

## Development of freezing-point regulators for cultured large yellow croaker

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Received: 19 December 2011 / Accepted: 22 May 2012  
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### RESEARCH PAPER

#### Abstract

The influence of temperature, freezing medium, water content and size of fish cakes on the freezing process of large yellow croaker (LYC) were investigated systematically. Then the effects of freezing point regulators such as sodium chloride, sucrose, sorbitol, vitamin C and glucose were analysed by response surface methodology. The variables ranges used according to the single factor experiment were 3-7% sodium chloride (w/w), 5-9% sorbitol (w/w) and 2-3% sucrose (w/w) and the responses were optimised by a numerical method and were found to be 5.16% sodium chloride (w/w), 7.15% sorbitol (w/w) and 2.96% sucrose (w/w). All the derived mathematical models for the responses were found to be a significant fit for the predicted data. The results show the freezing point of LYC decreased from -1.5 to -4.2 °C after adding the optimised freezing point regulators, and the texture parameters were not significantly affected in comparison with the control group.

**Keywords:** freezing point control, ice-temperature preservation, large yellow croaker, mathematical models

#### 1. Introduction

Large yellow croaker (LYC) (*Larimichthys crocea*) belongs to the family *Perciformes* and the genus *Sciaenidae*, and is one of the principal fish cultivated throughout the world for its fleshy edible meat and delicate flavour (Chen *et al.*, 2003; Wang *et al.*, 2009). It is used for human consumption as well as for health care (Xu *et al.*, 2004). LYC is rich source of proteins and contains appreciable amounts of selenium and vitamins. Selenium is the most active form of trace elements, which can clean the free radicals produced by human body metabolism, has anti-aging properties and can control efficacy for various cancers as well as having a beneficial effect on humans (Shi *et al.*, 2002; Ying *et al.*, 2009; Zhang *et al.*, 2006).

With the development of offshore net-gage cultivation, the quality of mariculture fish has greatly improved. Both the nutrition and meat quality of LYC cultured by offshore net-gage cultivation are close to that of wild LYC (Arkoudelos *et al.*, 2007; Rosnes *et al.*, 2006). In the fish preservation field, we still use traditional freezing methods for storing the fresh fish (Fernandezak *et al.*, 2009; Magnussen *et al.*,

2008). Although we achieve a long preservation period, there is a loss of nutrition after thawing and the needs of consumers pursuing fresh fish products cannot be met. In the 1970s, Shan and Zhang (1976) proposed the ice-temperature preservation (IPC) concept which was supported by further investigation. Subsequent research revealed that preserving food at ice temperature prevented the destruction of the cells, inhibited the activity of harmful microbes and prolonged the preservation period (Murase, 1994). IPC is a new storage technology, which stores the food around freezing point. On the present market, control accuracy designed for the processing industry is  $\pm 2$  °C but the previous experiments indicated that the freezing point zone of LYC is  $\pm 0.5$  around -1.5 °C (Han *et al.*, 2009; Lu *et al.*, 2007). This is narrow for IPC used in the LYC processing industry. Special IPC equipment is expensive and the running costs are high; most companies cannot afford to use it (Li *et al.*, 2008; Murase, 1994). There is significant value in enlarging the freezing point zone of LYC. In this research, we use various solutions to lower the freezing point of LYC in an effort to expand the storage temperature zone without ice crystal formation and to match the accuracy of control temperature in the industry.

## 2. Materials and methods

### Fish and reagents

Fresh samples of LYC were obtained from Zhoushan Marine Fisheries Aquaculture Companies of China, weighing  $280 \pm 15$  g. Sodium chloride, sucrose, sorbitol, vitamin C, glucose and other reagents were used as analytically pure.

### Main equipment

The following equipment was used: AR224CN precision balance from Ohuas Co., Ltd. (Shanghai, China); BZF-50 vacuum drying oven from Boxun Co., Ltd. (Shanghai, China); 539-WT refrigerator from Haier Co., Ltd. (Qingdao, China); MDF-U4186S ultra low temperature refrigerator from Sanyo Co., Ltd. (Tokyo, Japan); liquid quick freezing machine, self-made; JK-16U temperature recorder from Jking Co., Ltd. (Changzhou, China); HD-108 ice crusher from Hongda Co., Ltd. (Guangzhou, China); TMS-PRO food texture analyser from Food Technology Corporation (FTC), Ltd. (Sterling, VA, USA).

### Determination of large yellow croaker's freezing point

A schematic diagram of the experimental equipment is shown in Figure 1. Clean, sound LYC without any physical damage was selected. Three point metal shrouded hypodermic T-type probes were used for measuring the temperatures in the fore, middle and rear part of LYC. The sample was placed in an expanded polystyrene box, which was placed in a refrigerator under static conditions at  $-18$  °C. The freezing point is the temperature at which the slowest cooling rate is observed according to the typical cooling curves.

### Effect of different sizes on the freezing process

Clean, sound LYC without any physical damage was selected and the fish sample was cut into five fish cakes with  $1 \times 1$ ,  $2 \times 2$ ,  $3 \times 3$ ,  $4 \times 4$  and  $5 \times 5$  cm pieces. Freezing points were measured to discover the relationship between size and freezing point.

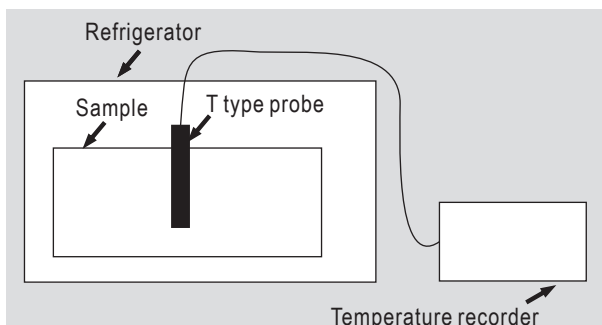


Figure 1. Schematic diagram of the experimental equipment.

### Effect of different water content on the freezing process

The treated fresh LYC was cut into five groups of  $2 \times 2$  cm pieces, then four groups of the fish cakes were put into the vacuum drying oven at  $30$  °C to let the relative water content decrease from 100% to 80%, 60%, 40% and 20%, respectively. Meanwhile, the freezing process of five fish cakes was observed under  $-18$  °C gas quick-frozen conditions. The calculation method of relative water content in fish cakes was as follows:

$$\text{relative water content} = b/a \times 100\%$$

Where a is the weight of fish cake before drying and b is the weight of fish cake after drying.

### Effect of different freezing temperature and freezing medium on the freezing process

The fish samples were cut into six groups with  $2 \times 2$  cm pieces. Then the freezing process of each group was observed at  $-18$ ,  $-25$  and  $-50$  °C under gas or liquid (100 g broken ice and 143 g  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ ) conditions, respectively.

### Development of freezing-point regulator for large yellow croaker

#### Experimental design

Response surface methodology was used in the experimental design and analysis, because of a wider range of different variables generally employed for the pre-treatment of LYC. A Box-Behnken experimental design with augmented points in three variables was used (Table 1) and the model is shown in Equation 1.

$$EY = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i < j=1}^3 \beta_{ij} X_i X_j + \sum_{i=1}^3 \beta_{ii} X_i^2 + \varepsilon \quad (1)$$

Where EY is the measured response and obtained according to Equation 2, it is the comprehensive effect,  $\beta_0$  is the intercept term,  $\beta_i$  and  $\beta_{ij}$  are the constant coefficients. The variables  $X_i$  and  $X_j$  represent the first-order interaction between  $X_i$  and  $X_j$  for  $j < i$ , and  $\varepsilon$  is the error term.

Table 1. Factors and levels in Box-Behnken experimental design.

Factor	Code	Level		
		-1	0	1
Sodium chloride	$X_{1(w/w)}$	3%	5%	7%
Sorbitol	$X_{2(w/w)}$	5%	7%	9%
Sucrose	$X_{3(w/w)}$	1%	3%	5%

$$EY = \frac{|\text{freezing point}| + \text{sensory evaluation scores}}{2} \quad (2)$$

### Single factor experiment

Different concentrations (1, 3, 5, 7, and 9%) of sodium chloride were added to the surface of the fresh LYC. After 3 h, the fish samples were placed in a refrigerator under static conditions at -18 °C and the temperature recorder was used to measure the freezing point in the geometric centre of LYC. Different freezing point regulators such as sucrose, sorbitol, glucose, vitamin C were used in the same way in the following groups. The cooling curve was observed to evaluate the freezing point regulators' ability to decrease the freezing point.

### Sensory evaluation

Sensory scores for the LYC were calculated using the quality index method shown in Table 2 by seven trained panellists from the laboratory staff. Each assessor scored for appearance, meat quality and flavour on a scale of 1 to a maximum of 8, where 8 represented the freshest quality and the scores decreased according to spoilage down to 1 for each parameter. Scores of separate characteristics were totalled to give a comprehensive sensory score.

### Test of large yellow croaker's quality and structure

Fish samples were assigned into two groups including the treated group and the control group; each group contained 3 fish samples. Fish sample quality and structures were assessed using a FTC texture analyser with 100 N load cell and computer software. A flat-ended cylindrical probe of stainless steel with 5 mm diameter was used for the

compression study. Due to non-homogeneity in the fish flesh the sampling area was selected from the upper portion with respect to the lateral line of the fish body. The sample was placed under the probe that moved downwards to a distance of 35 mm at a constant speed of 40 mm/min.

### Statistics

Design Expert (Stat-Ease, Inc., Minneapolis, MN, USA) and SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software were used for regression and graphical analysis of the data obtained. The optimum values of the selected variables were obtained by solving the regression equation and by analysing the response surface contour plots.

## 3. Results

### Determination of freezing-point of large yellow croaker and learning of freezing process

As can be seen from Table 3, the different parts of LYC were basically similar under -18 °C gas quick-frozen conditions during the freezing process. The freezing point of LYC was near to -1.5 °C. It took 46-48 min to decrease the temperature of LYC from room temperature to the freezing point and 85-87 min to pass the maximal ice crystal production area. The duration of the total freezing process was 173-176 min. All parts in the LYC showed no significant difference when under -18 °C gas quick-frozen conditions during the freezing process.

As can be seen from Table 4, there was no correlation between the fish cakes and freezing point for -18 °C gas quick-frozen conditions during the freezing process.

**Table 2. Sensory evaluation standards.**

Project	Good (7-8 points)	Slightly good (5-6 points)	Common (3-4 points)	Bad (1-2 points)
Appearance	Very bright	Bright	Slightly dull	Dull
Meat quality	High muscle elasticity	Slightly muscle elasticity	Low muscle elasticity, a slight taste of chewing additives	No muscle elasticity, an intense taste of chewing additives
Flavour	Neutral	Fishy	Stale	Spoiled

**Table 3. Effect of different part of large yellow croaker in freezing process.**

	Freezing point (°C)	Time to reach freezing point (min)	Time to get through the maximal ice crystal production area (min)	Time to reach -18 °C (min)
Front part	-1.4	48	87	175
Middle part	-1.5	46	86	173
Back part	-1.5	47	85	176

**Table 4. Effect of different size of fish cakes on freezing process.**

Size of fish cakes (cm)	Freezing point (°C)	Time to reach freezing point (min)	Time to get through the maximal ice crystal production area (min)	Time to reach -18 °C (min)
1×1	-1.5	8	19	40
2×2	-1.4	10	25	60
3×3	-1.5	14	29	70
4×4	-1.5	20	50	108
5×5	-1.4	30	55	115

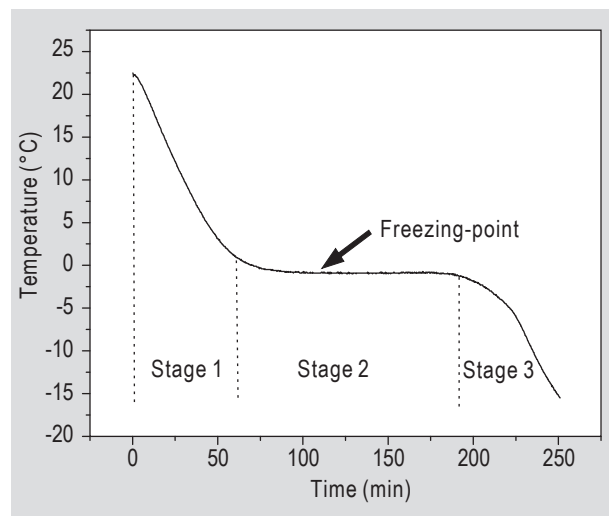
The freezing point of each group showed no significant difference. However, the time taken to reach the freezing point increased from 8 to 30 min when the size of fish cakes increased from 1×1 to 5×5 cm. The time taken to get through the maximum ice crystal production area showed the same phenomenon. This phenomenon indicates that the time for small fish cakes to get through the maximum ice crystal production area is much quicker than large ones, which is conducive to the quality assurance. However, if the size is too small it is difficult to maintain the sensory quality.

As can be seen from Table 5, the freezing point gradually decreased from -1.4 to -3 °C in conjunction with a moisture content decrease from 100 to 20%. There was a negative correlation between the freezing point and moisture content. If the fish cakes have a low moisture content, the surface of the fish meat will contract and dehydrate and finally affect the meat quality. But if the fish cakes contain excessive moisture, it will prolong the time to get through the maximum ice crystal production area, which will not benefit the fish meat quality. The appropriate moisture content was 60% according to the data obtained in the experiment.

As the data in Figure 2 show, the cooling curve of LYC generally occurs in three stages: the first is a decrease from room temperature to -1 °C. The temperature drops quickly because of the release of sensible heat during this stage. The second stage is from -1 to -3 °C. Most of the free and bound water in the fish samples turns to ice, with the latent heat

release. It is called the maximum ice crystal production area. The cooling curve is steep during the third stage because of both latent heat and sensible heat release but the emission is negligible.

As Table 6 shows, the time taken to get through the maximum ice crystal production area was quicker when the LYC was held under low quick-frozen conditions and this benefited product quality. The temperature recorder did not obviously find the inflection point when using the liquid freezing medium. The time to pass through the maximum

**Figure 2. The typical cooling curve.****Table 5. Effect of different water content of fish cakes on freezing process.**

Relative water content of fish cakes (%)	Freezing point (°C)	Time to reach freezing point (min)	Time to get through the maximal ice crystal production area (min)	Time to reach -18 °C (min)
20	-3	3	10	35
40	-2	5	14	40
60	-1.7	7	17	50
80	-1.5	8	20	57
100	-1.4	10	25	60

**Table 6. Effect of different freezing temperature and freezing medium of large yellow croaker on freezing process.**

Freezing temperature (°C)	Freezing medium	Freezing point (°C)	Time to reach the freezing point (min)	Time to get through the maximal ice crystal production area (min)	Time to reach -18 °C (min)
-18	gas	-1.5	10	25	60
-18	liquid	nd	nd	nd	25
-25	gas	-1.5	8	20	55
-25	liquid	nd	nd	nd	20
-50	gas	-1.6	4	12	27
-50	liquid	nd	nd	nd	10

nd = not detected.

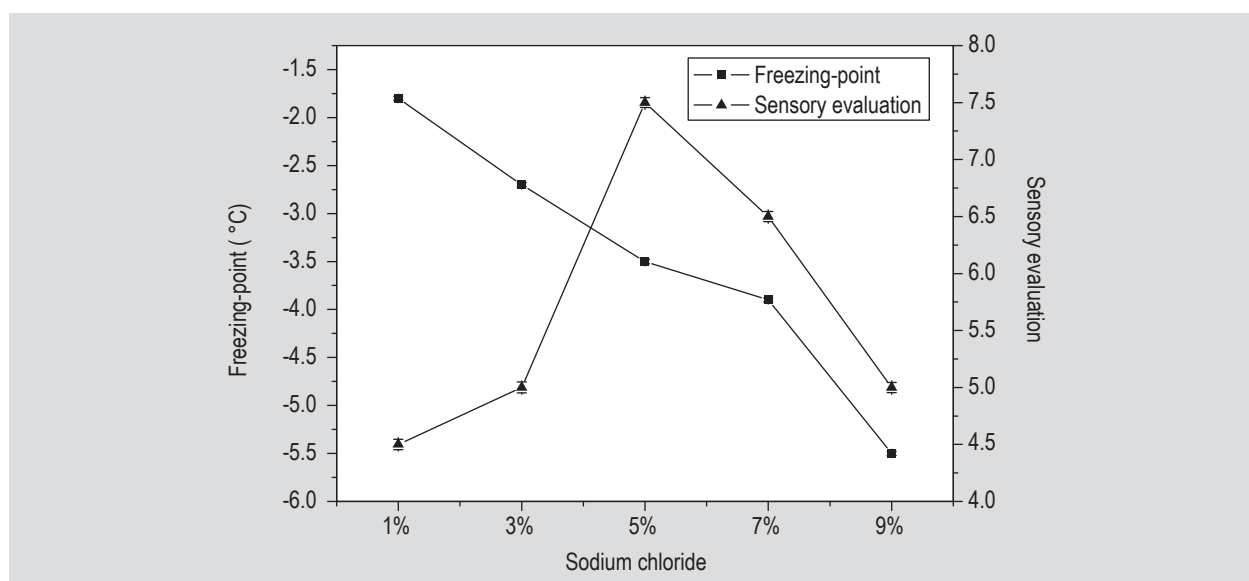
ice crystal production area was short. This phenomenon indicates that the conduction rate of liquid was quicker than gas at the same temperature. However, using liquid to freeze the fish cakes incurred high pre-treatment, instrument and equipment costs.

### Result of single factor experiment

As can be seen from Figure 3, sodium chloride had a large impact on decreasing the freezing point because of its small molecular size. It easily penetrated the muscle cell and increased intracellular osmotic pressure, which prompted the free water transformation into bound water. When the concentration of sodium chloride increased from 1 to 9%, the freezing point decreased from -1.9 to -5.5 °C. The relationship between sensory evaluation and sodium chloride concentration had a parabolic form and a maximum was found. When the sodium chloride concentration was 5%, the sensory evaluation reached the

highest score of 7.5. Adding a certain amount of sodium chloride could slow down the rate of yellowing of the LYC but if the concentration increased further, it would make the fish salty, and finally affect the sensory quality.

The relationship between sucrose concentration and freezing point is shown in Figure 4. The freezing point was -1.5 °C when the additive concentration was 1%, and if the additive concentration increased to 9%, the freezing point reached the lowest freezing point -2.2 °C. The results show that changing the sucrose concentration reduced the freezing point to a certain degree but the ability of sucrose to decrease the freezing point was less than sodium chloride. The molecular weight of sucrose is greater than sodium chloride and it does not easily penetrate the fish samples. Figure 4 shows that the sensory evaluation got the highest sensory score 7.5 when the sucrose concentration was 3%. But with further increases the fish samples became sweet and the quality of the fish meat was adversely affected.

**Figure 3. Effect of sodium chloride on decreasing the freezing point and sensory evaluation of large yellow croaker.**

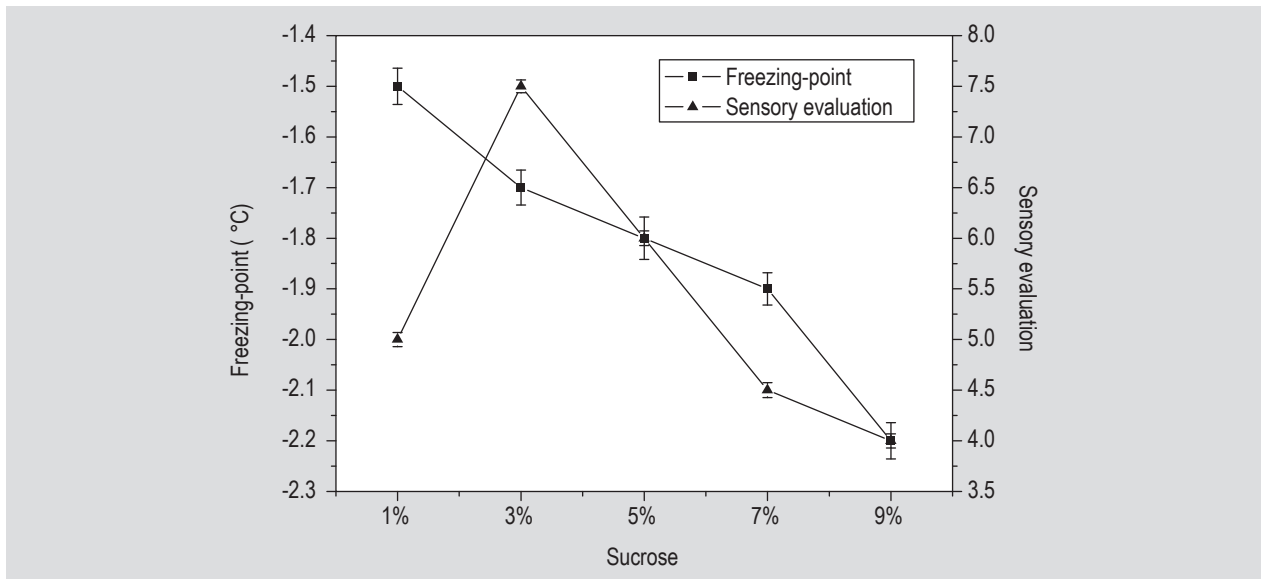


Figure 4. Effect of sucrose on decreasing the freezing point and sensory evaluation of large yellow croaker.

As can be seen from Figure 5, the effect of glucose on decreasing the freezing point was similar to that of sucrose. The results show that the glucose had a small impact on decreasing the freezing point when the concentrations were 1% and 3%. When the concentration increased from 5 to 9%, the freezing point began to decrease and finally reached -2.5 °C. The molecular weight of glucose is 180, which is larger than sodium chloride and other small molecules, so it is difficult for glucose to penetrate the fish meat, which is disadvantageous for depressing the freezing point. The sensory score reached its maximum of 6.5 when the concentration of glucose was 3%. If the additive concentration continued to increase, it made the

fish meat sweet. The impact of glucose was small and was not suitable for further processing.

As Figure 6 showed, vitamin C had a small impact on decreasing the freezing point; the freezing point was only -2.1 °C when the additive concentration was 9%. The molecular weight of vitamin C is large and it does not easily penetrate the LYC. The sensory evaluation reached the highest score of 7.5 when the concentration was 5% and if it increased further, the fish meat became sour and adversely influenced the sensory score.

As can be seen from Figure 7, the ability of sorbitol to decrease the freezing point was great. Sorbitol is a

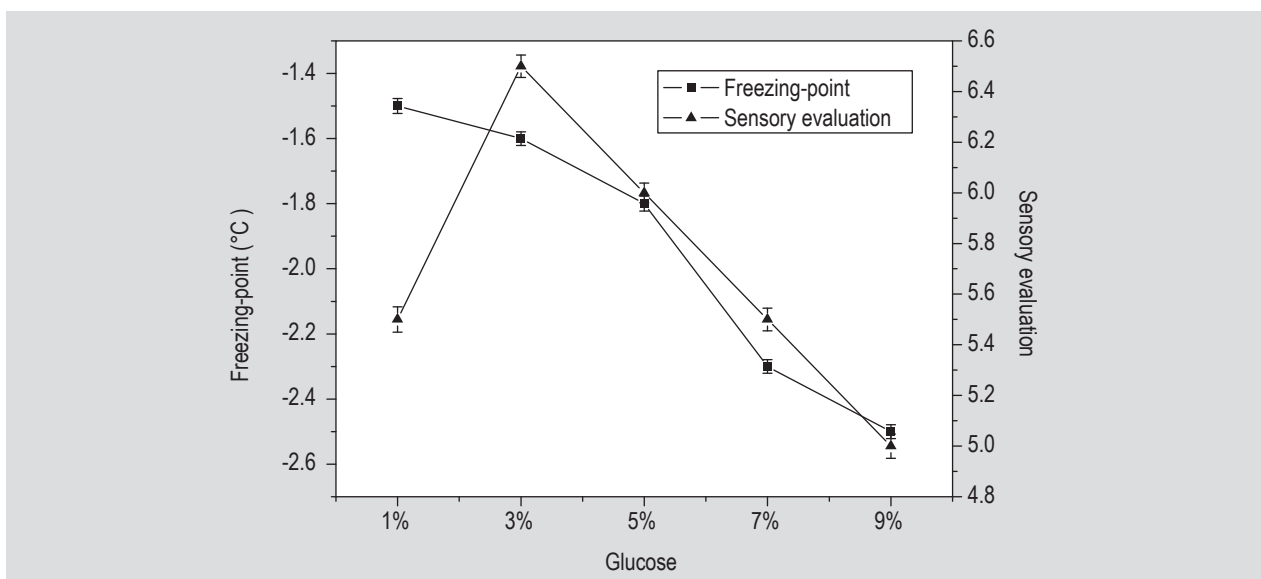


Figure 5. Effect of glucose on decreasing the freezing point and sensory evaluation of large yellow croaker.

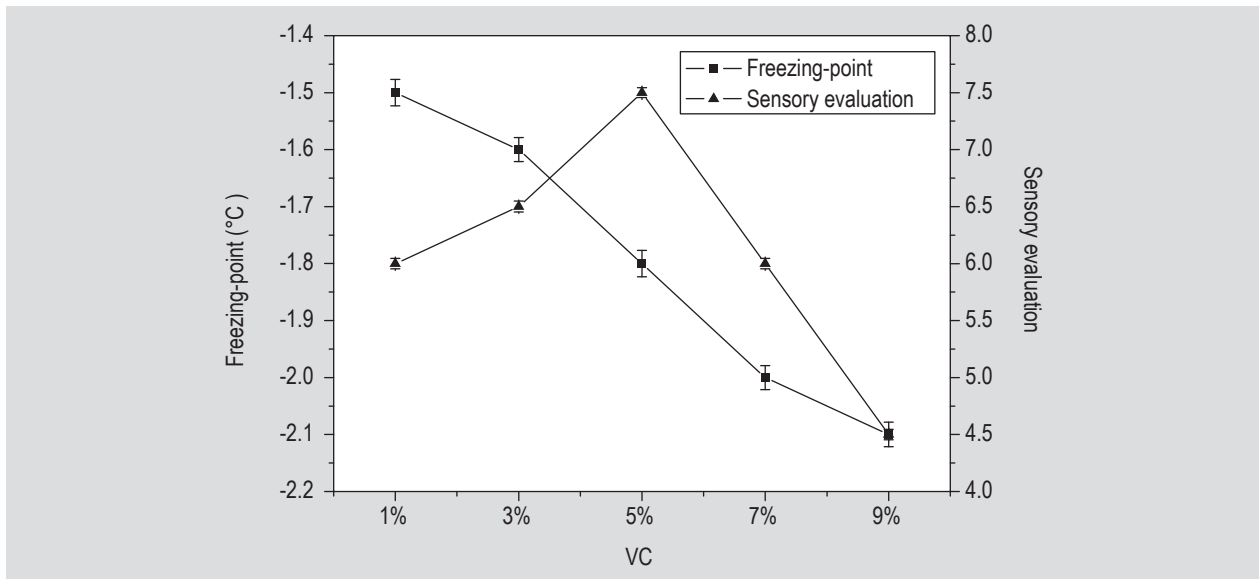


Figure 6. Effect of vitamin C on decreasing the freezing point and sensory evaluation of large yellow croaker.

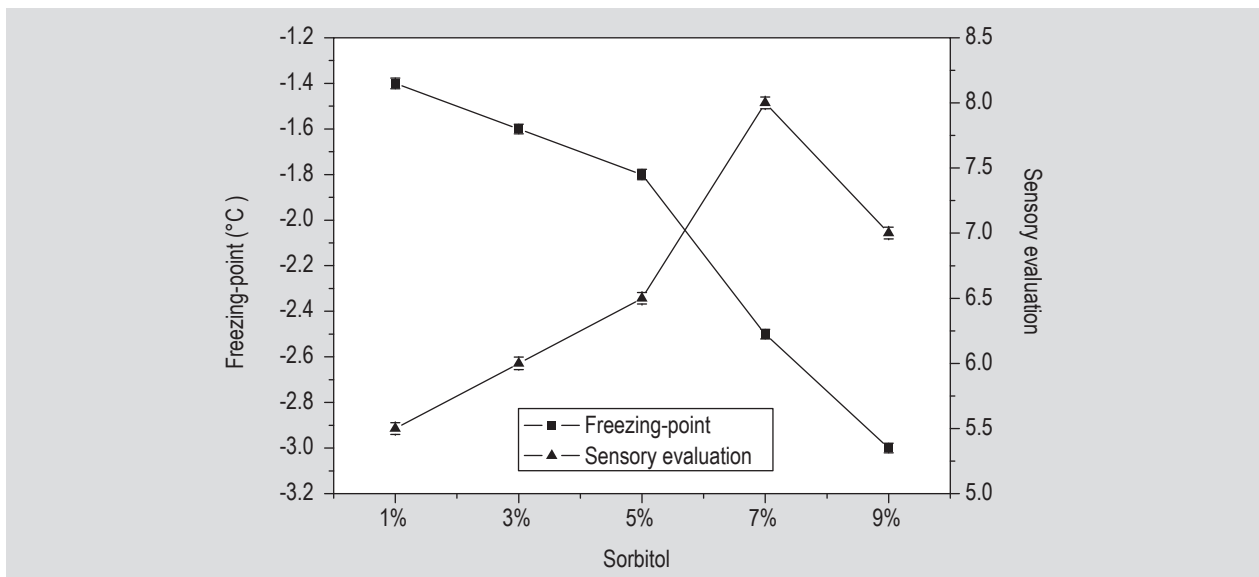


Figure 7. Effect of sorbitol on decreasing the freezing point and sensory evaluation of large yellow croaker.

hydrophilic material containing many OH groups and can transform the free water into bound water. The freezing point was  $-3^{\circ}\text{C}$  when the additive concentration was 9%. The sweetness of sorbitol is lower than sucrose and glucose, which makes it suitable for further processing. The sensory evaluation reached the highest score of 7 when the concentration was 7%. The fish meat was not affected at this concentration. Sorbitol creates a viscous material at higher concentrations and though the amorphous structure can slow down the speed of ice formation, it will have a negative impact on sensory quality.

Based on the single-factor test results, a three-factor quadratic regression orthogonal rotation combination

design was carried out to consider the effect of each freezing point regulator on freezing point and sensory quality. 3-7% sodium chloride (w/w), 5-9% sorbitol (w/w) and 1-5% sucrose (w/w) were taken as input variables. The influence of the three factors on comprehensive scores was analysed according to the establishment of a regression model and the response surface.

#### Building models and its significance test

Comprehensive scores for the freezing point experiments are listed in Table 7. The following equation is a regression model with the experimental results:

**Table 7. Box-Behnken experimental design and corresponding results.**

Test number	X <sub>1</sub> (w/w) (%)	X <sub>2</sub> (w/w) (%)	X <sub>3</sub> (w/w) (%)	Freezing point (°C)	Sense scores	Comprehensive scores
1	5	5	5	-3.7	5.0	4.35
2	3	7	1	-3.2	5.0	4.1
3	5	5	1	-3.6	5.5	4.55
4	3	7	5	-3.3	4.5	3.9
5	5	7	3	-3.6	8.0	5.8
6	7	7	5	-4.6	4.5	4.55
7	3	5	3	-3.1	5.5	4.3
8	5	9	5	-4.3	4.5	4.4
9	7	9	3	-4.8	4.5	4.65
10	5	7	3	-3.6	8.0	5.8
11	5	7	3	-3.7	7.5	5.6
12	7	7	1	-4.1	4.5	4.3
13	7	5	3	-4.0	4.5	4.25
14	5	9	1	-4.1	5.0	4.55
15	3	9	3	-3.4	5.5	4.45

$$EY = -6.8734 + 1.832 \times X_1 + 1.8239 \times X_2 + 0.9219 \times X_3 + 0.0156 \times X_1 \times X_2 + 0.0281 \times X_1 \times X_3 + 3.125 \times 10^{-3} \times X_2 \times X_3 - 0.0196 \times X_1^2 - 0.1339 \times X_2^2 - 0.1839 \times X_3^2$$

Statistical testing of the model was performed with analysis of variance (ANOVA). The results of the ANOVA for comprehensive scores are shown in Table 8. The quadratic regression shows that the model was significant because the value of F statistic (the ratio of mean square due to regression to mean square to real error) of 19.55 was greater

than F<sub>0.001,9,10</sub> (4.94). The value of the correlation coefficient (R<sup>2</sup>=0.9724) indicates that the equation had high credibility. The P-value of X<sub>1</sub><sup>2</sup> (P<0.01), X<sub>2</sub><sup>2</sup> (P<0.01) and X<sub>3</sub> (P<0.01) in Table 8 also implies that the second-order polynomial model fitted the experimental results well.

The optimal conditions were established by a Box-Behnken experimental design. Independent variables of sodium chloride (X<sub>1</sub>), sorbitol (X<sub>2</sub>) and sucrose (X<sub>3</sub>) were set in range while dependent variables were fixed at maximum.

**Table 8. Variance analysis for the developed quadratic regression model.**

Source	Sum of squares	DF	Mean square	F-value	P-value
Model	4.9	9	0.544	19.55	0.0022**
X <sub>1</sub>	0.12	1	0.125	4.49	0.0876
X <sub>2</sub>	0.045	1	0.045	1.62	0.2595
X <sub>3</sub>	0.011	1	0.011	0.40	0.5529
X <sub>1</sub> X <sub>2</sub>	0.016	1	0.016	0.56	0.4874
X <sub>1</sub> X <sub>3</sub>	0.051	1	0.051	1.82	0.2353
X <sub>2</sub> X <sub>3</sub>	6.25×10 <sup>-4</sup>	1	6.25×10 <sup>-4</sup>	0.02	0.8867
X <sub>1</sub> <sup>2</sup>	2.28	1	2.277	81.83	0.0003***
X <sub>2</sub> <sup>2</sup>	1.06	1	1.058	38.03	0.0016**
X <sub>3</sub> <sup>2</sup>	2.00	1	2.00	71.75	0.0004***
Residual	0.14	5	0.028		
Lack of fit	0.11	3	0.038	2.81	0.2732
Pure error	0.027	2	0.013		
Corrected total	5.04	14			

\*\* P<0.01; \*\*\* P<0.001.

The resulting solution by Design Expert (Figures 8, 9 and 10) was sodium chloride 5.16% (w/w), sucrose 2.96% (w/w), and sorbitol 7.15% (w/w). The predicted values of comprehensive scores and the freezing point were 5.76 and -4.2 °C and the model can clearly determine the conditions.

### Texture analysis

The variation in textural parameters of the fish sample is presented in Table 9. There was a slight variation in fish hardness between the control group and the treated

group, which was 22 N for the control group and 19.76 N for the treated group. The brittleness of the fish sample was 22 N for the control group and 21 N for the treated group. Other texture indexes were the same as the hardness and brittleness and there was no significant difference ( $P>0.05$ ) between the treated and control group according to a mathematical statistic using SPSS 17.0.

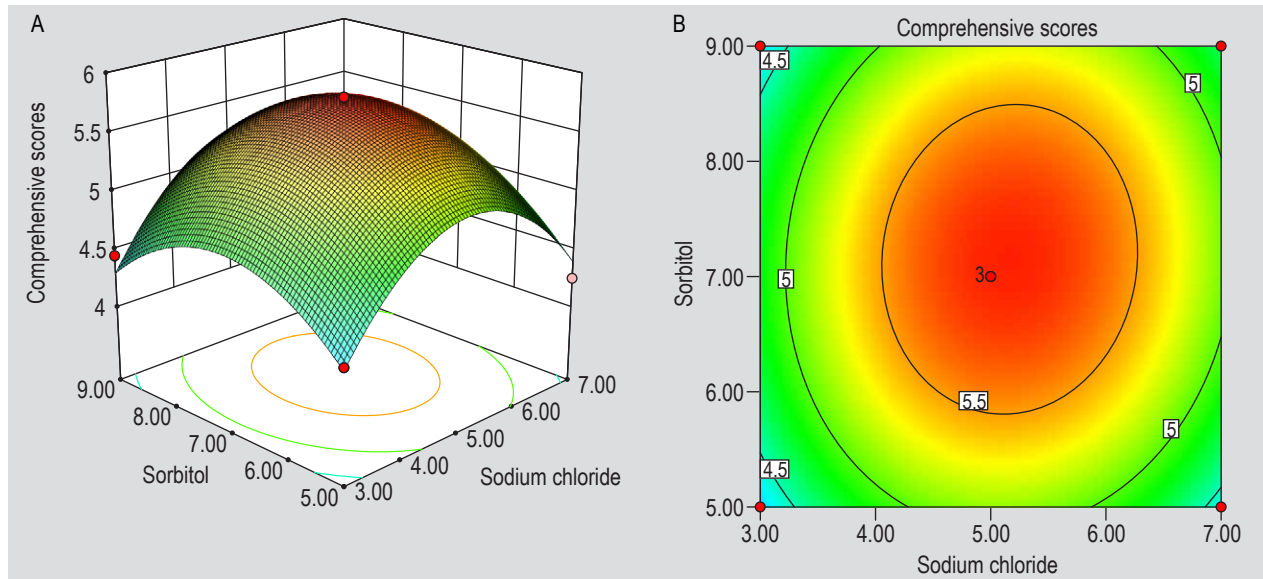


Figure 8. (A) Response surface and (B) contour plots illustrating the interactive effects of sorbitol and sodium chloride on comprehensive score.

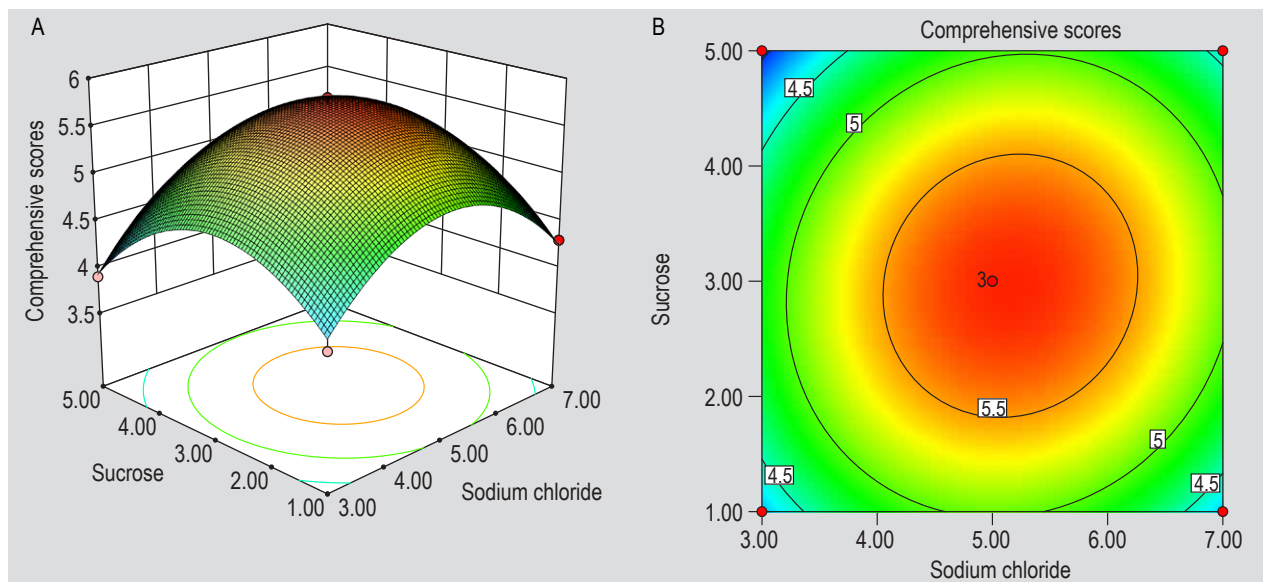


Figure 9. (A) Response surface and (B) contour plots illustrating the interactive effects of sucrose and sodium chloride on comprehensive score.

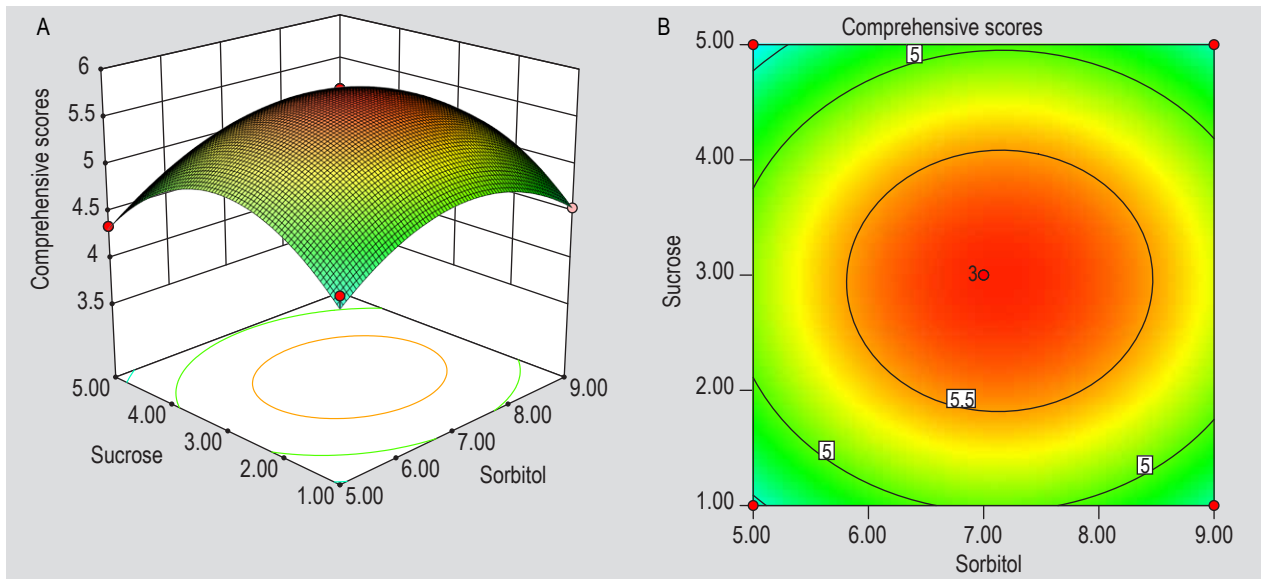


Figure 10. (A) Response surface and (B) contour plots illustrating the interactive effects of sucrose and sorbitol on comprehensive score.

Table 9. Texture analysis of fish cakes.

	Hardness (N)	Brittleness (N)	Viscosity (mj)	Cohesion (P)	Elasticity (mm)	Tackiness (N)	Chewiness (mj)
Control group	22	22	0.18	0.35	1.65	7.60	12.56
Treated group	19.76	21	0.12	0.40	1.80	7.86	15.31

### 4. Conclusions

Food preservation is very important for the safety and reliability of the product, and temperature is the major factor for determining the shelf life and quality of food products. Storing food at ice temperatures has three distinct advantages: maintaining food freshness, retaining high food quality and suppressing the growth of harmful microbes. The primary role at ice temperature is expanding the ice crystals, which bring benefits to the proteins, lipids and texture of food products (Sivertsvik *et al.*, 2003). The effects of single freezing point regulators such as sorbitol, glucose and sucrose on the freezing point of fresh-cut litchi pulp have been studied and the results revealed that the freezing point was decreased from -2.8 to -3.5 °C (Wu *et al.*, 2011). In our study, response surface methodology was used for establishing a mathematical model of the relationship between the freezing-point regulators and operating parameters. The regression model was highly significant and had good predictability. We obtained a larger ice temperature zone comparing with other studies by using compound freezing point regulators. The optimal conditions are obtained as follows: 5.16% sodium chloride (w/w), 2.96% sucrose (w/w), 7.15% sorbitol (w/w). Compared to the control group, fish samples treated with

optimal freezing-point regulators can reduce the freezing point from -1.5 to -4.2 °C without affecting the meat quality traits and so meet the requirements for industrial production.

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