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Full Length Research Paper

Modeling growth of specific spoilage organisms in tilapia: Comparison Baranyi with chi-square automatic interaction detection (CHAID) model

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Tilapia is an important aquatic fish, but severe spoilage of tilapia is most likely related to the global aquaculture. The spoilage is mostly caused by specific spoilage organisms (SSO). Therefore, it is very important to use microbial models to predict the growth of SSO in tilapia. This study firstly verified *Pseudomonas* and *Vibrio* as the SSO of tilapia, then established microbial growth models based on Baranyi and chi-square automatic interaction detection (CHAID) models and compared their effectiveness. The results showed that both Baranyi model and CHAID model are appropriate for predicting the growth of microorganism. Baranyi model fits the microorganism growth better than CHAID model overall though CHAID model fits well at stationary phase. CHAID model predicts the microorganism growth accurately when the rate of change of the experiment data is big.

Key words: Specific spoilage organisms (SSO), tilapia, chi-square automatic interaction detection (CHAID), Baranyi, shelf-life.

INTRODUCTION

Tilapias are one of the most important food fishes in the world. Native to Africa and the Middle East, tilapia offers the possibility of commercial and home-grown protein sources because of their superior culture facilities when wild capture fisheries are becoming increasingly depleted in the world (Ahmed et al., 2005). The world outputs of tilapia are exceeding 3 million tons at 2010. As the world's largest tilapia production base, the output of China accounts for 50% of the globe.

Tilapia is undemanding, omnivorous, fast growing, easily bred in captivity under a wide variety of water and climate conditions and suggested as more disease resistant than other fishes. Tilapia can tolerate, grow and even reproduce in saline waters, although, this capacity is somewhat offset under high salinity conditions. These exclusive features make tilapia ideal aquaculture species and explain why it has become one of the most important domesticated fishes around the world (Mark et al., 2010). But new severe tilapia spoilage is most likely related to the global intensification of aquaculture.

Shelf life is used to describe the quality of fresh fish as well as the spoilage level (Ghaly et al., 2010). The shelf life of fresh fish is affected by many factors and some direct methods (chemical and sensory) for assessing the quality of fish have been used in the past few decades. But they are limited by test time and sensitivity. It has been found that the presence and performance of microorganisms in the fish products are closely related to the spoilage of fish and the remaining shelf life, especially the specific spoilage organisms (SSO). SSO can be able to survive and increase on the products and their microbial metabolites make corrupt stench. With increasing spoilage, establishing model of SSO to predict shelf life becomes a highly essential concern (Koutsoumanis, 2009).

It is known that time and temperature is two of the most important physical factors affecting the growth of SSO in foods. Therefore, modeling with the effect of temperature

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Abbreviations: SSO, Specific spoilage organis; **CHAID,** chi-square automatic interaction detection.

for the growth of SSO can help to predict and estimate potential growth of SSO reliably during processing and storage.

In this study, the samples of Tilapia were captured from the warm water of the Eastern Pacific. At the end of the shelf life, the percentage of *Pseudomonas* and *Vibrio* is 58% (Ahmed et al., 2005), which are both the dominant SSO of tilapia. So we decided to establish the growth model of *Pseudomonas* and *Vibrio* to predict the shelf life of tilapia.

Growth predictive models are currently accepted as informative tools that assist in rapid and cost-effective assessment of microbial growth for product development, risk assessment, and education purposes (Ross et al., 1994; Theofania et al., 2011; Antonios et al., 2011). More recent, predictive microbiology has been used to forecast the growth of spoilage microorganisms in order to predict the shelf life of a food product. The spoilage of food causes significant economic losses. Although, industrial standards have been greatly improved in the last years, food spoilage which is caused by SSO is still a major concern for both food producers and regulatory agencies (Panagou et al., 2009).

At present, the main microbial growth predictive model given by research at home and abroad is microbial growth kinetics model. In many cases, the growth of micro-organism can be described by a growth curve which consists of three parts: Lag phase, exponential phase and stationary phase. In recent years, the main models used in microbial growth include linear model, Logistic equation, Gompertz model, Baranyi model, etc, in particular, Baranyi model is widely used in microorganism research because of four reasons: ease of use; dynamic environment used; suitable for a variety of circumstances; model parameters having physiological significance. A research on tilapia had established growth kinetics model of specific spoilage organisms and established a shelf life prediction model (Xu et al., 2005). Food College of Tasmania University invented Food spoilage predictor based on *Pseudomonas* growth kinetics model.

Other models also were used to establish microbial models. Neural network was applied as a non-linear modeling technique in food mycology. It was found that neural networks offered an alternative and powerful technique to model microbial kinetic parameters and could thus become an additional tool in predictive mycology and it could build up a model of the joint effect of water activity, pH level and temperature (Panagou *et al.,* 2009). But neural network model had bigger error than other microbial growth-predictive model. Bayesian modeling of *Clostridium perfringens* growth in beef-in-sauce products was established. A Bayesian approach was proposed to model the overall uncertainty regarding parameters during the germination, outgrowth and lag phase (Jaloustre et al., 2010). The microbial growth-predictive model based on Bayesian model was cockamamie.

The aforementioned models assumed that the growth of microorganism had a regular growth pattern and experiment data were relatively complete.

This study used CHAID method to establish growth model based primarily on data without assuming growth regular and complete data.

This study attempted to introduce CHAID model and microbial growth kinetics model to fit microorganism growth and compared the differences of the two models. Taking tilapia for example, firstly, its specific spoilage microorganisms were identified and recognized; secondly, CHAID and Baranyi models were used to process experimental data and fit the growth of specific spoilage organisms; thirdly, two models were compared and evaluated.

MATERIALS AND METHODS

Bacteria identification and data acquisition

Samples

The samples of tilapia were captured in Fujian Province of China, and cold shocked in ice water. Selected individuals basically of the same size (300 ~ 400 g/tail) were brought into the laboratory at the temperature between 0 - 1℃.

Isolation of bacteria

Bacterial strains were isolated from the intestine of tilapia with aseptic operation at each sampling. The bacterial colonies were divided into different types according to the colony characteristics of shape, size, elevation, structure, surface, edge, color and opacity, and the number of colonies of each recognizable type was counted. Three to five representatives of each colony type were then streaked on additional plates repeatedly until pure cultures were obtained (Chen et al., 2011).

Biochemical identification

By using various substances, different microorganisms were able to produce different metabolites because of different metabolism types and enzymes. Therefore, patterns or other features could be identified by specific biochemical reactions. Microbial biochemical reaction is an important basis for classification and identification. This study used common bacteria identification handbook - Berger's classification system to preliminarily identify microorganisms.

Pseudomonas identification combination: Ribose, erythritol, inositol, xylose, glucose and urine acid.

Vibrio identification combination: Inositol, mannitol, sorbitol, salicin, rhamnose, xylose, melibiose, lactose, galactose, arabinose, mannose, glucose, peptone water, hydrogen sulphide, amylase, urine acid dehydrogenase, lysine dehydrogenase, arginine dehydrogenase, 0% NaCl peptone water, 3% NaCl peptone water, 6% NaCl peptone water, 8% NaCl peptone water and 10% NaCl peptone water.

Enumeration of microorganisms

One species of *Pseudomonas* and *Vibrio* were chosen, stored at 5 and 10℃ and inoculated two kinds of bacteria per 24 h. All the inoculated plates were incubated at 30℃ for 48 h and colony

forming units (cfu) were counted with a Quebec Darkfield Colony Counter (Leica, Inc., Buffalo, New York) equipped with a guide plate ruled in square centimeters. The temperature and incubation time used were found to be suitable for the growth of the investigated bacteria. Readings obtained with≥30 to 300 colonies on a plate were used to calculate bacterial population numbers, recorded as cfu per unit of sample (Ahmed et al., 2005).

The growth models of microorganism

Baranyi model

The Baranyi model (Baranyi et al., 1994) is used to fit the microbial growth at constant temperature conditions. The formula is

$$y(t) = y_0 + \mu_{\max} F(t) - \ln\left(1 + \frac{e^{\mu_{\max} F(t)} - 1}{e^{(y_{\max} - y_0)}}\right) \quad (1)$$

where

$$F(t) = t + \frac{1}{v}\ln\left(e^{-vt} + e^{-h_0} - e^{(-vt-h_0)}\right)$$

Where, y(t) is the number of microorganisms (ln cfu / g) at the time t; y_0 is the number of microorganisms (ln cfu / g)at the time 0; y_{max} is the maximum cell number (ln cfu / g); μ_{max} is the maximum specific growth rate; vis the growth rate of the microorganism in the limited substrate, assumed to be a constant and h_0 is equal to $\mu_{max}\lambda$.

The parameter h_0 should be approximately constant in situations where the pre-inoculation history of the cells was identical according to Baranyi and Roberts. In reality, this parameter varied. Therefore, the growth data at each temperature was first fitted with the Baranyi model to obtain four parameters y_0, y_{max}, h_0, and μ_{max}. A mean value for the parameter h_o for all growth curves was determined. After the mean value of h_0 was determined, the growth data were fitted again with the Baranyi model to obtain the values of y_0, y_{max}, and μ_{max} for each growth curve, after fixing the value of h_0 with the mean value, ħ (Vijay et al., 2009).

CHAID model

CHAID which is short for chi-square automatic interaction detection is a method with classification tree (Chae et al., 2001). It is initially used for market research and is a relatively new method in data classification. The three methods, RFM, CHAID and logistic regression had been compared and found that CHAID method was the most effective to deal with defective data (McCarty et al., 2007).

The decision tree of CHAID shows and expounds interrelations between a given variable of interest (target variable) and a chosen set of predicting variables as dendrograms. In order to be handled like ordinary scaled variables, metrically scaled variables have to be grouped into intervals. The response variable can be of any scale type. The interrelations between the response variable and the respective predictor variables are determined by methods of inferential statistics. A null hypothesis is set up assuming that there is no relations between response and predictor variable. The probability of error (*p*-level) is determined according to the scale type of the response variable. The algorithm merges the characteristics of the predictor variables in a set of as few as possible statistically different classes which are in turn ranked on the basis of their statistical association with the response variable to build the model tree. In this way, that predictor with the lowest *p*-level is chosen to split the sample into the respective subgroups (Magidson et al., 1993;Mevlut, et al., 2009). The statistical calculations were done with SPSS clementine 12.0. The CHAID method has three steps:

a. Select the experiment data of microorganism growth, and then cross-classify the explanatory variable (time) and the target variable (microorganism number) to generate a series of two-dimensional table.
b. Calculate chi-square (χ^2) statistic or the likelihood statistic of the two-dimensional table to compare the statistical p-value. Use the largest statistical p-value of two-dimensional table as the best initial classification and use the explanatory variables classify target variables continuously based on the best classification.
c. Repeat the process of the previous step until the classification conditions that p-value is bigger than the split level (α_{split}).

Evaluation methods of growth models

The regression coefficient can reflect whether the model fits the growth of microbiology or not. It is used as an overall measure of the predict level. The higher its value ($0 < R^2 < 1$), the more accurate of predict model.
Mean square error (MSE) is used to measure the change of numerical value. It is equal the square of the remaining value dividing degree of freedom. The lower its value (0 < MSE), the more accurate of predict model.

$$MSE = \frac{\sum \left(\mu_{observed} - \mu_{predicted}\right)^2}{n} \quad (2)$$

N stands for the number of measurements; $\mu_{observed}$ stands for the measured value and $\mu_{predicted}$ stands for the predicted value.
Bias factor indicates the distance between numerical value and the curve. The higher its value (*BF*<1), the more accurate of predict model.

$$bias\ \ factor = 10^{\left(\frac{\sum \log\left(\frac{\mu_{observed}}{\mu_{predicted}}\right)}{n}\right)} \quad (3)$$

Accuracy factor means the distance between numerical value and the equivalent line. The lower its value (1<*AF*), the more accurate of predict model.

$$\text{accuracy}\ \ factor = 10^{\left(\frac{\sum \log\left|\frac{\mu_{predicted}}{\mu_{observed}}\right|}{n}\right)} \quad (4)$$

RESULTS AND DISCUSSION

The results of physiological and biochemical identification

All the purified isolates were observed for cell shape, motility, flagellation, spores and Gram staining. The isolates were then subjected to biochemical tests following the criteria described in the common bacteria identification handbook for identification to genus or species level. The results are as follows:

Table 1 shows 6 conventional biochemical reactions.

Table 1. Biochemical identification results of *Pseudomonas.*

Item	1	2	3	4	5	6	7
Ribose	+	+	+	+	+	+	+
Red Xianchun	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-
Xylose	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Urine Acid	+	-	-	+	+	-	+

+ stands for positive reaction, - stands for negative reaction. / stands for having no biochemical experiments.

Table 2. Biochemical identification results of *Vibrio.*

Item	1	2	3	4	5	6	7	8	9	10	11	12
Inositol	+	+	+	+	+	+	+	+	+		+	+
Mannitol	+	+	+	+	+	+	+	-	+	+	+	+
Sorbitol	+	+	-	-	-	-	-	+	+	+	+	+
Salicin	+	/	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	/	-	-	-	-	-	+	+	+	+	/
Xylose	/	/	+	-	-	/	+	-	+	-	+	/
Melibiose	-	/	-	-	-	/	-	-		/		/
Lactose	+	+	+	-	/	+	+	+	+	-	+	+
Galactose	+	/	-	-	-	-	-	/		-	-	-
Arabinose	+	+	+	-	-	-	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Peptone Water	+	/	+	+	+	+	+	+	+	+	+	+
Hydrogen Sulphide	+	+	+	-	-	+	+	+	+	+	+	+
Amylase	-	/	+	/	-	/	-	-	+	+	+	+
Urine Acid Dehydrogenase	+	-	-	-	-	-	+	+		-	-	-
Lysine Dehydrogenase	-	+	-	+	+	+	+	+	+	+	+	+
Arginine Dehydrogenase	-	-	+	-	-	+	+	-	-	+	+	-
0%NaCl Peptone Water	+	+	+	+	+	+	+	+	+	+	+	+
3%NaCl Peptone Water	+	+	+	+	+	+	+	+	+	+	+	+
6%NaCl Peptone Water	+	+	+	+	+	+	+	-	+	-	+	/
8%NaCl Peptone Water	+	+	-	+	+	+	+	+	+	+	-	+
10%NaCl Peptone Water	+	+	-	-	+	+	+	+	-	+	-	-

According to the results of the biochemical reactions, 7 strains of *Pseudomonas* are identified by checking with the standard in "common bacteria identification handbook ".Table 2 shows 23 conventional biochemical reactions. According to the results of the biochemical reactions, 12 species of *Vibrio* are identified by checking with the standard in "common bacteria identification handbook".

One species of *Pseudomonas* and *Vibrio* are chosen to establish microbial models about CHAID and Baranyi model.

The results of Baranyi model

SPSS 16.0 was used to analyze the experiment data. The estimated values for the parameters in the Baranyi model are shown in Table 3. The number of initial bacteria is different at lag phase for different temperatures, but it is basically constant at stationary phase. Different bacteria have different maximum number of bacteria. The maximum specific growth rate increases with the increasing of temperature. v has little change because the medium

Table 3. The estimated parameters in the Baranyi primary model.

Tem	y_0	y_{max}	μ_{max}	v	h_0
Pseudomonas at 5℃	5.951	20.445	0.021	0.162	2.944
Pseudomonas at 10℃	6.973	21.241	0.196	0.156	2.721
Vibrio at 5℃	9.022	19.042	0.039	0.141	2.509
Vibrio at 10℃	7.663	19.863	0.152	0.145	2.627

y_0 is the number of microorganisms (ln cfu / g)at the time 0; y_{max} is the maximum cell number (ln cfu / g); μ_{max} is the maximum specific growth rate; v is the growth rate of the microorganism in the limited substrate, assumed to be a constant and h_0 is equal to $\mu_{max}\lambda$.

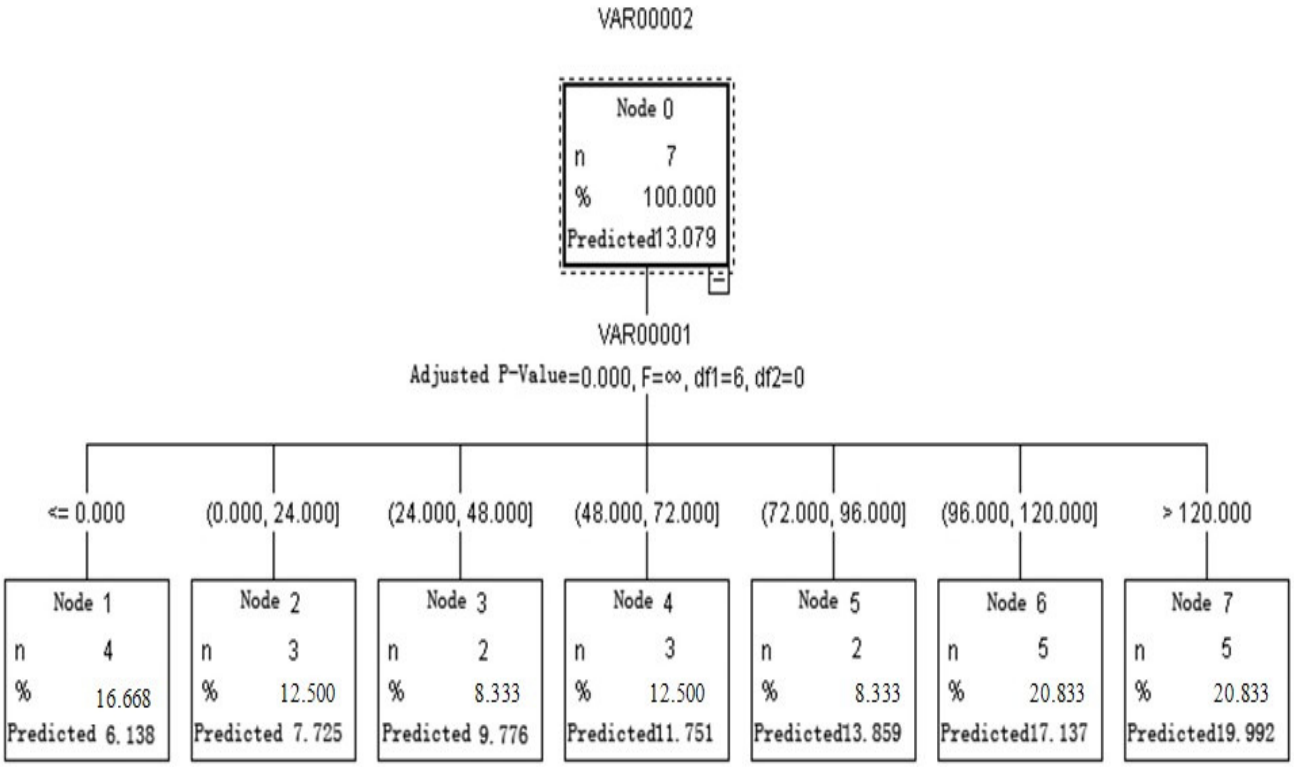

Figure 1. The estimated values in the CHAID model of *Pseudomonas* at 5℃.

supply sufficiently in the experimental conditions. h_0 which is related to the critical substance necessary for growth is constant because the critical substance is abundant at experimental conditions.

The results of CHAID model

SPSS Clementine12.0 was used to analyze the experiment data, the results were as follows:

Adjust p-value, F and df stand for test statistic in the Figures 1-4. N stands for the number of measurements, *%* stands for the percentage. *Predicted* stands for predictive value.

Taking Figure 1 for example, the grid *N* stands for the total measurements. *Predicted* stands for the overall predictive value. The following grid *n* stands for the measurements of each class. *%* stands for the measurements percentage of each class in total measurements. Predicted stands for predictive value after the measured

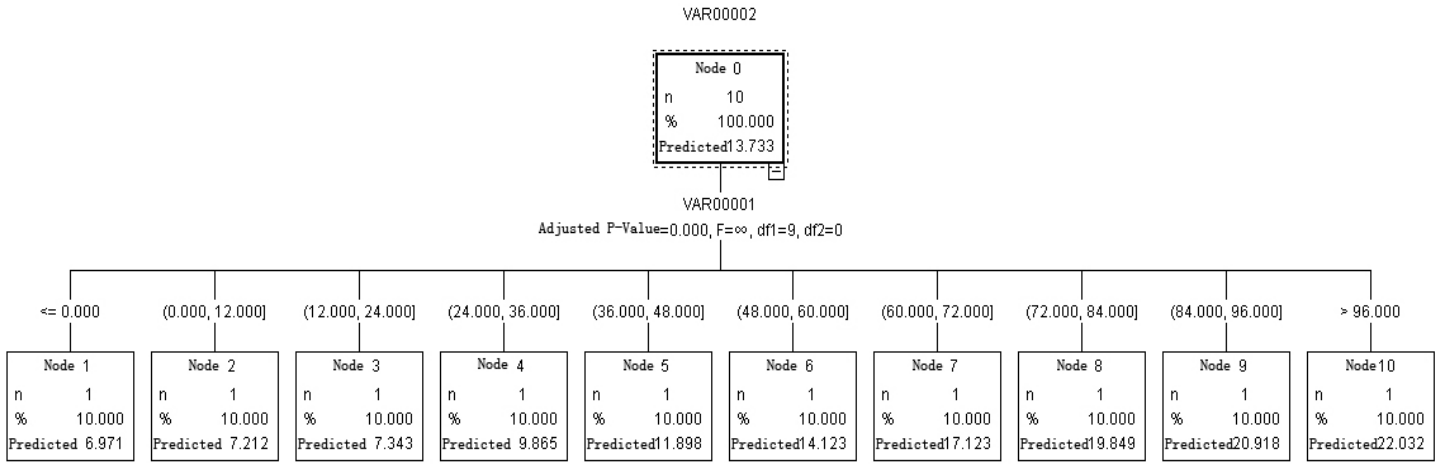

Figure 2. The estimated values in the CHAID model of *Pseudomonas* at 10℃.

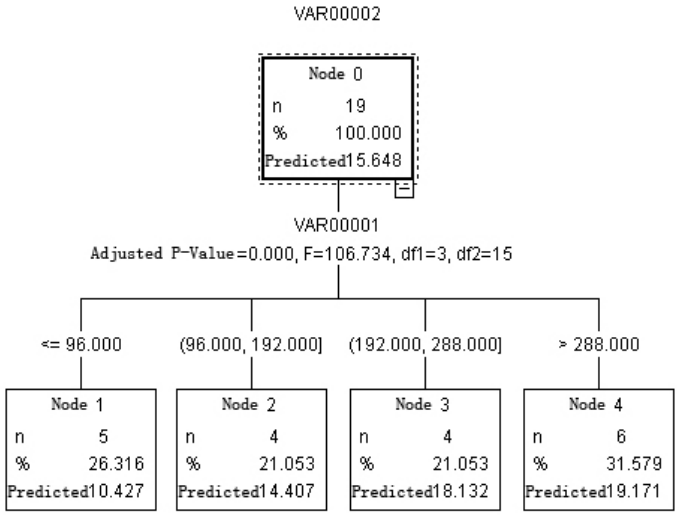

Figure 3. The estimated values in the CHAID model of *Vibrio* at 5℃.

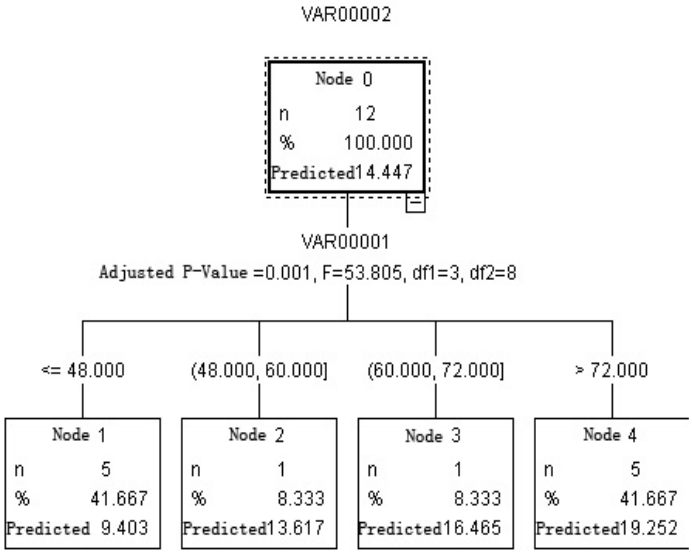

Figure 4. The estimated values in the CHAID model of *Vibrio* at 10℃.

values are classified calculated.

The comparing of the growth curves about CHAID and Baranyi models

Baranyi model and CHAID model both have the ability to predict the microbial growth, but Baranyi model fits the curve better than CHAID model overall. Baranyi model has better fitting ability for growth of microorganisms at both lag phase and exponential phase than CHAID model which somewhat fits deviant; however, Baranyi model does not fit accurately at stationary phase. CHAID model forms trapezoidal curve, have large deviations and predict the scope of microorganism. As a mechanistic model, Baranyi is more versatile and capable of fitting curves without stationary phase. CHAID model is more accurate at stationary phase (Figures 5-8).

The reliability of growth model

Tables 4 and 5 shows the values of the regression coefficient (R^2), mean square error (*MSE*), accuracy factor (*AF*), and bias factor (*BF*), obtained from the experimental models based on the CHAID and the Baranyi models.

Tables 4 and 5 shows that Baranyi model is better to fit microbial growth than CHAID model because evaluating index (R^2, *MSE, BF* and *AF)* values of Baranyi model are better than CHAID model. But it is opposite at 10℃ for the growth of *Pseudomonas* since the rate of change is bigger.

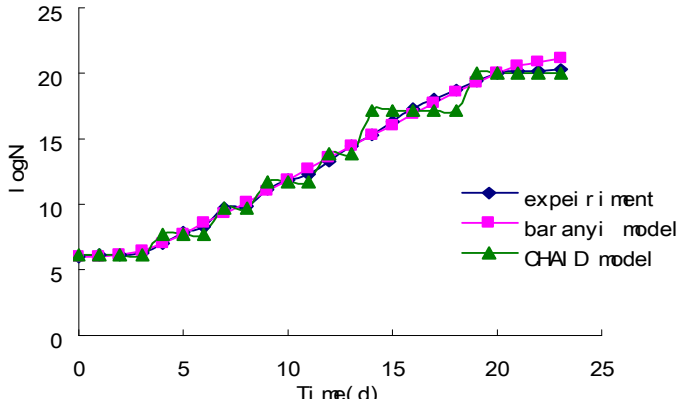

Figure 5. Predictive values of Baranyi model, predictive values of CHAID model compared with measured values of *Pseudomonas* at 5℃.

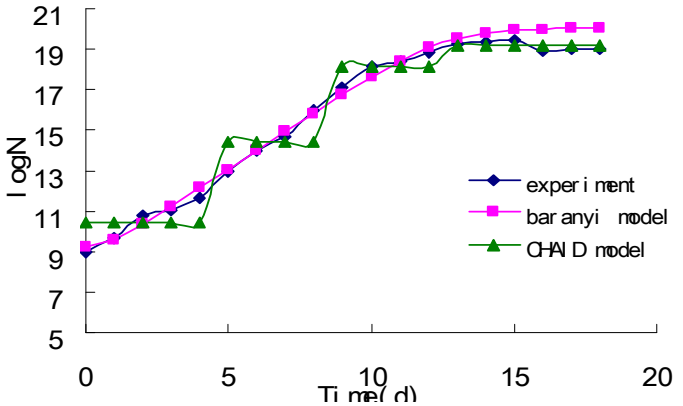

Figure 6. Predictive values of Baranyi model, predictive values of CHAID model compared with measured values of *Vibrio* at 5℃.

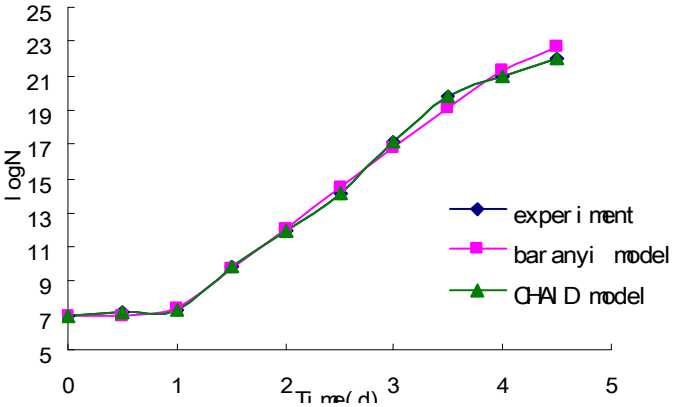

Figure 7. Predictive values of Baranyi model, predictive values of CHAID model compared with measured values of *Pseudomonas* at 10℃.

The prediction model is more accurate when the experiment data is more. Comparing the observations and the growth curve, Baranyi model has good prediction ability at lag phase and exponential phase, while CHAID model fits very well at stationary phase. Overall, Baranyi model is better than CHAID model, considering Baranyi model fitted well but CHAID model has large deviations at the exponential phase.

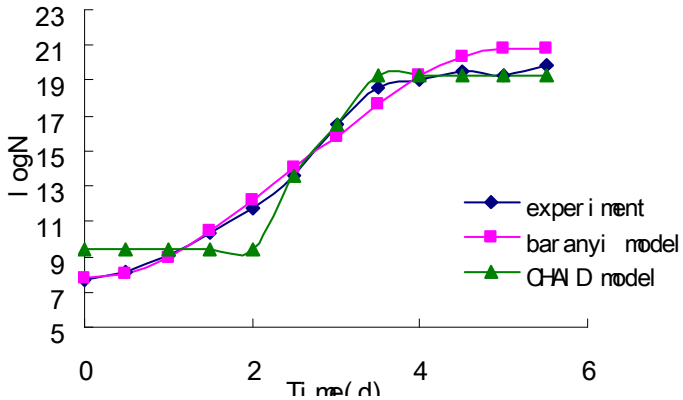

Figure 8. Predictive values of Baranyi model, predictive values of CHAID model compared with measured values of *Vibrio* at 10℃

Conclusion

The results of this study demonstrate that *Vibrio* and *Pseudomonas* are the SSO of Tilapia. They shorten the shelf life of Tilapia at normal temperature seriously. The growth curve has different lag phase, exponential phase and stationary phase for different microorganism at different temperatures. Baranyi model and CHAID model can primarily predict the growth conditions of micro-organisms. Baranyi model has better fitting ability at lag phase and exponential phase than CHAID model. CHAID model has a good fitting at stationary phase though it is not as accurate as Baranyi model. CHAID model predicts the microorganism growth very well when the rate of change of the experiment data is big.

Further research advice: combination of predicting model containing advantages of two models is establishes, which can predict the growth of SSO better; It's difficult to predict microbial growth in actual production, logistic, storage and consumption based on constant temperature because temperature is fluctuant so randomly in actual sense that we cannot directly use mathematics to describe the variation of temperature. So, it is very important to establish the growth model at variable temperature.

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Table 4. *Pseudomonas* evaluation results.

Parameter	R^2	MSE	BF	AF
5℃ Baranyi model	0.975	0.059	0.998	1.014
5℃ CHAID model	0.865	0.315	0.999	1.033
10℃ Baranyi model	0.996	0.237	0.996	1.022
10℃ CHAID model	0.994	0.001	0.998	1.002

R^2, Regression coefficient; MSE, mean square erroe; AF, accuracy factor; BF, bias factor.

Table 5. *Vibrio* evaluation results.

Parameter	R^2	MSE	BF	AF
5℃ Baranyi model	0.981	0.255	0.922	1.024
5℃ CHAID model	0.955	0.596	0.908	1.046
10℃ Baranyi model	0.978	0.483	0.987	1.033
10℃ CHAID model	0.953	1.022	0.915	1.069

R^2, Regression coefficient; MSE, mean square erroe; AF, accuracy factor; BF, bias factor.

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