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REGULAR ARTICLE

Shelf life prediction and Bacterial flora for the fresh and lightly salted *Pseudosciaena crocea* stored at different temperatures

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ABSTRACT

The active dominant spoilage organisms in the farmed *Pseudosciaena crocea* would cause short shelf life. This study focused on an identification of spoilage bacteria and the development of predictive model of shelf life in *Pseudosciaena crocea* both fresh and lightly salted during the storage at low, ambient and fluctuating temperature conditions. Microbiological, chemical changes in *Pseudosciaena crocea* were assessed to ensure the shelf life; Phenotypic and 16S rRNA were used to identified dominated strains from different conditions. Shelf life, remaining shelf life were predicted by predictive models including exponential, school-field and Square-root equation. The results showed that Shewanella spp. was the dominant organism at low temperature, while *Vibrio* spp. and *Enterobacter* were the dominant organisms at ambient and fluctuating temperatures.While the *Proteus vulgaris, Vibrio alginolytica* and *serratia* were the dominant group at low, ambient and fluctuating environment of the lightly salted *Pseudosciaena crocea* .The shelf-life of fresh *Pseudosciaena crocea* were 5.4 ~ 17.8 days, 1.1days and 4.6 ~ 9.3 days under low, ambient and fluctuating temperature; while the shelf life of lightly salted *Pseudosciaena crocea* were 12.2 ~ 49.1days, 4.2day and 20.5 ~ 23.5 days under low, ambient and fluctuating temperature. The evaluation index showed that School-field model and Exponential model in fresh fish were better, Exponential model was better to predict the shelf life in lightly salted fish. The results can facilitated the establishment of spoilage bacterial Hazard Analysis Critical Control Point during the processing of the *Pseudosciaena crocea* products.

Keywords: Fresh and lightly salted; *Pseudosciaena crocea;* Dominant spoilage organisms; Shelf-life prediction; Storage temperature

INTRODUCTION

Pseudosciaena crocea, or called Large Yellow Croaker(LYC),with succulent and nice flavor, is a kind of economic valuable fish species in East China Sea(Xie et al.,2011).Its common consumption patterns are fresh and lightly salted measures. However, due to its abundant proteins, spoilage bacteria is extremely easy to cause corruption, which leads to a relatively short shelf life compared the *LYC* with other marine fish products (Li et al.,2012). It is well known that fresh fish spoilage is the result of microbial activity of Specific Spoilage Organisms (SSO), a fraction of the total fish microbial that degrades the fish into biochemical components, which is usually perceived the loss of fishy freshness by the consumer(Garcia et al.,2015). It is important to identify these organisms in order to indicate the shelf life of the product. Species of the genera *Pseudomonas* spp., *Shewanella* spp., *Carnobacterium* spp., *Lactococcusare* spp. were well known as SSOs in fish products(Dalgaard et al.,2003, Koutsoumanis et al.,2000, Nieminen et al.,2016). Currently, compare with the Bacterial colony morphology, cell morphology and physiological biochemical characteristics, the sequence analysis of the 16S rRNA gene is the most common and accuracy methods for studying seafood microbial that grown on plates(Alfaro et al.,2013)

Fish quality and the growth of the (SSO) may be affected by many factors related to the environment, so it is important to explore a evaluation system through monitoring environmental conditions of the packaged foods during transport and storage to provide food

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quality and safety information, in order to guarantee food quality, reduce losses and consumer complaints(Heredia et al.,2009). The predictive microbiology is the theoretical basis of the development of the food safety Intelligent evaluation system, which takes the microorganisms in food as the object of study, use integrated mathematics, statistics and computer interdisciplinary (Palino,2007) to reflect the factors of microbial activity at external condition by mathematical model, which is an important point in food processing to ensure the quality and safety(Berger,2007). Predictive microbiology is using mathematical models to make the quantitative prediction of dynamic changes of harmful microbes in food under certain conditions(PéRez-RodríGuez et al.,2013), so it is not only a method for preventing food-borne pathogens from damaging the food products and an effective early alarming tool in the food safety, but also an effective means to prevent food quality from decreasing caused by spoilage. Compared with time-consuming and cumbersome traditional evaluation methods, the preservative and intelligent evaluation methods are fast, accurate, being able to optimize the preservation of aquatic products(Mcmeekin et al.,2008).

The aim of the present investigation was to determine (i) the shelf life by sensory, microbiological and chemical changes,(ii) the initial and spoilage microbial of fresh and lightly salted LYC during the storage by 16S rRNA gene sequencing analysis and (iii) develop the mathematical models to predict and remain the shelf life, which can provide the supports for digital describing mechanics of targeted microorganism and intelligently evaluating the safety and risk of fishery products.

MATERIALS AND METHODS

Materials and Storage

The fresh samples were from Ningde City, Fujian Province Sandu Bay, use ice refrigerated trucks for transportation (0 ~ 1 ° C), 6 ~ 9 h delivered to the laboratory. The lightly salted samples were from NINGDE WEIJIAHAO company, 450g products, which the salt content was 2.0%, moisture content was 60% and water activity was around 0.96 with vacuum package.

After sample arrived at laboratories, then kept in low temperature (0 ~ 10 ° C), into the precision low temperature incubator (MIR-553, Sanyo, Japan), set inside temperature 2 ~ 3 ° C and timely add ice.

Fresh and lightly salted *LYC* are usually transported and stored with chilled chain, 0°C is often regarded as the reference temperature, 5°C is common cold storage temperature and 25°C is the ordinary ambient temperature, so both of the Fresh and lightly salted *LYC* 0 °C,5 °C,25 °C was chosen to built the shelf life models. While Fresh samples were stored at 7,10 ° C which in the arrange of 0~25°C for verification, and fluctuating temperature (A: 4 °C (50 h) → 10 °C (28.5 h) → 5 °C (48 h).; B: 4 °C (50 h) → 10 °C (28.5 h) → 15 °C (24 h) → 25 °C (5 h). for verification of the remaining shelf life models. While the lightly salted samples were stored 10 ° C, 15 ° C which in the arrange of 0~25°C for verification, and fluctuating temperature (C: 10 °C (96h) → 15 °C (96h) → 5 °C.; D: 10 °C (240 h) →5 °C. for verification of the remaining shelf life models, which the temperature were chosen according to the process simulation that may occur during transportation with the arrange 0~25°C and were the most common temperature in the daily life.

Took the low temperature samples every 24h, took the samples at ambient temperature and fluctuating temperature every 8h. Used the time temperature recorder (Test 175-T2, Testo, Germany, accuracy ± 0.5 ° C, the range of -20.0 ~ 70.0 ° C) to measure the fish temperature.

Sample Preparation

According to the experiment plan and storage chain, took 3 parallel experiments under each temperature section, low temperature, ambient temperature and fluctuating temperature every time. Every time selected 3 fish randomly, take off the scales, gutted and gills of the fish and sterile cut along the spine, the flesh of fish were cut and broken with a homogenizer (basic panoramic, IUL, Spain) to the fish paste state and evenly divided into two parts for the total number of colonies (total viable counts, TVC), as well as volatile basic nitrogen (total volatile basic nitrogen, TVBN) measurement.(He et al.,2015)

Test Method

Quality index

TVBN measuring methods used the international method(Quanyou et al.,2006).

Bacterial count: Weigh the broken fish 10.0 g, added 90mL 0.1% Peptone sterile saline, after high-speed oscillation to 10-fold dilution of fish paste, took three appropriate concentration of dilution 0.1mL, with automated microbiology plate spiral pipettes (Eddy Jet, IUL, Spain) was applied on nutrient agar medium and iron agar (iron agar, IA) flat surface, each dilution was applied two plates, cultivate at 25 ° C for 48 h, counted the bacteria cultured on agar colonies.

TVBN and TVC were regarded as the index that can show the shelf life, when the TVC was over 7 or the TVBN

was over 30 $mg100^{-1}g^{-1}$, the products reached the end of its shelf life.

Bacterial phenotypic Identification

Took the plates at the end of the shelf life, chose the appropriate total number of colonies, colony flat on the whole plate or certain areas (usually 30 ~ 100CFU colonies), according to colony morphology, Gram stain, cell morphology, spores or without sports and oxidation/ fermentation characteristics, using bacteria classification grouping method, each colonies picked at least 2 ~ 3CFU colony, isolated and purified the colonies, cultured under 25 ° C for 24 ~ 48 h. Bacterial colony morphology, cell morphology and physiological and biochemical characteristics were analyzed using Sensitire (trek diagnostic system Ltd, UK) and MIDI (MIDI Inc., Newark, Del, USA) classification multiphase systems, which divide the spoilage bacteria into several groups, choose the high proportion bacteria groups which the proportion was over 20% for further 16S rRNA gene partial sequence-based Identification.

16S rRNA gene partial sequence-based Identification

A total of 12 pure cultures were chosen according 2.3.2 and recovered from TSA plates used for genetic Identification. Bacterial DNA extraction and purification were performed on pure cultures using the Bacterial Genomic DNA extraction kit (Sangon, China) according to the manufacturer's instructions and get the target 16S rRNA gene. The primers used for PCR were 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-CCC GGG AAC GTA TTC AC CG-3'), the final volume of the PCR mixture was 50 µl, which contained 1µl DNA, 5µl 10×buffer, 1µl $MgCl_2$, 4µl dNTP, 1 U of Taq polymerase 0.5µl, 27 F 1µl, 1492R 1µl and add ddH_2O to 50µl(Tiangen, China). Samples were analyzed in a Instrument for Polymerase Chain Reaction (Biometra, Goettingen, Germany) with the following program: 3 min at 94 °C, 35 cycles of 30s at 94 °C, 45s at 50 °C and 100 s at 72 °C and a final step of 7 min at 72 °C, cold down to 4 °C. The products were than for gel electrophoresis at 110v for 30min with 1% gel, and saw if there was a target band in the gel imager around 1500bp. The PCR products with target band were sent to Shanghai Biological Engineering technology Services Company for 16S rRNA sequencing. For the detection of closest relatives, all sequences were compared with the BLAST function on NCBI with> 99% relative result and then submit to NCBI which the submit number were showed in Fig 1. Sequence data were aliened and phonotypes were defined as the data showed>99% similarity to each other. All relative unique phonotypes and sequences were obtained from GenBank, and the maximum likelihood statistical method was used to build phylogenetic tree through the MEGA7.0 software. Bootstrapping was performed with 500 replicates to assign confidence levels to the tree topology.

Effect of storage temperature on the shelf life of *Pseudosciaena crocea*

Construction of the relative rate model of corruption

Data on the rates of spoilage determined as the reciprocal of shelf-life (RS, day) from Fresh and lightly salted sampled were calculated and three empirical models: exponential model (1) school-field model (2)Square-root (3) were fitted to the combined RS-data. In this study the shelf life data of 0,5,25°C were used to built the RRS models.

$$In(RRS) = a \times (T - T_{ref}) \tag{1}$$

$$In(RRS) = \frac{-E_a \times 10^3}{R} \times (\frac{1}{T+273} - \frac{1}{T_{ref}+273}) \tag{2}$$

$$\sqrt{RRS} = \frac{T - T_{\min}}{T_{ref} - T_{\min}} \tag{3}$$

Where a is the coefficient; Ea is the in the apparent activation energy, kJ/mol, T is the storage temperature, R is 8.314 $J/(mol \cdot K)$, T_{min} is the temperature coefficient characteristics, the theoretical minimum temperature.

Construction shelf life prediction model

LYC are usually transported with chilled chain, 0°C is often regarded as the reference temperature of fresh fish during the storage period. Combined with the relative corruption rate which is defined through the experiment, developed Exponential, School-field and Square-root shelf life prediction model is as follows:

$$SL_T = \frac{SL_{ref}}{\exp[aT]} \tag{4}$$

$$SL_T = \frac{SL_{ref}}{\exp[\frac{-E_a}{R} \times (\frac{1}{T+273} - \frac{1}{273})]} \tag{5}$$

$$SL_T = \frac{SL_{ref}}{(T/T_{\min} + 1)^2} \tag{6}$$

Where SL_T is the shelf life, T, is the temperature, R is 8.314 $J/(mol \cdot K)$, SL_{ref} is the reference temperature

Construction of the remaining shelf life prediction model

To predict the remaining shelf life, the time-temperature history is divided into a very short time under the set temperature interval, the time experienced at different temperatures is converted to a reference temperature 0°C, The difference of shelf life under the reference temperature was calculated, so that it could export the remaining shelf life at different temperatures as equations:

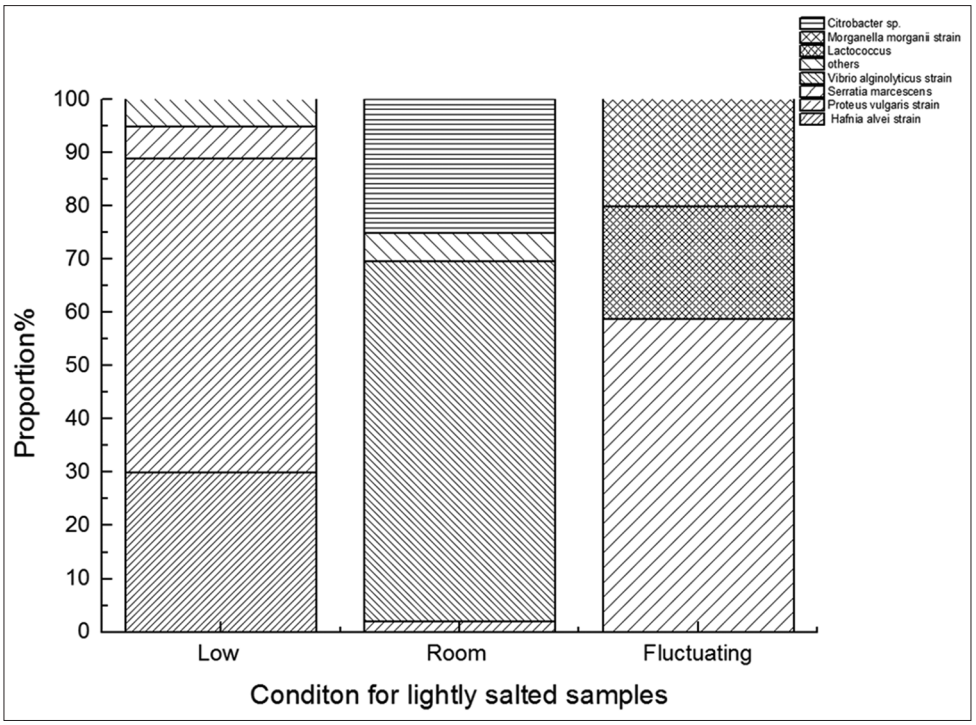

Fig 1. The proportion of the bacteria in fresh and lightly salted samples

$$RSL_T = \frac{SL_{T_{ref}} - \sum_{i=1}^{n}\left(ST_n \times RRS_{T_n}\right)}{\exp\left(a \times T\right)} \quad (7)$$

$$RSL_T = \frac{SL_{T_{ref}} - \sum_{i=1}^{n}\left(SL_{T_n} \times rrs_{T_n}\right)}{\exp\left[\frac{-E_a \times 10^3}{R} \times \left(\frac{1}{T+273} - \frac{1}{T_{ref}+273}\right)\right]} \quad (8)$$

$$RSL_T = \frac{SL_{T_{ref}} - \sum_{i=1}^{n}\left[SL_{T_n} \times RRS_{T_n}\right]}{\left(1+a \times T\right)^2} \quad (9)$$

Where RSL_T is the remaining shelf life of the temperature T, SL_{Tref} is the shelf life of the temperature T_{ref}, which is the reference temperature shelf life; ST_{Tn} is the storage time of the temperature T_n, RRS_{Tn} relative corruption rate of the temperature T_n.

Data Processing

Viable counts, kinetic parameters(μ_m,*lag*),TVBN, values were statistically tested by SPSS(21.0). The data of sequence-based Identification was used MEGA7.0 and all the pictures were made by origin 9.0.

Evaluation Model

To compare the performance of the model, the most effective means is to detecting the difference between the predicted value and the real data, the coefficient of determination R^2, residual sum of squares (residual sum of squares, RSS), the degree of deviation (bias factor, BF), accuracy (accuracy factor, AF), RMSE (root mea n square error, RMS) goodness of fit of the model (goodness of fit) were evaluated. In this study the data in 7,10,15 and fluctuating temperature were used for Evaluation.

$$RSS = \sum_{i=1}^{n}\left(x_p - x_0\right)^2 \quad (10)$$

$$BF = 10^{\frac{\sum_{i=1}^{n}\lg\left(x_p / x_0\right)}{n}} \quad (11)$$

$$AF = 10^{\frac{\sum_{i=1}^{n}\left|\lg\left(x_p / x_0\right)\right|}{n}} \quad (12)$$

$$RMS = \sqrt{\frac{\sum_{i=1}^{n}\left(x_p - x_0\right)}{n}} \quad (13)$$

In the formula, n is the number of sample, x_p and x_0 were predicted and actual values.

RESULTS

Quality characters of the initial and chemical changes

Tab.1 showed the evolutionary TVC of fresh and lightly salted *LYC* which stored at different temperatures. Respectively, TVBN values increased throughout the storage. As expected, the rate of change of the chemical

indicator was higher at high temperature than at low temperature. The freshness lost gradually in *LYC* is dependent on its own physiological, biochemical, microbiological and other factors, so evaluating quality characteristics is essential to reduce the risk of corruption during the supply chain. From Table 1, provided the initial storage of fresh and lightly salted *LYC* samples, TVBN were (8.04 ± 1.07) and (8.93±1.07) mg/100g and the total number of colonies of bacteria were (4.21 ± 0.25) and (5.72±0.25) lg (CFU/g), obviously the light salted samples value was higher. Besides, at low temperature (0 ~ 10 °C), the total number of colonies of bacteria were (6.63~6.72) and (6.03~7.98) mg/100g at the end of shelf life storage, while at ambient temperature storage of fresh and lightly salted LYC, the total number of colonies of bacteria were (6.51 ± 0.24) and (7.12±0.33) lg (CFU/g), TVBN were (24.03 ± 0.38) and (30.84±0.25) mg/100g.

Due to the different spoilage bacterial activity during the storage, there were significant differences between the fresh and lightly salted samples in TVC and TVBN (Sig1> 0.05). So that Bifidobacterium classification grouping method and 16s RNA were both used for deep identification.

IDENTIFICATION OF THE SPOILAGE MICROBIOLOGY

Indigenous microbiology Microbiological analysis and 16S rRNA gene partial sequence-based identification

At the end point of shelf lives of fresh samples, from 0 ~ 10 °C, 280 bacteria were isolated; from 25 °C and fluctuating temperature 81 and 63 strains were isolated and identified.

The results showed in Figs. 1 and 2, at low temperature (0 ~ 10 °C) *Shewanella (Shewanellaputrefaciens)* was the specific spoilage bacteria, the bacterial ratio was 67.5%, and the result was the same as *Birte FV* (Vogel et al.,2005) reported that the *Shewanella* existing in seawater was the main spoilage bacterial of fish in cold storage. And at 25°C , the proportion of *Vibrio (Vibronaceae)* and *Enterobactershare* were 53.1% and 25.9%, while at the fluctuating temperature, *Vibrio (Vibronaceae)* was the dominant group with the ratio of 42.9%. At the end of storage, *Shewanella, Enterobacter* and *Vibrio* grew significantly, which mainly caused the spoilage of the fresh samples.

At the end of shelf lives of lightly salted samples, from 0 ~ 15°C ,150 bacteria were isolated, from 25 °C and fluctuating temperature, both 50 strains were isolated and identified. The results were showed in Figs. 1 and 3, at low temperature (0 ~ 15°C) *Proteus vulgaris* was the specific spoilage bacteria, the bacterial ratio was 58.9%, at 25°C , *Vibrio alginolytica* shared the proportion of 67.64%.While at the fluctuating temperature, *serratia* was the dominant group with the ratio of 58.8%, which mainly caused the spoilage of lightly salted samples.

SHELF-LIFE AND PREDICTION MODEL

Construction and verification of the relative rate model of corruption

From the quality characteristics, chemical and microbiological changes, the shelf life of fresh samples was 5.4~17.8 days (0-10°C), 1.1days (25°C) and 4.6~9.3 days (Fluctuating temperature);the shelf life of lightly salted samples was 12.2~49.0days (0-15°C), 4.2days (25°C) and 20.5~23.5 days (Fluctuating temperature). So it showed that salted progress can extend the shelf life of the fresh samples.

According to the measured data of shelf life, three RRS model formulas in Table 3 were used to derive the relative corruption rate of RRS data at 0°C ~ 25°C (Table 2). Regarded 0°C as the reference temperature and chose 0°C , 5°C and 25°C RRS data to fit the Exponential, School-field and Square-root RRS models which were

Table 1: Quality characters of the initial and the end of shelf life of *LYC* stored at different temperature

Storage condition/ fresh samples	Temperature/ °C	TVC/ lg (CFUg^{-1})	TVBN/ (mg100^{-1}g^{-1})	Storage condition /lightly salted samples	Temperature/ °C	TVC/lg (CFUg^{-1})	TVBN/ (mg100-1g^{-1})
Initial	-	4.21±0.25	8.04±1.07	initial	-	5.72±0.25	8.93±1.07
Low temperature	0	6.64±0.49	30.12±2.06	Low temperature	0	6.037±0.49	30.24±0.84
	5	7.60±0.25	29.58±3.19		5	7.98±0.25	30.56±0.19
	7	6.63	26.3		10	6.64	29.66
	10	6.72	28.26		15	7.6	31.08
Ambient	25	6.51±0.24	24.03±0.38	Ambient	25	7.12±0.33	30.84±0.25
Fluctuating temperature	A	7.23±0.01	27.40±0.04	Fluctuating temperature	C	7.92±0.21	29.23±0.44
	B	6.67±0.01	26.42±0.09		D	7.95±0.33	30.1±0.13

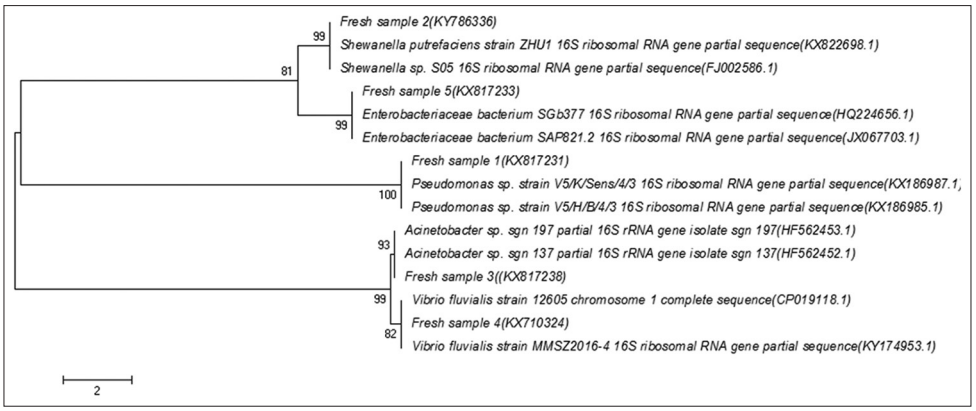

Fig 2. Phylogenetic analysis for fresh samples

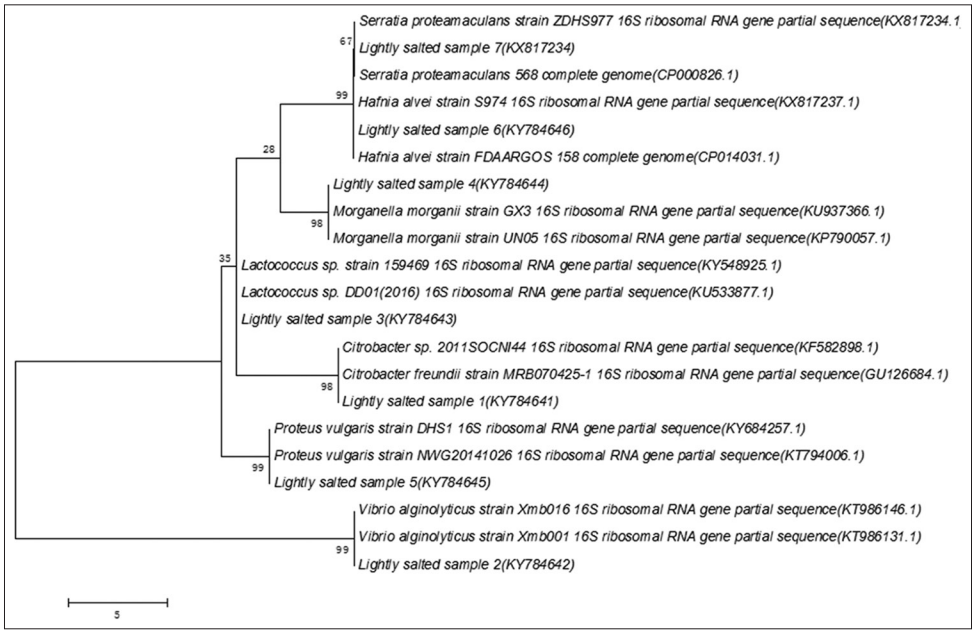

Fig 3. Phylogenetic analysis for lightly salted samples

Table 2: Validation on shelf life predictive models of *Pseudosciacna crocea* stored under different temperatures

Storage conditions/ fresh samples	Temperature/°C	Shelf-life/d	Relative corruption rate	Storage conditions/lightly salted samples	Temperature/°C	Shelf-life/d	Relative corruption rate
Low temperature	0	17.8±2.5	1	Low temperature	0	49±1.1	1
	5	9.3±1.1	1.91		5	29.1±1.0	1.684
	7	7.2	2.47		10	18.5	2.65
	10	5.4	3.24		15	12.2	4.016
	Total	5.4~17.8	-		Total	12.2~49.0	-
Ambient temperature	25	1.1	14.8	Ambient temperature	25	4.2	11.667
Fluctuating temperature	A	9.3	-	Fluctuating temperature	C	20.5	-
	B	4.6	-		D	23.5	-
	Total	4.6~9.3	-		Total	20.5~23.5	-

showed in Table 3, resulted that the model parameters: the temperature characteristic coefficient a_1 = 0.11 and a_2 = 0.098; Ea_1= 74.00kJ/mol and Ea_2= 66.44kJ/mol; $Tmin_1$ = - 10.00°C and $Tmin_2$ = -10.52°C .

The Evaluation of predictive performance of models

In this study, the data at 7, 10, 15°C were used for RRS model evaluation. Use the BF, AF, RSS and RMS to evaluate the performance of RRS Exponential, School-field and Square-root model. Since the BF of these 3 models were 0.75-1.25, showed the model was acceptable to fresh samples and lightly salted samples in Table 4. Besides, AF of these models were all <1.30, which also indicated the results were acceptable. Including AF, BF, RSS and RMS and other evaluation parameters, three kinds of equations were acceptable, the RSS and RMS parameters of School-field RRS model were the lowest for fresh *LYC*, and the RSS and RMS parameters of Exponential RRS model were the lowest. So the result indicated that the performance of School-field RRS model was superior to Exponential RRS model and Square-root RRS model in fresh samples, while the Exponential RRS model showed great good of fitness in lightly salted samples.

Construction and validation of the shelf life predictive model

According to the parameters of the RRS models, the formulas from 4 to 6 were used to predict the shelf life of fresh fish at 7 and 10°C , and at 10 and 15°C of lightly salted fish. The predicted values were compared with the observed data. Table 5 showed the predicted values of the Exponential, School-field and Square-root shelf life prediction model, and their relative error in fresh samples were 7.3% and 13.03%, 1.8% and 9.38%,-18.2% and -14.45%, respectively, indicating that the formula Exponential model and the School-field model could better predict the shelf life of the fresh product at a constant temperature than the Square-root model. For the lightly salted samples, their relative error were -0.90% and -8.30%, -12.56% and -5.85%,-31.8% and -30.40%, indicating that the formula Exponential model could better predict the shelf life of the lightly salted sample than the other two models. It was shown that the three models were different when used to predict the shelf life in the two kinds of fish samples.

Development and validation of the remaining shelf life prediction model

According to the parameters of the RRS models, the formulas from 7 to 9 were used to predict the remaining

Table 3: RRS model for fresh/light salted samples

	RRS model for fresh samples	R^2	RMSE	RRS model for lightly salted samples	R^2	RMSE
Exponential	$In(RRS)=0.11\times T$	0.99	0.07	$In(RRS)=0.098\times T$	0.99	0.02
School-field	$In(RRS)=\frac{-74\times10^3}{8.314}\times(\frac{1}{T+273}-\frac{1}{273})$	0.99	0.03	$In(RRS)=\frac{-66.44\times10^3}{8.314}\times(\frac{1}{T+273}-\frac{1}{273})$	1	0.003
Square-root	$\sqrt{RRS}=0.1\times T+1$	0.96	0.21	$\sqrt{RRS}=0.095\times T+1$	0.99	0.12

Table 4: Evaluation of predictive performance of three RRS models

RRS Model of fresh samples	RRS predict value						RRS Model of lightly salted samples	RRS predict value					
	7°C	10°C	BF	AF	RSS	RMS		10°C	15°C	BF	AF	RSS	RMS
Exponential	2.15	2.98	0.928	1.077	0.36	0.347	Exponential	2.678	4.382	1.033	1.033	0.135	0.212
School-field	2.26	3.17	0.963	1.038	0.049	0.127	School-field	2.813	4.593	1.067	1.067	0.359	0.346
Square-root	2.89	4	1.13	1.13	0.754	0.501	Square-root	3.81	5.89	1.116	1.156	4.857	1.272

Table 5: Validation on shelf life predictive models of fresh and lightly salted *LYC* stored aerobically under isothermal conditions

Models	Shelf life prediction (days) for fresh samples						
	7°C	Relative error%	10°C	Relative error %	R^2	RMSE	RMS
Exponential	8.279	13.03	5.973	7.3	0.995	0.07	0.705
School-field	7.876	9.38	5.615	1.8	0.999	0.004	0.41
Square-root	6.159	–14.45	4.45	–18.2	0.961	0.22	0.814
Models	**Shelf life prediction (days) for lightly salted samples**						
	10°C (days)	Relative error %	15°C	Relative error %	R^2	RMSE	RMS
Exponential	18.334	–0.9	11.18	–8.3	1	0.021	0.483
School-field	17.417	–5.85	10.667	–12.56	1	0.004	0.992
Square-root	12.859	–30.4	8.311	–31.8	0.991	0.128	3.891

Note: relative error = $(S_{Lprd}- S_{Lobs}) \times 100\%/S_{Lobs}$

shelf life of the fresh and lightly salted fish. After resuming A (fresh fish): 4°C (50 h) → 10°C (28.5 h) → 5°C (143 h), the final shelf life was 9.3d. The equations from 7 to 9 were used to calculate remaining shelf life at different temperatures with the range of 0 ~ 25°C , the shelf life of the predicted value in this invention of three models were 9.6, 9.2 and 7.3 d, relative error of 3.3%,- 0.9% and -22.1%, showed in Table 6. After resuming B(fresh fish): 4°C (50 h) → 10°C (28.5 h) → 15°C (24 h) → 25°C (5 h) and finally arrived the end of shelf life (4.6d), the remaining shelf life prediction value were 4.6, 4.7 and 4.5 d, relative errors were 0%, 0.2% and 0.2% (Table 6). By comparison of the relative error of the remaining shelf life prediction model of these three kinds, it showed the performance of Exponential and School-field remaining shelf life were much more excellent, which could quickly and efficiently predict the remaining shelf life of a range of 0 ~ 25°C of the fresh fish. The lightly salted samples after resuming C (C: 10°C (96h) → 15°C (96h) →5°C), finally reached the end of shelf life with 20.5 days; and 23.5 days of storage in the treatment of D (10°C (240 h) →5°C), and the relative errors of Exponential remaining shelf life prediction model was the lowest (0.97 and 6.5%), which meant that these models could best predict the remaining shelf life of the lightly salted samples within the range of 0 ~ 25°C .

DISCUSSION

The present study aimed at evaluating the quality change of *LYC* stored at different temperature conditions with different treatments. The initial total aerobic counts obtained in this study (4.21 log CFU/g) were quite similar to the raw Atlantic salmon stored at 4°C , 10°C , 21°C (Miks-Krajnik et al.,2016), but higher than those reported by Zhu (Zhu et al.,2016) around 3.55 log cfu/g, and reaching up to 7.61 log cfu/g after 5 days in 4°C LYC.\In this study the total average aerobic counts, while reaching up to 7.60, 6.51 and 7.23 at low, room and variable temperature in this paper, among the range of 6–9 log CFU/g, which are always used as a reference method of shelf life estimation(Miks-Krajnik, et al.,2016, Zhu, et al.,2016). As for the fresh and lightly salted *LYC*, respectively, the product had a much longer shelf life between 5.4~17.8 and 12.2~49.0 days under low temperature, while only 1.1day and 4.2days under ambient temperature and 4.6~9.3 and 20.5~23.5days under the fluctuating temperature. Which was similar to other research, J. Emborg(Emborg et al.,2002) explored the Microbial spoilage and formation of biogenic amines in fresh and thawed modified atmosphere-packed salmon (Salmosalar) which showed it could keep 14~21days at 2°C. And Dalgaard (Dalgaard et al.,2006)found the shelf life was15 days, 9 days in raw chilled garfish under air condition at 0°C and 5°C . It could be concluded that the shelf life and the total aerobic counts were highly relative to the storage temperature, lower temperature could keep a longer shelf life but the total aerobic counts at the end of storage had no obviously difference among the different storage temperature. And Salt processing could keep the product for a longer shelf life.

For raw fish stored aerobically, many bacteria were regarded as potential SSOs, including *Pseudomonas* spp.*, Shewanella putrefaciens, Photobacterium phosphoreum, psychrotolerant bacteria, Serratia spp., Yersinia spp.*(Alfaro,Hernandez,2013, Emborg et al.,2005, Koutsoumanis,Nychas,2000, Zhu, et al.,2016). The comparison were showed in Table 7.With 16S rRNA gene sequencing, it was confirmed that *Shewanella* was the predominant genera in the spoiled sample according to microbial count under low temperature, which was similar to the report by GU (Gu et al.,2013)and ZHU(Zhu, et al.,2016). The *Shewanella* genus currently includes 48 identified species. *S. baltica* and *S. putrefaciens* were the two main spoiling species of *Shewanella* in *LYC*, which are typical SSOs in fresh marine

Table 6: Remaining Shelf life predictive models of fresh/lightly salted samples stored under non-isothermal conditions

Temperature changes	*Slobs/d*	Exponential (remaining shelf life prediction model)		School-field (remaining shelf life prediction model)		Square-root (remaining shelf life prediction model)	
		SL_{pre}/d	Relative error /%	SL_{pre}/d	Relative error /%	SL_{pre}/d	Relative error /%
A	9.3	9.6	3.3	9.2	–0.9	7.3	–22.1
B	4.6	4.6	0	4.7	0.2	4.5	–0.2
C	20.5	20.7	0.97	19.5	4.87	12.71	38
D	23.5	23.63	6.5	22.38	4.76	15.04	36

Fresh samples: A: 4°C (50 h)→10°C (28.5 h)→5°C (143 h)…;B: 4°C (50 h)10°C (28.5 h)→15°C (24 h)→25°C (8 h)… ;Lightly salted : (C: 10°C (96h) →15°C (96h) → 5°C .; D: 10°C (240 h) →5°C, SLobs is the measured shelf life values, SLprd is the shelf life prediction value, relative error = (SLprd- SLobs) × 100%/SLobs

Table 7: The comparison of the potential SSOs

Sample type	Storage temperature	Potential SSOs in this paper	Potential SSOs in compared reference
Fresh *LYC*	Low temperature	*Shewanella putrefaciens*	*Shewanella putrefaciens* [Gu, 2013, Zhu, 2016]
	Ambient temperature	*Vibrio fluvialis Enterobacter* spp.	*Vibrio* spp. in marine products[(Parlapani ,2013]
Lightly salted *LYC*	Low temperature	*Proteus vulgaris*	*Proteus vulgaris*[zhang, 2011] *Brevundimonas diminute*
	Ambient temperature	*Vibrio alginolytica serratia*	*Vibrio spp.* in marine products[Parlapani ,2013]

fish (Parlapani et al.,2013). Under ambient temperature, *Vibrio* and *Enterobac*ter were the dominant groups, while *Vibrio spp* shared the main part at non-isothermal temperature in this paper, which suggested that *Vibrio* was frequently present in marine products(Seminario et al.,2011). The competition and interactions between the different bacteria in the spoilage flora therefore played a major role in determining the rate and the extent of fish spoilage. As for the lightly salted *LYC Proteus vulgaris*.

Brevundimonas diminute were potential SSOs under low temperature(Zhang et al.,2011).In this study, *Shewanella, Vibrio* and *Enterobacter* were observed to be the predominant microorganisms in fresh samples, and *Proteus vulgaris, Vibrio alginolytica* and *serratia* were the dominant microgranisms in light salted samples at different storage temperatures. Therefore, it might be an important measure to control the growth of *Shewanella, Vibrio, Proteus vulgaris* and *Enterobacter* these 4 kinds of microorganisms in order to extend the fish shelf life.

The shelf life prediction models was verified to predict the shelf life of the marine products. However, according to the relative error, the two models, the School-field model and the Exponential model, were superior to the Square-root model, they could quickly and effectively predict the shelf life at the range of 0°C ~ 25°C in fresh LYC and the Exponential model was the best choose to predict the shelf life and the remaining shelf life of the lightly salted *LYC* among the three models. While García(Garcia, et al.,2015) used QIM model to reflect the quality of the retailed fresh hake, Zhu (Zhu, et al.,2016) used the modified Gompertz model to reflect the growth of the SSOs in order to predict the shelf life of the refrigerated *LYC*, and Powell and his team(Powell et al.,2015) chose a Belehradek-type model to reflect the growth data of spoilage bacteria on modified atmosphere packaged Atlantic salmon produced in Australia. There were many models being chosen to reflect the quality of marine products, to reflect the growth of the spoilage bacteria, or to predict the shelf life of the marine products. However, different marine products with different treatments and different storage conditions need specific models to implement the different function. This paper showed that, among the three models -- the School-field model, the Exponential model and the Square-root model, which are most traditional and common methods for predicting the shelf life, the School-field model and the Exponential model were more suitable for the *Pseudosciaena crocea* than the Square-root model.

CONCLUSION

This paper studied the spoilage characteristics of *Pseudosciaena crocea* with two treatments – fresh and lightly salted, which were mainly about the microbiological and chemical changes in the *Pseudosciaena crocea* during the storage at 0~10°C , 25°C , fluctuating temperature with different storage time. Determined the dominated spoilage organisms and predicted the shelf life of the *LYC* with the mathematical models which were constructed with intelligent evaluation system.

(1) The result showed that in fresh *LYC*, *Shewanella* was the dominant group at low temperature, while *Vibrio* spp. and *Enterobacter* were the dominant group at ambient temperature and at non-isothermal temperature. *Vibrio* shared the main proportion, while in lightly salted *LYC*, *Proteus vulgaris, Vibrio alginolytica* and *serratia* were the dominant group at low, ambient and fluctuating environment through 16S rRNA sequencing and traditional Identification method.

(2) The shelf life of fresh *LYC* were 5.4 ~ 17.8 days, 1.1 days and 4.6~9.3 days under the low, ambient and variable temperature, while the lightly salted *LYC* were 12.2~49.1 days, 4.2 days and 20.5~23.5 days under the low, ambient and fluctuating temperature.

(3) The School-field model and the Exponential model could predict the shelf life at the range of 0 ~ 25°C more quickly and effectively in the fresh *LYC* than Square-root model, while the Exponential model was better to predict the shelf life than the other two models of lightly salted *LYC*

This paper can provide the supports for Digital mechanics of targeted microorganism and intelligently evaluating the safety and risk of fishery products.

AUTHORS' CONTRIBUTIONS

All the authors contributed equally to the writing of this paper, they were also involved in the overall work of experiments

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