	Mud	7.73 ^a	11.20ª	9.67ª	8.27 ^a	10.20ª	6.13 ^a	12.33ª
4°C	Ammonia	11.67ª	14.60 ^a	13.93ª	11.40 ^a	15.07ª	8.93ª	11.73ª
4ºC	Mouldy	3.53 ^a	3.40 ^a	8.00 ^a	5.20 ^a	5.80 ^a	2.93ª	4.87 ^a
	Rancid	3.27 ^a	2.93ª	7.87ª	5.73 ^a	4.47 ^a	2.33ª	7.33ª
	Rotten	6.00 ^a	4.73 ^a	5.27ª	5.93ª	5.27ª	6.87ª	4.73 ^a

a) Odor Table 3.7. Changes in odor scores of tilapia fillets during storage at 1±1°C and 4±1°C

Note: on the same row, different letters (if available) show statistically significant differences (p < 0.05) in the average scores among storage durations.

The results showed that there was no significant modification in the odor of tilapia fillets when they were stored at $1 \pm 1^{\circ}$ C and $4 \pm 1^{\circ}$ C.

Table	able 3.8. Changes in color scores of tilapia fillets during storage of 1 ± 1 °C and 4 ± 1 °C										
	Color	0	72	144	192	216	240	264			
1ºC	Light-Dark	52.67ª	47.34 ^{ab}	25.60 ^b	50.47 ^{ab}	44.80 ^{ab}	39.47 ^{ab}	51.73 ^{ab}			
	Smooth-Rough	37.67 ^{ab}	47.93 ^b	20.93ª	43.27 ^{ab}	26.13 ^{ab}	33.47 ^{ab}	33.27 ^{ab}			
	Color	0	48	96	120	144	168	192			
4⁰C	Light-Dark	53.27ª	47.87 ^a	54.40 ^a	39.53ª	43.27 ^a	44.53 ^a	47.40 ^a			
	Smooth-Rough	32.07 ^a	39.73ª	42.00 ^a	36.93ª	42.40 ^a	35.87ª	36.93ª			

b) External color

At $1 \pm 1^{\circ}$ C and for 144 hours of storage, the score for color of fillets was 25.60%, and that for the smoothness of fillet surface was 20.93%, the lowest level. At 4 ± 1 °C, the score for the smoothness of fillet surface ranged from 32.07-42.4%.

c) Flesh texture

Table 3.9. Changes in texture scores of tilapia fillets during storage at $1\pm1^{\circ}$ C and $4\pm1^{\circ}$ C.

	Texture	0	72	144	192	216	240	264
	Flake	35.40 ^a	38.33ª	30.20 ^a	37.20 ^a	37.40 ^a	33.67ª	37.33ª
	Softness	48.00 ^a	51.60ª	38.00 ^a	52.13ª	44.47 ^a	48.27 ^a	50.13ª
1 °C	Fibre	41.20 ^{ab}	45.33 ^{ab}	28.60 ^a	50.73 ^b	44.27 ^{ab}	42.13 ^{ab}	50.67 ^b
	Mushy	29.07ª	32.47 ^a	27.53ª	35.27ª	38.20 ^a	33.00 ^a	33.93ª
	Chewy	40.60 ^a	54.80ª	55.53ª	45.33ª	54.47ª	46.60 ^a	43.80 ^a
	Juicy	31.13 ^a	33.00 ^a	44.67ª	36.60 ^a	38.07 ^a	42.20 ^a	34.67 ^a
	Texture	0	48	96	120	144	168	192
	Flake	41.20 ^a	44.20 ^a	45.27ª	38.53ª	50.33 ^a	50.53ª	56.13ª
	Softness	32.93ª	41.13 ^a	40.80 ^a	41.27ª	47.80 ^a	51.67ª	46.20 ^a
4 °C	Fibre	37.40 ^a	44.13 ^a	43.67ª	48.73ª	50.40 ^a	52.07 ^a	43.93ª
	Mushy	35.53ª	38.07 ^a	33.40 ^a	37.60 ^a	44.00 ^a	45.13 ^a	41.33 ^a
	Chewy	53.33ª	43.40 ^a	43.33ª	46.73ª	39.73 ^a	47.60 ^a	38.07ª
	Juicy	47.20 ^a	39.20 ^a	40.07 ^a	29.67ª	35.53ª	30.33 ^a	30.47 ^a

Note: on the same row, different letters show statistically significant differences (p < 0.05) in the average scores of texture among storage durations.

At $1 \pm 1^{\circ}$ C, the scores for texture of steamed fillets rose up over storage time. However, at $4 \pm 1^{\circ}$ C, in terms of 6 texture properties, there were no statistically significant differences (p>0.05) in the texture scores of steamed fillets among storage durations.

d) Taste

-								
	Flavour	0	72	144	192	216	240	264
	Sweet	48.67ª	43.87ª	39.00ª	29.07ª	32.20ª	38.53ª	26.00 ^a
	Fatty	9.27ª	10.27ª	13.27ª	4.60 ^a	12.87ª	9.07ª	4.73ª
1ºC	Sour	3.07 ^a	1.20 ^a	2.53ª	7.93ª	4.47 ^a	3.80 ^a	4.33ª
	Rancid	0.53ª	1.47ª	0.00 ^a	0.47ª	0.07 ^a	0.67ª	0.53ª
	Rotten	0.07 ^a	0.07 ^a	0.00 ^a	5.47ª	0.00 ^a	0.33ª	0.40 ^a
	Flavour	0	48	96	120	144	168	192
	Sweet	44.20 ^b	39.60 ^{ab}	37.93 ^{ab}	25.67 ^{ab}	35.87 ^{ab}	27.47 ^{ab}	15.33ª
	Fatty	8.67ª	5.80 ^a	10.33 ^a	5.47ª	4.53 ^a	10.53ª	2.13 ^a
4ºC	Sour	1.13 ^a	3.67 ^a	3.20 ^a	1.87ª	4.20 ^a	4.27ª	1.53 ^a
	Rancid	0.00 ^a	0.33ª	3.27ª	0.60 ^a	0.53ª	2.40 ^a	1.73 ^a
	Rotten	0.00 ^a	0.13ª	2.93ª	0.33ª	0.27ª	0.80 ^a	0.60ª

Table 3.10. Changes in taste scores of tilapia fillets during storage at $1\pm1^{\circ}C$ and $4\pm1^{\circ}C$

Note: on the same row, different letters show statistically significant differences (p < 0,05) in the average scores of texture among storage durations.

It is obvious that there was a downward trend in scores for protein sweetness as storage time increased. As analyzed in the item 3.3.1.2, Nile tilapia fish had less lipids, and there was no considerable modification of lipids taking place. Therefore, fillets received a very low score for rancidity, with figures of 0.53% and 1.73% at $1 \pm 1^{\circ}$ C and $4 \pm 1^{\circ}$ C, respectively.

3.3.2.3. Investigation of changes in the sensory quality of tilapia fillets during cold storage at $1 \pm 1^{\circ}C$ and $4 \pm 1^{\circ}C$ by Torry scale

The assessment of the sensory quality of steamed fatty fillets was made by using the Torry scale, and the results are revealed in Figure 3.6.



Figure 3.6. Torry scores of tilapia fillets for different periods of time during cold storage at $1 \pm 1^{\circ}$ C và $4 \pm 1^{\circ}$ C

Note: at the same storage temperature, different letters show statistically significant differences (p<0.05) in the average Torry scores among various storage periods of time.

At $1 \pm 1^{\circ}$ C, the Torry score recorded at the initial stage of storage was 8.05, but fell to 7 after 264 hours. At $4 \pm 1^{\circ}$ C, the score was 7.85 at hour 0, with the figure falling down to only 6.4 after 192 hours.

3.3.3. Investigation of the bacterial growth in tilapia fillets at stable cold temperatures over storage time

3.3.3.1. Investigation of the growth of coliforms in tilapia fillets at stable cold temperatures over storage time



Figure 3.7. Counts of coliforms in tilapia fillets at stable cold temperatures of 1, 4, 9, 15, 19, 25, 29 ± 1°C and ambient temperatures of 30 – 33.5°C

Note: different letters show statistically significant differences (p<0.05) in the average counts of coliforms among storage periods of the same temperature

The levels of coliforms were recorded at different temperature conditions,, with figures of 6.22 log CFU/g at $1 \pm 1^{\circ}$ C; 8.16 log CFU/g at $4 \pm 1^{\circ}$ C; 9.32 log CFU/g at $9 \pm 1^{\circ}$ C, 8.15 log CFU/g at $15 \pm 1^{\circ}$ C; 11.39 log CFU/g at $19 \pm 1^{\circ}$ C; 14.55 log CFU/g at $25 \pm 1^{\circ}$ C; 10.44 log CFU/g at $29 \pm 1^{\circ}$ C; and 9.25 log CFU/g at ambient temperatures of about $30 - 33.5^{\circ}$ C.

3.3.3.2. Investigation of the growth of E.coli in tilapia fillets at stable cold temperatures over storage duration



Figure 3.8. Counts of *E.coli* in tilapia fillets at stable cold temperatures of 1, 4, 9, 15, 19, 25, 29 ± 1°C and ambient temperatures of 30 – 33.5°C

Note: different letters show statistically significant differences (p < 0.05) in the average counts of E.coli among storage periods of the same temperature

None of *E.coli* bacteria was detected in fillets stored at either $1 \pm 1^{\circ}$ C or $4 \pm 1^{\circ}$ C. According to regulations of the Health Ministry, the *E.coli* counts for frozen aquatic products must be less than 2 log CFU/g. Accordingly, tilapia fillets will be expired after 24 hours of storage at $9 \pm 1^{\circ}$ C, 24 hours at $15 \pm 1^{\circ}$ C, 20 hours at $19 \pm 1^{\circ}$ C, 14 hours at $25 \pm 1^{\circ}$ C, 5 hours at $29 \pm 1^{\circ}$ C, and only 2 hours at ambient temperatures of around 30 $- 33.5^{\circ}$ C.

3.3.3.3. Investigation of changes in the TPC of cold-tolerant bacteria in Nile tilapia fillets at stable cold temperatures over storage time



Figure 3.9. Changes in the TPC of cold-tolerant bacteria in tilapia fillets at stable cold temperatures of 1, 4, 9, 15, 19, 25, $29 \pm 1^{\circ}$ C and ambient temperature of $30 - 33.5^{\circ}$ C

Note: different letters show statistically significant differences (p < 0.05) in the average TPC among storage periods of the same temperature

The initial TPC in tilapia fillets was recorded at 5.6 log CFU/g. Based on TCVN 5289:2006, fish must be discarded if the value of TPC is higher than the regulated threshold if 6 log CFU/g. For that reason, tilapia fillets will be expired after 144 hours of storage at $1 \pm 1^{\circ}$ C, 96 hours at $4 \pm 1^{\circ}$ C, 72 hours at $9 \pm 1^{\circ}$ C, 48 hours at $15 \pm 1^{\circ}$ C, 20 hours at $19 \pm 1^{\circ}$ C, 14 hours at $25 \pm 1^{\circ}$ C, 5 hours at $29 \pm 1^{\circ}$ C, and 4 hours at $30 - 3.5^{\circ}$ C.

3.3.3.4. Investigation of the growth of Pseudomonas spp. in tilapia fillets at constant cold temperatures over storage time



Figure 3.10. Counts of *Pseudomonas* spp. in tilapia fillets at stable cold temperatures of 1, 4, 9, 15, 19, 25 and $29 \pm 1^{\circ}$ C and aient temperatures of $30 - 33.5^{\circ}$ C

Note: different letters show statistically significant differences (p < 0.05) in the average count of Pseudomonas spp. among storage periods of the same temperature

The inital number of Pseudomonas spp. in tilapia fillets was about 4.22 log CFU/g. The figures recorded at the end of storage period were 8.42 log CFU/g at $1 \pm 1^{\circ}$ C, 8.72 log CFU/g at $4 \pm 1^{\circ}$ C, 8.64 log CFU/g at $9 \pm 1^{\circ}$ C, 8.69 log CFU/g at $15 \pm 1^{\circ}$ C, 8.67 log CFU/g at $19 \pm 1^{\circ}$ C, 6.25 log CFU/g at $25 \pm 1^{\circ}$ C, 6.74 log CFU/g at $29 \pm 1^{\circ}$ C, and 8.96 log CFU/g at ambient temperatures of $30-33.5^{\circ}$ C.

3.4. Establishment of the models for the growth of spoilage microorganisms in tilapia fillets at stable storage temperatures over time

3.4.1. Establishment of primary models for the growth of spoilage microorganisms in tilapia fillets at constant temperatures over storage time

3.4.1.1. The primary model for the TPC of cold-tolerant aerobic bacteria in tilapia fillets at constant storage temperatures over time

Temperature	R ²	SE of	Y_0	λ	μ_{max}	Y_{max}
		ГЦ	(log Cr U/g)	(gio)	(1/gl0)	(log Cr U/g)
$1\pm1^{\circ}\mathrm{C}$	0.82	0.62	5.50 ± 0.34	67.05 ± 43.45	0.02 ± 0.003	10.27 ± 3.88
$4\pm1^{o}C$	0.83	0.67	5.57 ± 0.35	51.07 ± 24.87	0.03 ± 0.007	9.54 ± 0.26
9 ± 1^oC	0.82	0.73	5.09 ± 0.40	9.48 ± 28.58	0.03 ± 0.005	10.54 ± 3.35
$15 \pm 1^{\circ}C$	0.81	0.83	5.08 ± 0.47	15.51 ± 15.95	0.06 ± 0.015	10.01 ± 0.38
$19\pm1^{o}C$	0.86	0.59	5.46 ± 0.34	5.77 ± 8.62	0.08 ± 0.02	9.57 ± 0.18
$25\pm1^o\mathrm{C}$	0.90	0.55	5.69 ± 0.28		0.21 ± 0.03	10.17 ± 0.14
29 ± 1^oC	0.83	0.60	5.87 ± 0.24	5.01 ± 1.74	0.32 ± 0.07	9.03 ± 0.17
30-33.5°C	0.91	0.62	5.59 ± 0.34	1.40 ± 1.38	0.46 ± 0.06	11.26 ± 0.42

Table 3.11. Parameters of the primary model for the growth of TPC in tilapia fillets at stable storage temperatures

From the model of Baranyi & Roberts presented in Table 3.11, it can be seen that the TPC of the initial prediction (Y0) ranged from 5.50 to 5.59 log CFU/g. At all constant temperature conditions, that number increeased to reach the peak of about 9.03-11.26 log CFU/g (Y_{max}). The growth rate (µmax) of the TPC at $1 \pm 1^{\circ}$ C was 0.02 (1/hour) which climbed to 0.46 (1/hour) at ambient temperatures of 30 – 33.5°C. The duration of lag phase (λ) decreased from 67.05 ± 43.45 hours at $1 \pm 1^{\circ}$ C to 1.4 ± 1.38 hours at 30-33.5°C. The duration of the lag phase reduced as storage temperature increased. This demonstrates that the bacterial growth rate goes rapidly when they are living in favourable conditions, helping bacteria to reach their highest level (Ymax).

3.4.1.2. The primary model for the growth of Pseudomonas spp. in tilapia fillets over storage time and temperature

Table 3.12 describes parameters of the primary model for the growth of spoilage bacteria, *Pseudomonas* spp. that was built based on the model of Baranyi và Roberts.

mites at constant storage temperatures									
	Temperature	R ²	SE of Fit	Y ₀ (log CFU/g)	λ (hour)	μ _{max} (1/hour)	Y _{max} (log CFU/g)		
	1 ± 1^oC	0.80	0.88	3.87 ± 0.36	143.79 ± 20.57	0.06 ± 0.03	7.81 ± 0.25		
	4 ± 1^oC	0.80	0.82	4.18 ± 0.45	41.33 ± 31.85	0.03 ± 0.01	8.91 ± 0.51		
	9 ± 1^oC	0.92	0.49	4.24 ± 0.23	41.83 ± 13.46	0.04 ± 0.01	8.65 ± 0.29		
	15 ± 1^oC	0.83	0.67	4.22 ± 0.38	20.39 ± 10.14	0.09 ± 0.02	8.20 ± 0.18		
	19 ± 1^oC	0.95	0.39	4.14 ± 0.22	16.44 ± 3.22	0.14 ± 0.02	8.53 ± 0.10		
	$25\pm1^o\mathrm{C}$	0.81	0.59	4.17 ± 0.29	-	0.19 ± 0.03	7.52 ± 0.15		
	29 ± 1^oC	0.81	0.59	4.23 ± 0.27	3.58 ± 1.69	0.38 ± 0.11	7.30 ± 0.14		
	30-33.5°C	0.82	0.83	4.08 ± 0.46	1.13 ± 1.61	0.63 ± 0.17	8.71 ± 0.25		

Table 3.12. Parameters of the primary growth model for *Pseudomonas* spp. in tilapia fillets at constant storage temperatures

From the model stated in Table 3.12, the growth rate of *Pseudomonas* spp. rose as storage temperatures increased. Specifically, *Pseudomonas* spp. reached 0,06 (1/hour) at $1 \pm 1^{\circ}$ C, and went up to 0.63 (1/hour) at 30-33.5°C. The duration of lag phase reduced from 143.79 ± 20.57 hours at $1 \pm 1^{\circ}$ C to only 1.13 ± 1.61 hours at ambient temperatures of about 30 – 33.5°C.

3.4.2. Establishing the secondary model for the growth of spoilage microorganisms in tilapia fillets based on storage time

 Table 3.13. Parameters of the secondary growth model for TPC and Pseudomonas

 spp. in tilapia fillets

	<i>b</i> (°C ⁻¹ hour ^{-1/2})	<i>T</i> _{min} (°C)	R ²
TPC	0.015 ± 0.002	0.23 ± 0.05	0.91
Pseudomonas spp.	0.016 ± 0.004	$\boldsymbol{6.48 \pm 19.92}$	0.83

Tmin is the minimum temperature for bacterial growth. The secondary model shows that parameters obtained from the growth model of TPC had $T_{\rm min} = 0.23 \pm 0.05$ oC and $b = 0.015 \pm 0.002$ (°C-1giò-1/2), while those of Pseudomonas spp. had $T_{\rm min} = 6.48 \pm 19.92$ °C and $b = 0.016 \pm 0.004$ (°C-1giò-1/2). The value of R² was relatively high, with 0.91 for TPC and 0.83 for *Pseudomonas* spp, indicating the feasible application of the model into estimating the TPC of bacteria living at low temperatures and *Pseudomonas* spp. at fluctuating temperatures.

3.4.3. Testing of the predictive model for the TPC of aerobic microorganisms in tilapia fillets stored at fluctuating temperatures simulating storage conditions of supply chains



Figure 3.11. Observed and predicted growth of TPC in tilapia fillets during storage at dynamic temperatures regines (1) and dynamic (2)

Note: different letters show statistically significant differences (p < 0.05) in the average TPC among storage durations

The shelf life of tilapia fillets stored at temperatures regines dynamic 1 should not be longer than 32 hours of storage when the TPC exceeded the allowed limit of 6 log CFU/g. At temperatures regines dynamic 2, the initial TPC was 5.55 log CFU/g which climbed up to 11.32 log CFU/g after 168 hours of storage. There was a rapid rise in the TPC after 22 hours of storage, and the figure for 50 hours of storage was 7.45 log CFU/g, higher than the regulated threshold.

Six out of 8 recorded values of actual TPC were within prediction range (ASZ) at temperatures regines dynamic 1, making up 75%, while the figure for temperatures regines dynamic 2 was 7 out of 9, accounting for 75%. The model is regarded as reliable if at lest 70% of observed values are within the prediction range.

3.4.4. Testing the application of the model for the TPC of aerobic microorganisms in tilapia fillets at the end of supply chains.



Figure 3.12. Changes in the TPC of cold-tolerant bacteria in tilapia fillets during storage in real-world conditions of supply chains

Note: different letters show statistically significant differences (p < 0.05) in the average TPC among storage durations

It can be seen from Figure 3.12 that there was a upward trend in the TPC during storage, and the TPC figures quickly exceeded the allowed limit. Specifically, the initial TPC was about 5.45 log CFU/g which increased over storage time and exceeded the regulated threshold of 6.39 log CFU/g after 22 hours of storage. After 7 days (168 hours) of tilapia fillet storage, the TPC was extremely high, with the figure of 11.71 log CFU/g.

Figure 3.12 also shows that 8 out of 9 values of actual TPC were within the prediction range (ASZ), accounting for 88.89%, leaving only 1 value of actual TPC (11.11%) to be in the prediction range for "fail-safe".

After applying the predictive model for the growth of TPC at low temperatures, the results show that the application of the predictive model for bacterial growth in tilapia fillets during supply chains provided high efficacy. Therefore, based on the history of storage temperature and time, the TPC of Nile tilapia fillets can be quickly identified at any point of time during supply chains, which makes it possible to predict a safe shelf life of tilapia fillets for consumers through the bacterial growth model. This finding is also helpful for suppliers in controlling their product quality in a quick, accurate and cost-effective manner, allowing them to meet today's real demands.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. The preliminary findings regarding the fillet quality show that Nile tilapia fillets had a relatively high content of nutrients.

2. Nile tilapia fillets defrosted by cold air (7 \pm 1°C) had the best quality compared to those defrosted by ambient temperature, cold air (8 \pm 1°C), and water of normal temperature.

3. The study results indicate that temperature and time of storage greatly affected chemical composition, sensory quality, and especially bacterial growth.

The observed changes in the chemical composition during the storage of tilapia fillets at various temperatures demonstrate that although the contents of TVB-N quickly went up as storage temperatures increased, the TVB-N levels were still within the limit of 25 mgN/100 g.

The QI scores at all different temperature conditions had the linear correlation with storage time. In addition, the QIM was able to clearly identify the difference between newly preserved samples and those which were already stored for long period.

Based on the TPC, Nile tilapia fillets will be exprired after 144 hours of storage at $1 \pm 1^{\circ}$ C; 96 hours at $4 \pm 1^{\circ}$ C; 72 hours at $9 \pm 1^{\circ}$ C; 48 hours at $15 \pm 1^{\circ}$ C; 20 hours at $19 \pm 1^{\circ}$ C; 14 hours at $25 \pm 1^{\circ}$ C; 5 hours at $29 \pm 1^{\circ}$ C; and 4 hours at 30-33.5°C. At this storage time, the TPC exceeded the permit limit of (>106 CFU/g according to regulations of the Health Ministry.

4. Based on the data on the changes in bacterial counts found at different stable temperatures, the growth models for the TPC of cold-tolerant aerobic bacteria and *Pseudomonas* spp. were developed, with the relatively high value of R^2 . The findings obtained from the testing of the established models by fluctuating temperatures of simulated and real-world conditions show that the models could be used for predicting the bacterial counts based of records of storage temperatures, helping to identify the shelf life of products during supply chains.

Recommendations

Further in-depth studies about changes in lipids and their composition during the storage of Nile tilapia fillets are needed.

It is essential to continously update data about microbial growth in Nile tilapia fillets, and improve the predictivity of kinetic models to ensure that these models are as close to real-world conditions as possible during supply chains.

Build predictive models for the growth of hygiene indicator microorganisms during chilled/frozen storage of Nile tilapia fillets.

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