

Research Article

Formulation Optimization of a Composite Biological Preservative for *Scophthalmus maximus*

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Abstract: Based on the study of single biological preservative, a composite biological preservative composed of pullulan, ϵ -polylysine and protamine was developed using orthogonal array design. The quality of *Scophthalmus maximus* during cold storage was evaluated based on the parameters of Total Bacterial Counts (TBC), pH, Total Volatile Basic Nitrogen (T-VBN) and Trimethylamine (TMA). The significance of various factors on cold storage was in the order of pullulan, ϵ -polylysine and protamine and the optimum ratio was 1.5% pullulan, 0.6% ϵ -polylysine and 0.6% protamine. The *Linnaeus* treated with this biological preservative of storage at (4 \pm 1) $^{\circ}$ C showed better results of TBC, pH, T-VBN and trimethylamine than the untreated control group. The shelf-life of *Linnaeus* was prolonged for 4~5 days, indicating a good fresh-keeping effect.

Keywords: Evaluation, prediction models, *Scophthalmus maximus*, shelf life

INTRODUCTION

Scophthalmus maximus (Linnaeus) belongs to *Pleuronectiformos*, *Bothidae*, *Scophthalmus* and the English name is turbot, commonly known as the European flounder, it is one of the world recognized quality flounder. *Scophthalmus maximus* subcutaneous tissue and fins are rich in gelatine, head and tail fin are small, fin for cartilage; body without the ossicles, visceral mass is small, with high rate of meat and white muscle. *Scophthalmus maximus* is not only rich in amino acids, but also rich in Highly unsaturated fatty Acids (DHA and EPA). It is a kind of sea-fish which benefits to the human heart, delay aging and promote children growth (Jilin *et al.*, 2008). In addition, being rich in major and trace elements, such as calcium, magnesium, iron, zinc, copper, manganese, chromium and selenium, it is also beneficial to the human body growth and health care (Wang *et al.*, 2003). As a result of high moisture and protein content, it is easy to grow microorganisms and cause spoilage during storage and transportation of the *Scophthalmus maximus*, which seriously restricted the production.

Biological preservative refers to a class of substances obtained from the extraction of plant, animal and microorganisms. It can be achieved naturally or using biological engineering technology. Pullulan is a kind of edible packaging material. It has been widely used in food preservation for its non-toxic, colorless and tasteless, film forming properties, gas barrier properties, plasticity, soluble in water and some other characteristics (Xu *et al.*, 2003; Leather, 2003; Cheng *et al.*, 2012; Zhang *et al.*, 2011). ϵ -polylysine was

approved by the FDA for its safety as food preservative in October, 2003. It has many advantages in the field of preservatives including high safety, broad antimicrobial spectrum, good water solubility and good thermal stability and it has been widely applied for food preservation in many countries (Hiraki *et al.*, 2003; Muto, 2009; Yu *et al.*, 2007). Protamine is a natural preservative, which has the advantages of high safety, strong antibacterial ability, wide antibacterial spectrum and good stability and has attracted attentions in recent years (Li *et al.*, 2007; Hu *et al.*, 2011).

In view of perishable problem of *Scophthalmus maximus*, this work optimized a composite biological fresh-keeping agents based on the study of single chemical preservative, which provided some basic reference for its further application and substitution for biological preservative.

MATERIALS AND METHODS

Materials: *Scophthalmus maximus*: Purchased from Qinhuangdao market, fresh, about 1.5 kg for each.

Sodium chloride (AR), Methylene blue (AR), Tianjin Kermel Chemical Reagent Co., Ltd.; Anhydrous ethanol (AR), Trichloroacetic acid (AR), Anhydrous potassium carbonate (AR), Tianjin Kaitong Chemical Reagent Co., Ltd.; Boric acid (AR), Methyl red (AR), Tianjin Chemical Reagent, Department 3; Plate count agar, Beijing Luqiao Technology Co., Ltd.; Protamine (99%), Pullulan (99%), ϵ -polylysine, Shanghai Quangwang biological science and Technology Co., Ltd. Tissue triturator, Jiangsu Jintan Yitong Electronics Co., Ltd.; Biochemical incubator (YLD-6000), Jiangsu

Table 1: Orthogonal test factors level table

Level	Factor/%		
	Pullulan /A	ε-polylysine/B	Protamine/C
1	1.0	0.2	0.4
2	1.5	0.4	0.6
3	2.0	0.6	0.8

Dingtian Honghua Instrument; Acidity meter (PHS-3C), Shanghai Precision Scientific Instrument Co., Ltd.; Aseptic operation table (SW-CJ-2F), Suzhou Antai Air Technology Co., Ltd.

Test methods: Experimental of raw materials: *Scophthalmus maximus* were killed immediately after they were shipped to the laboratory. Then the lab assistant washed it cleanly, took out the meat under room temperature, cut the meat into blocks of uniform size, washes them with saline, drained and randomly divided into two groups. One group was soaked for 10 min in the composite preservative and the other group was dipped in physiological saline as blank control. After taken out and drained at room temperature, they were immediately loaded into a sterile fresh-keeping bag (120 g/bag) and stored in the freezer with the temperature of at (4±1)°C. The TBC, pH, T-VBN and TMA were determined every other day and each sample was measured 3 times in parallel.

Selection of optimum ratio of the composite biological preservative: Pullulan, ε-polylysine and protamine were selected for the preservation of *Scophthalmus maximus*. According to the results of the single factor test, 3 mass fractions were selected for each kind of preservative for the L₉ (3³) orthogonal test, the optimum compound ratio was selected depending on the result of variance analysis. The Orthogonal test factors and levels are shown in Table 1.

Determination of total bacterial counts: National food safety standard GB/T 4789.2-2010 «Food

microbiological examination: Aerobic plate count», Pour plate method, 3 parallel for each dilution.

pH determination: Method refers to Xie and Yang (2011) with a few modifications. Briefly, an amount of *Scophthalmus maximus* meat was triturated with a tissue triturator. Then 5 g of the sample was transferred into a conical flask, 45 mL distilled water was added, static for 30 min after homogeneous, then filtering and the pH value was finally determined using a precision acidity meter.

Determination of T-VBN: National food safety standard GB/T5009.44-2003 «Method for analysis of hygienic standard of meat and meat product» .

Determination of TMA: Method refers to Zhao (1986).

Data processing: Drawing with Excel and data analysis using SPSS 17.0 software. Data analysis between groups was performed by t-test, when p<0.01 indicating extremely significant differences, 0.01<p<0.05 for the significant difference and when p>0.05 there is not significant difference.

RESULTS AND DISCUSSION

Optimum ratio of the composite biological preservative: The L₉ (3³) orthogonal test results of the sixth day for *Scophthalmus maximus* cold storage are shown in Table 2. The antibacterial effect assessed by TBC was in the following order: A>B>C, namely the pullulan, ε-polylysine and protamine. The composition of A₂B₃C₃ gave a best antibacterial effect. As to the TVB-N content, the effect was in the order of A>B>C, namely the pullulan, ε-polylysine and protamine and the best composition was A₂B₃C₂. From Table 3 we can see that pullulan and ε-polylysine have significant

Table 2: Results of *Scophthalmus maximus* fresh-keeping orthogonal experiment L₉ (3³) on the 6th day

	A	B	C	TBC/lg (CFU/g)	TVBN/mg/100 g)
1	1	1	1	6.87	26.89
2	1	2	2	6.93	25.82
3	1	3	3	5.94	25.78
4	2	1	2	6.06	22.61
5	2	2	3	5.15	22.46
6	2	3	1	5.03	21.17
7	3	1	3	5.67	24.68
8	3	2	1	5.98	23.28
9	3	3	2	5.52	22.59
TBC					
K ₁	19.74	18.61	17.94		
K ₂	16.24	18.07	18.51		
K ₃	17.17	16.55	16.76		
R	3.50	2.06	1.75		
T-VBN					
K ₁	78.49	74.18	71.34		
K ₂	66.24	71.56	71.02		
K ₃	70.55	69.54	72.92		
R	12.25	4.64	1.90		

Table 3: Variance analysis of *Scophthalmus maximus* fresh-keeping orthogonal experiment $L_9(3^3)$ on the 6th day

	Parameter	S.S.	df	M.S.	F	p
A	TBC	2.128	2	1.064	62.129	0.016
	T-VBN	25.742	2	12.871	1589.04	0.001
B	TBC	0.757	2	0.379	22.101	0.043
	T-VBN	3.608	2	1.804	222.733	0.004
C	TBC	0.532	2	0.266	15.527	0.061
	T-VBN	0.690	2	0.345	42.584	0.023
Deviation	TBC	0.034	2	0.017		
	T-VBN	0.016	2	0.008		

S.S.: Sum of square; M.S.: Mean square

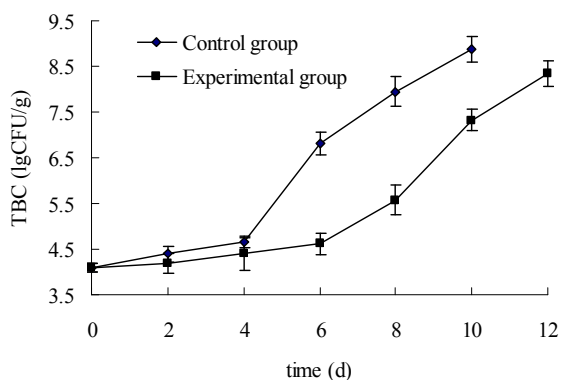


Fig. 1: Changes of TBC of *Scophthalmus maximus* during storage at $(4\pm 1)^\circ\text{C}$

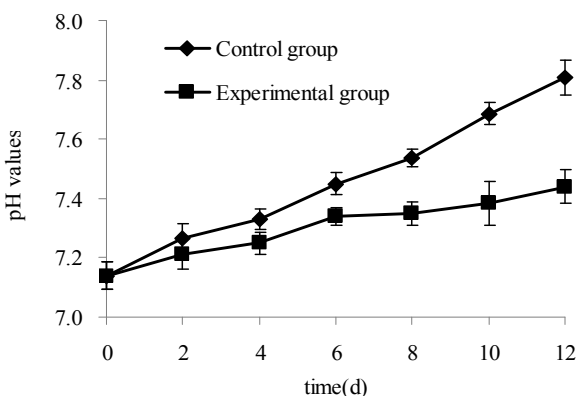


Fig. 2: Change of pH of *Scophthalmus maximus* during storage $(4\pm 1)^\circ\text{C}$

effects on TBC ($p < 0.05$) and the effect of protamine was not significant ($p > 0.05$); pullulan, ϵ -polylysine and protamine all have significant effects on T-VBN content significantly ($p < 0.05$). Since the optimal compositions were not accordant according to indicators analyzed separately, a comprehensive consideration according to the significance of different parameters and the optimum composition of the biological preservative was selected, which is $A_2B_3C_2$, namely, the optimum ratio of composite biological antistaling agent is pullulan 1.5%, ϵ -polylysine 0.6% and protamine 0.6%.

Changes of TBC of *Scophthalmus maximus* during cold storage: There is a very close relationship

between the microorganisms and the spoilage of aquatic products, that is the number of bacteria can reflect the degree of decay of aquatic products. The determination of TBC was used to determine the degree of food bacterial contamination. It reflects whether the food hygienic conditions meet the requirements of food production, so as to make an appropriate hygienic evaluation for the detected sample and it directly reveals changing process of *Scophthalmus maximus*. Change of TBC of *Scophthalmus maximus* during storage is shown in Fig. 1, in which the TBC of control group and experimental group both showed a rising trend as the storage time went, but the TBC of the experimental group were lower than that of control group. In the condition of $(4\pm 1)^\circ\text{C}$, storage time and the composite biological preservative, both have significant effects on the total number of *Scophthalmus maximus* TBC ($p < 0.05$). The TBC of the control group on the sixth day were 6.82 Lg (CFU/g) and were 8.87 Lg (CFU/g) on the tenth days. The value of the experimental group on the sixth days was only 4.61 Lg (CFU/g) and 7.31 Lg (CFU/g) on the tenth day. The composite biological preservative can obviously inhibit the growth of bacteria and keep the *Scophthalmus maximus*.

Changes of pH values of *Scophthalmus maximus* during cold storage: After the death of animal, protein decomposes as time prolonging and alkaline compounds are generated, thus a pH value rises (Zhao, 1986). Changes of pH of *Scophthalmus maximus* during storage was shown in Fig. 2. PH values of the control group and experimental group both raised as time prolonging, but the pH of the experimental group was lower than the pH of the control group. In the condition of $(4\pm 1)^\circ\text{C}$, storage time and the composite biological preservative, both have significant effects on pH ($p < 0.05$). The pH of the control group on the eighth day was 7.53 and the pH of the experimental group was 7.35 on the eighth day and 7.44 on the 12th day. Therefore, the biological preservative is very effective in reducing the rate of decay.

Changes of T-VBN of *Scophthalmus maximus* during cold storage: T-VBN is alkaline nitrogen compounds (ammonia, primary amine, *et al.*) generated by protein decomposing in storage of animal food, which is conducted by mildew and bacteria, thus

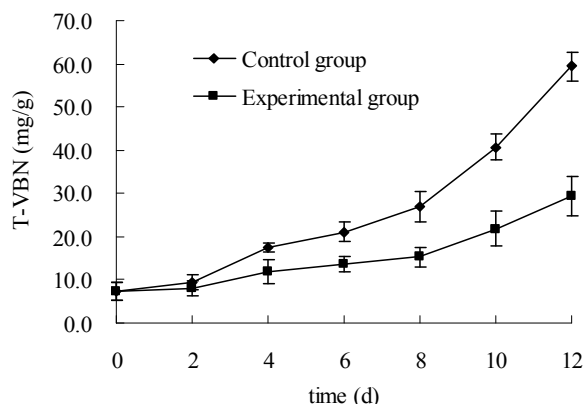


Fig. 3: Changes of T-VBN of *Scophthalmus maximus* during storage (4±1)°C

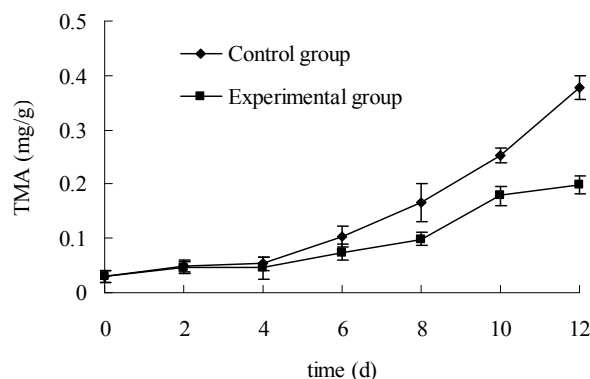


Fig. 4: Change of TMA of *Scophthalmus maximus* during storage at (4±1)°C

T-VBN is also an index for the decay of aquatic products. Change of T-VBN of *Scophthalmus maximus* during storage was shown in Fig. 3. T-VBN of control group and experimental group both raised as time prolonging, but T-VBN of the experimental group was lower than T-VBN of the control group. In the condition of (4±1)°C, storage time and the composite biological preservative, both have significant effects on T-VBN ($p < 0.05$). The T-VBN of the control group on the fourth day was 17.49 mg/100 g and was 21.49 mg/100 g on the 6th day, nearing the limit of the second freshness. But T-VBN of the experimental group was 21.83 mg/100 g on the 10th day. Therefore, the composite biological preservative can obviously inhibit the growth of bacteria and protein decomposing, making the growth rate of TVB-N in the experimental group was obviously lower than the control group.

Changes of TMA value of *Scophthalmus maximus*: Trimethylamine oxide in sea-fish was reduced to TMA by bacteria during the storage. TMA is a kind of volatile amines and can be used as an indicator of fish spoilage. With the extension of storage time, fish quality change can be assessed based on the TMA

value, which can be accumulated in fish (López-Caballero *et al.*, 2002). Change of TMA of *Scophthalmus maximus* during storage at (4±1)°C is shown in Fig. 4. The TMA values of the control group and experimental group both showed a rising trend as the extension of storage time and the value of the control group increased quicker than the experimental group. In the condition of (4±1)°C, storage time and the composite biological preservative, both have significant effects on the TMA value of *Scophthalmus maximus* bacterial counts ($p < 0.05$). The TMA value was 0.165 mg/100 g on the 8th day and this is much higher than the value of experimental group, which was 0.098 mg/100 g.

CONCLUSION

The optimum ratio of composite biological preservative, pullulan 1.5%, ε-polylysine 0.6% and protamine 0.6%, were selected based on the L₉ (3³) orthogonal test. The preservative can obviously inhibit the increases the TBC, T-VBN and TMA values and the shelf-lives were prolonged from about 5 to 4-5 days. The fresh-keeping effect of the proposed composite biological preservative is better than that of the single preservative. The composite biological preservative expands the antimicrobial spectrum compared to the single one and it has no residue of toxic substances. It was proved to be a safe and high-efficient preservative with good development prospect.

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