

Original Research Paper

Optimization of Key Technology for Instant Sea Cucumber Processing through Fuzzy Evaluation and Response Surface Methodology

^{1,2}Jianfeng Sun, ¹Tianshu Yang, ¹Qian Liu and ¹Zhaoping Sun

¹College of Food Science and Technology, Agricultural University of Hebei, Baoding 071000, China

²Engineering Technology Research Center for Processing of Agricultural Products of Hebei Province, Baoding 071000, China

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Corresponding Author:

Jianfeng Sun

College of Food Science and Technology, Agricultural University of Hebei, Baoding 071000, China

Email: causunjf@126.com

causunjf@hebau.edu.cn

Abstract: The original flavor of instant sea cucumber products can be retained and these products consequently show good market potential. In this study, the key processing technique for instant sea cucumber was optimized on the basis of single factor through Box-Behnken response surface test method by using collagen and polysaccharide contents and fuzzy mathematics sensory score as response values. Results showed that cooking time, cooking temperature, sterilization time and sterilization temperature significantly affected the overall quality and sensory quality of instant sea cucumber products. The optimum processing conditions were as follows: Cooking time, 17 min; cooking temperature, 83°C; sterilization time, 10 min; and sterilization temperature, 103°C. Under these conditions, the collagen content, polysaccharide concentration and sensory evaluation were 5.263±0.020%, 1.021±0.068% and 97.27±0.85, respectively and these findings were consistent with the predicted values. This study revealed that the nutrient components of instant sea cucumber are efficiently preserved and thus provided technical guarantee for the processing and production of instant sea cucumber.

Keywords: Instant Sea Cucumber, Fuzzy Evaluation, Response Surface Method, Collagen, Acid Polysaccharide

Introduction

Sea cucumber (*Stichopus japonicus*), which is an invertebrate belonging to Holothuroidea under Echinodermata, is a well-known Chinese traditional seafood because of its rich nutritional value and dietary health function (Eriksson and Clarke, 2015; Bai *et al.*, 2013; Wen *et al.*, 2010). More than 1,100 species of sea cucumber exist worldwide; of these species, about 140 kinds are found in China and 20 kinds are used as food sources (Conand and Byrne, 1993). Sea cucumber is composed of high protein, low fat and low cholesterol contents and highly bioactive ingredients (Lawrence *et al.*, 2010; Bordbar *et al.*, 2011; Mamelona *et al.*, 2009), including saponins, cerebrosides (La *et al.*, 2012), collagen and acid polysaccharides. These bioactive components exhibit anti-tumor, anticoagulation, anti-aging, anti-fungal, hypolipidemic and anti-diabetes properties; these components can also alleviate pain, improve immunity, prevent and treat rheumatoid arthritis and induce other beneficial effects (Bordbar *et al.*, 2011). Sea cucumbers exposed to high temperatures or

exogenous conditions, such as oil, water, or strong oxidants, contain autoenzymes that can cause a “skin” reaction (Zheng *et al.*, 2010). Therefore, these organisms should be handled immediately after capture. The original flavor of instant sea cucumber products can be retained, they can be easily consumed and stored and they can provide good market prospects (Toral-Grand *et al.*, 2008).

With fast-paced lifestyle and improvements in quality of life, demands for high-quality instant food have become urgent (Charunuch *et al.*, 2011). Key processing technologies for instant seafood products include cooking, stew system, heavy soup cooking and other methods. These processes optimize the texture, sensory or nutrition and other single indicators of sea cucumber products but fail to improve their palatability, nutrition and appearance. For example, high-temperature and high-pressure processing easily degrades collagen gel and consequently induces nutrient loss through water exchange. Collagen constitutes 70% of proteins in sea cucumber and polysaccharide is an important bioactive ingredient of sea cucumbers (Saito *et al.*, 2002). Sensory score is the relevant index of the general optimization process. In our

study, the appropriate parameters of instant sea cucumber processing were determined through single-factor and response surface analysis with Box–Behnken design by using collagen protein and polysaccharide contents and sensory scores as response values.

Materials and Methods

Fresh sea cucumbers were purchased from the aquatic product market of Qinhuangdao in Hebei Province. L-hydroxyproline (Batch number: B21928) and D-fucose (Batch number: B25632) standards were obtained from Shanghai Source Leaves, Biological Technology Co., Ltd. Hydrochloric acid, sulfuric acid, perchloric acid, isopropyl alcohol, two of chloramine T dimethylamino benzaldehyde, citric acid, 95% ethanol and 30% hydrogen peroxide were of analytical grade.

Processing Technology for Instant Fresh Sea Cucumber

The internal organs of fresh sea cucumber were removed (from the abdomen to the anus to the front of the body to cut 1/4-1/3), hot and stereotypes (85-95°C, 2 min). The sea cucumbers were then seasoned and cooked. After the sea cucumbers were sterilized through back pressure by using an RS-50L intelligent pressure high-temperature cooking pot (Guangzhou Lonbon Science and Technology Co., Ltd.), the product was vacuum-packed with a DQB-360W double chamber vacuum packaging machine (Shanghai Qingpa Food Packaging Machinery Co., Ltd.). The effects of different processing conditions on the sensory traits and quality of the sea cucumber products were investigated. Secondary seaweeds of the sea cucumber were removed and placed in a dressing solution at 60, 70, 80, 90 and 100°C in cooking liquid for 5, 10, 15, 20 and 25 min, respectively. They were then vacuumed through 9.0×10^4 Pa vacuum packaging at 95, 105, 115 and 121°C back pressure sterilization and pot sterilization for 5, 10, 15 and 20 min to prepare instant sea cucumber products. The following parameters were set to season the pretreatment sea cucumbers: Water ratio of 1:3, 42.0 g per 1 kg seasoning liquid containing white sugar; 6.0 g MSG; 24.0 mL soy sauce; 24.0 g salt; 3.6 g ginger powder; 3.6 g allspice; 4.8 g star anise; 6 mL white vinegar; 9.6 mL cooking wine; and 6 g chicken bouillon. The collagen and polysaccharide contents of the sea cucumbers were prepared and analyzed (Yao, 2013).

Determination of Collagen and Polysaccharide Contents

Preparation of L-Hydroxyproline Standard Curve

The appropriate amount of L-hydroxyproline was weighed and formulated with $100 \mu\text{g mL}^{-1}$ of the stock solution. The stock solution was absorbed at 0.00, 0.60,

1.20, 1.80, 2.40 and 3.00 mL in a 10 mL volumetric flask and constant volume with solution concentrations of 0.0, 6.0, 12.0, 18.0, 24.0 and $30.0 \mu\text{g mL}^{-1}$, respectively. In brief, 1 mL of this solution was separated and added with 1 mL of buffer solution (pH 6.0) and 1 mL of chloramine T solution. The new solution was shaken and kept at room temperature for 20 min. Afterward, 1 mL of perchloric acid solution was added and the solution was shaken and kept at room temperature for 10 min. Next, 1 mL of the reagent was added and the solution was shaken again. The reaction was conducted at 65°C for 20 min. The samples were cooled down to room temperature and spectrally scanned at 900-190 nm. Absorbance at 560 nm was scanned and recorded and the standard curve was plotted to obtain the regression equation (Yao, 2013).

Preparation and Determination of Collagen in Sea Cucumber

The chopped sea cucumber samples weighing about 1.0 g were placed in 20 mL jaws, added with 2 mL of 6 mol L^{-1} HCl solution and digested at 130°C for 4 h. After the samples were cooled to room temperature, pH was adjusted to 6.8-7.2. The solution was transferred in a 100 mL volumetric flask and 1 mL of the sample solution was obtained and treated according to the hydroxyproline standard. Absorbance values were measured and recorded and the collagen content was calculated according to the resulting L-hydroxyproline standard curve.

Preparation of D-fucose Standard Curve

The appropriate amount of D-fucose was weighed and formulated with $100 \mu\text{g mL}^{-1}$ of the stock solution. The stock solution was absorbed at 0.00, 0.60, 1.20, 1.80, 2.40 and 3.00 mL in a plug test tube and added with 1 mL of water, 0.6 mL of phenol solution and 3.00 mL of concentrated hydrochloric acid. The solution was mixed by vortexing and allowed to stand for 20 min until it cooled down to room temperature. The samples were spectrally scanned in the range of 900-190 nm. The absorbance at 490 nm was recorded after scanning and the standard curve was plotted to obtain the regression equation (Yao, 2013).

Preparation and Determination of Acid Polysaccharides in Sea Cucumber

The chopped sea cucumber samples weighing about 3.0 g were placed in a 100 mL cone bottle, added with 40 mL of 3% KOH solution and leached for 2 h by ultrasound. Their pH was adjusted to 6.8-7.2 by adding 2 M acetic acid and centrifuged at 8500 r/min and 4°C for 30 min. The supernatant was removed and slowly added with twice the volume of 95% ethanol. The resulting solution was allowed to stand overnight and then centrifuged at 8000 r/min for 15 min. The precipitate

was extracted and added with 10 mL of distilled water. Afterward, 2 mL of hydrogen peroxide was added for decolorization. The solution was added with 24 mL of 95% ethanol, allowed to stand overnight and centrifuged at 8000 r/min for 15 min. The sample was baked in an oven with a constant temperature of 50°C and the dried sample was prepared as a 200 µg mL⁻¹ solution. Then, 1 mL of the sample was obtained and processed according to the fucose standard. The absorbance values were measured and recorded and the polysaccharide content was calculated according to the measured D-fucose standard curve.

Fuzzy Evaluation

The instant sea cucumber samples were randomly placed in identical sensory panels coded with randomly selected 3-digit numbers prior to the random monadic presentation to each of the 10 evaluator professionals with expertise in sensory assessment. Traditional evaluation results are inaccurate because of the lack of objectivity. Some subjective factors significantly affect sensory evaluation results. However, fuzzy mathematics for comprehensive evaluation can involve different factors in scoring during sensory evaluation and consequently enhance the accuracy of results. Fuzzy mathematics for statistics and data analysis can also reduce accidental errors. Therefore, the sensory evaluation of sea cucumber was performed through fuzzy mathematics comprehensive analysis. The fuzzy evaluation method changes the traditional average scoring system and requires a choice for the evaluation domain $U = (U_1, U_2, U_3) = (\text{color, flavor, flesh elasticity})$, comment domain $V = (V_1, V_2, V_3, V_4) = (\text{very good, fine, ordinary, poor})$, fuzzy weight vector X

$= (X_1, X_2, X_3) = (0.45, 0.2, 0.35)$, grade evaluation matrix $P = (P_1, P_2, P_3, P_4) = (100, 80, 60, 40)$ and sensory evaluation indexes (Table 1) (Hu and Xia, 2011).

In this study, fuzzy mathematics comprehensive evaluation was performed through the following three-step membership grade. (i) The sensory evaluation results of each evaluation domain were calculated to build the multiple objective evaluation membership matrix $R_i = (r_{i1}, r_{i2}, r_{i3}, r_{i4})$ and obtain the fuzzy evaluation matrix $R = (R_{i1}, R_{i2}, R_{i3})^T$. (ii) The comprehensive grading vector was $B_k = X \times R = (X_1, X_2, X_3)(R_{i1}, R_{i2}, R_{i3})^T$ and the specific operations of (i) and (ii) were conducted by referring to previous studies (Gao and Fu, 2011; Liu *et al.*, 2012; 2014). (iii) The comprehensive evaluation score of each sample was $S_j = P \times B_k^T = (P_1, P_2, P_3, P_4)(B_{k1}, B_{k2}, B_{k3}, B_{k4})^T$, which can be applied to rank the competition of sea cucumber. In this study, the weight of the color, flavor and flesh elasticity of the sea cucumber represents the average of the mandatory scoring, sequential evaluation and statistical evaluation methods, respectively (Ji, 1991). The analyses were repeated thrice.

Response Surface Test

The effects of cooking time, cooking temperature, sterilization time and sterilization temperature as independent variables were selected by using single factor. Based on Box-Behnken design principle, four-factor and three-level response surface analyses were conducted by considering collagen and polysaccharide contents and sensory scores as evaluation indexes (Song *et al.*, 2012; Zhu *et al.*, 2016). The factor level design is shown in Table 2 and the analyses were repeated thrice.

Table 1. Sensory evaluation table

Grade	Label	Color	Flavor	Elasticity
Great	A	Black	Rich seafood flavor	Soft medium
Good	B	Brown	Slightly seafood flavor	Poor palatability
Ordinary	C	Yellow-brown	Slightly fishy	General taste
Bad	D	Yellow	Fishy	Poor hardness

Table 2. Factors and levels used in response surface design

Factor	Level		
	-1	0	1
Cooking time/min	10	15	20
Boiling temperature/°C	70	80	90
Sterilization time/min	5	10	15
Sterilization temperature/°C	95	105	115

Table 3. Regression equation of L-Hydroxyproline and D-fucose

	Regression equation	R ²	Application scope (µg/mL)
L-hydroxyproline	$Y = 0.0780X + 0.0816$	0.9938	0.00-15.00
D-fucose	$Y = 0.0093X - 0.0069$	0.9986	0.00-30.00

Results and Analysis

L-hydroxyproline and D-fucose Standard Curve

The absorbance was measured at 560 and 490 nm according to the mass concentration gradient of L-hydroxyproline and D-fucose standards. Using the mass concentration ($\mu\text{g/mL}$) as the abscissa and the absorbance as the ordinate, we plotted the standard curve. The regression equation is shown in Table 3.

Single Factor Test

Figure 1 illustrates the effect of different processing conditions on collagen and polysaccharide contents and sensory evaluation. Figure 1A shows that the collagen and polysaccharide contents of instant sea cucumber decreased as sterilization time was prolonged at a cooking time of 20 min, cooking temperature of 80°C and sterilization temperature of 121°C. As cooking time is extended and temperature is increased, collagen fibers gradually thicken, condense, gradually split and disintegrate (Xue, 2006). The sensory score of instant sea cucumber initially increased, subsequently decreased and reached the optimum at a sterilization time of 10 min. Thus, the sterilization time was set to 10 min.

Figure 1B indicates that the collagen and polysaccharide contents of instant sea cucumber initially remained unchanged and then decreased as sterilization temperature was increased at a cooking time of 20 min, cooking temperature of 80°C and sterilization time of 5 min. The collagen and polysaccharide structures of instant sea cucumber were gradually destroyed and broken into small peptides and monosaccharides or disaccharides as sterilization temperature increased. Consequently, collagen and polysaccharide lost their potency. The sensory score of sea cucumber also gradually increased. The elasticity, hardness and palatability of sea cucumber improved possibly because of the high-temperature and high-pressure stew system. Therefore, the sterilization temperature of 115°C was appropriate.

Figure 1C reveals that the collagen and polysaccharide contents of the instant sea cucumber initially increased and then decreased as cooking time was prolonged at a cooking temperature of 80°C, germination time of 5 min and sterilization temperature of 121°C. Higher levels were obtained when cooking time was set to 15 min because of the increase in collagen and polysaccharide levels due to the accumulation and contraction of sea cucumber in early cooking stages. The moisture content of instant sea cucumber almost remained unchanged and the collagen and polysaccharide contents gradually decreased as cooking time was prolonged. The collagen and polysaccharide contents of sea cucumber were not significantly different when the cooking time

was 10 and 15 min. The sensory score of instant sea cucumber continuously increased as cooking time was extended. The collagen and polysaccharide contents of instant sea cucumber were sharply reduced when cooking time was more than 15 min. Therefore, the suitable cooking time was 10 min.

Figure 1D shows that the collagen and polysaccharide contents and sensory score of instant sea cucumber initially increased and then decreased as cooking temperature increased at a cooking time of 20 min, germination time of 5 min and sterilization temperature of 121°C. The polysaccharide content of instant sea cucumber no longer changed when cooking temperature reached 80°C. The highest collagen content and sensory score of instant sea cucumber were obtained when the temperature reached 70 and 90°C. These values subsequently declined. Therefore, the appropriate cooking temperature was 80°C.

Response Surface Design and Result Analysis

According to the single factor test, the significant test factors were greater. Based on Box-Behnken principle, four factors and three levels of cooking time (29 test points) included cooking temperature, sterilization time and bactericidal temperature as independent variables, whereas collagen and polysaccharide contents and sensory score were set as response values. The experimental design and results are shown in Table 4.

Variance analysis was performed on the regression model and the results are shown in Table 5. Three groups of model factors were $p < 0.0001$, which indicated that the factor regression model exhibited a significant difference ($p < 0.01$). In the loss of the proposed $P = 0.0832$ (collagen), $P = 0.08718$ (polysaccharide) and $P = 0.1282$ (sensory evaluation), the difference was not significant and these data suggested that the model was stable and the error was random. The three models could predict the effects of different processing conditions on the collagen and polysaccharide contents and sensory score of instant sea cucumber.

Establishment of Mathematical Model and Significance Test

The polynomial regression model of the collagen and polysaccharide contents, sensory score and relevant factors were obtained through the multiple fitting regression of the experimental data. The collagen and polysaccharide contents and sensory score of instant sea cucumber were used as the response values, whereas cooking time (X_1), cooking temperature (X_2), sterilization time (X_3) and sterilization temperature (X_4) were considered as independent variables. The secondary regression model is shown in Table 6.

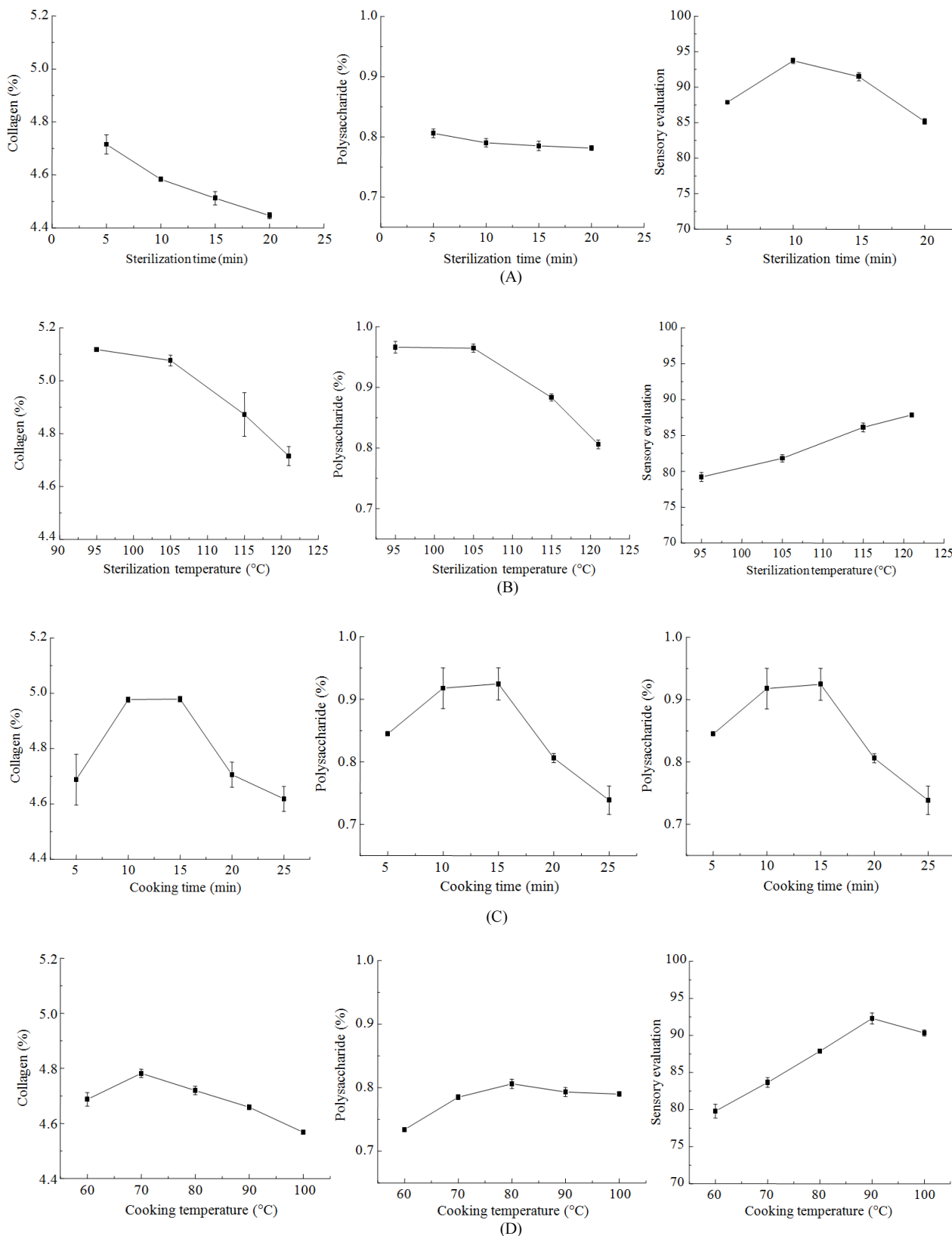
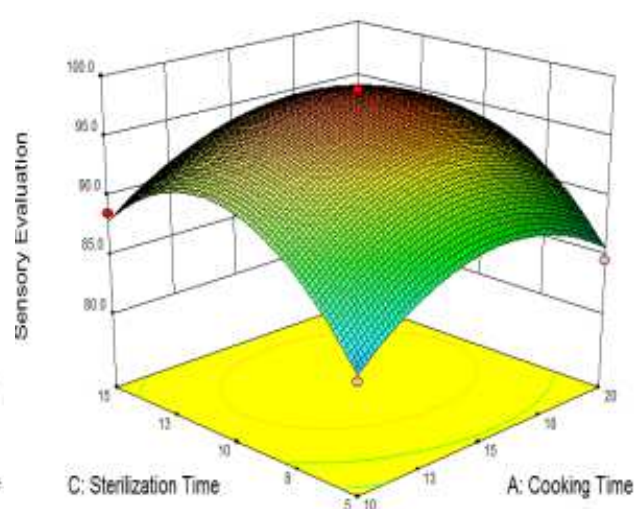
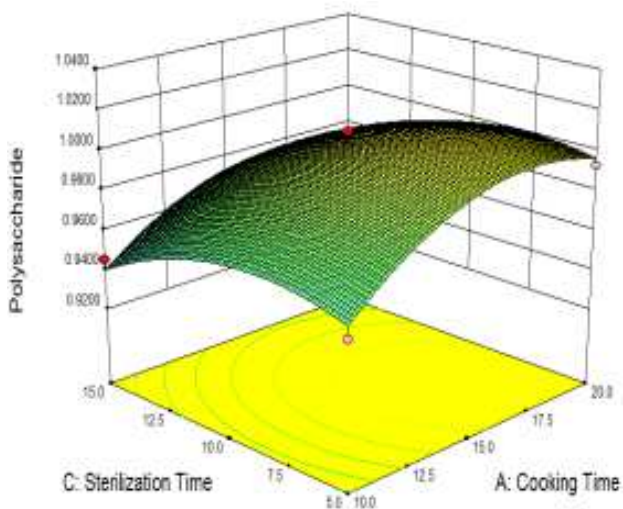
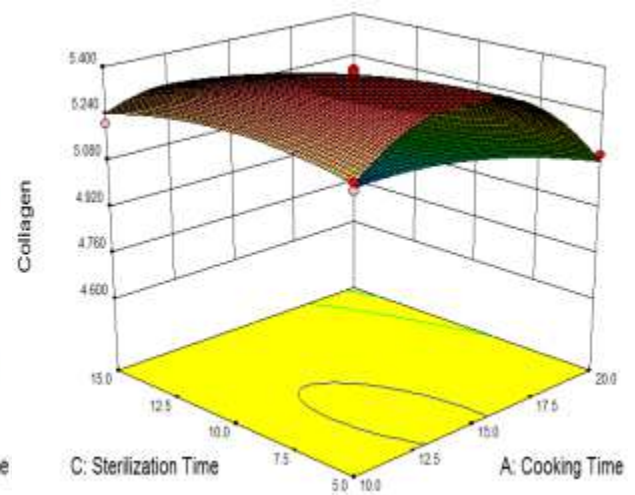
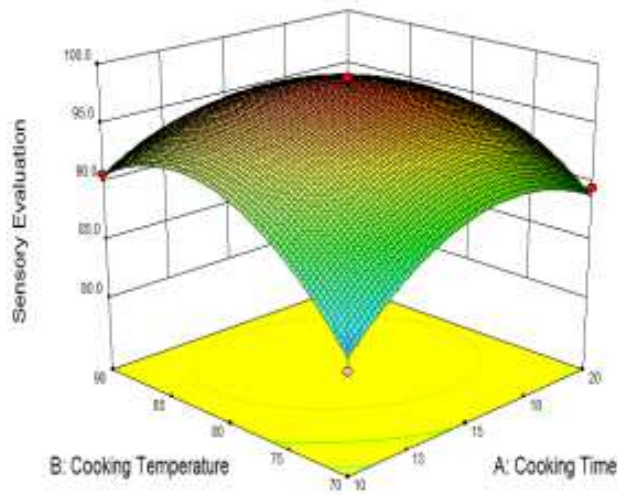
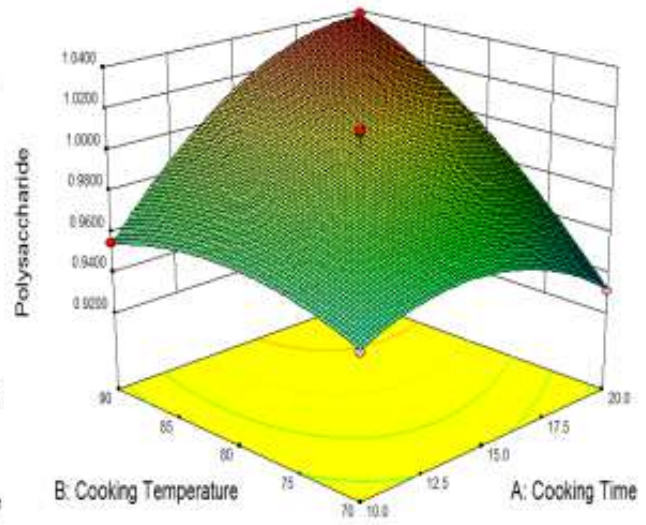
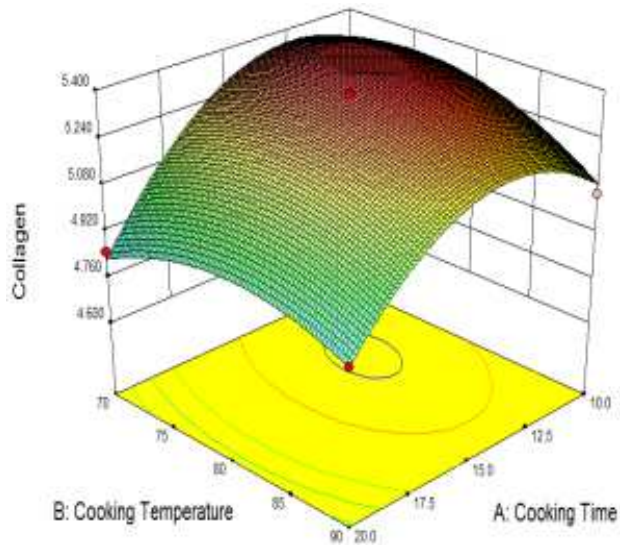
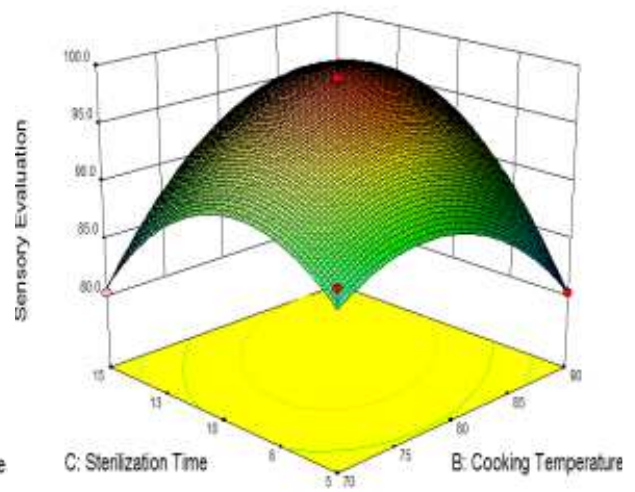
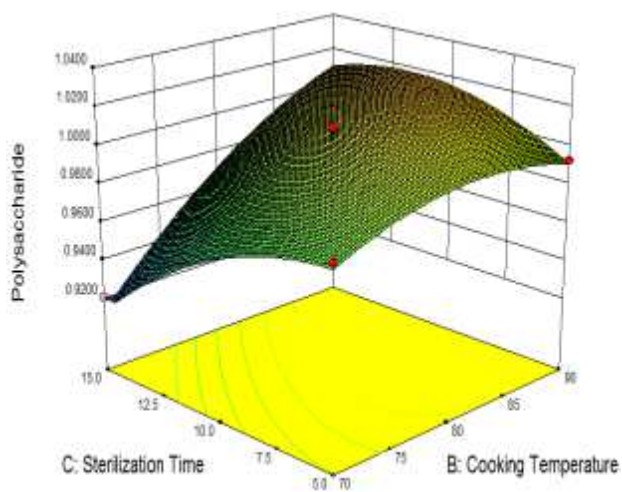
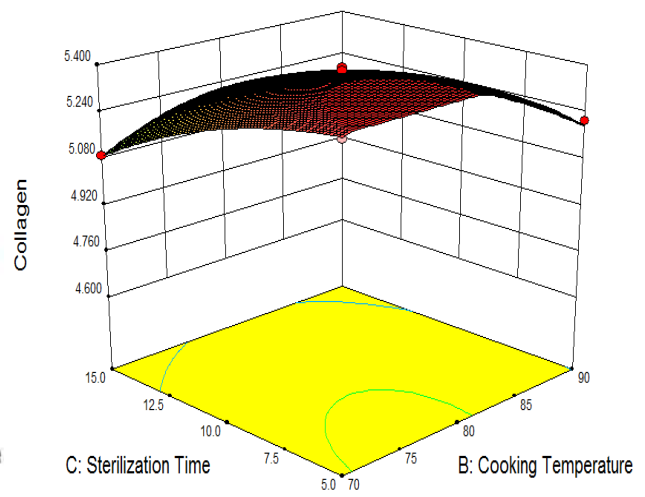
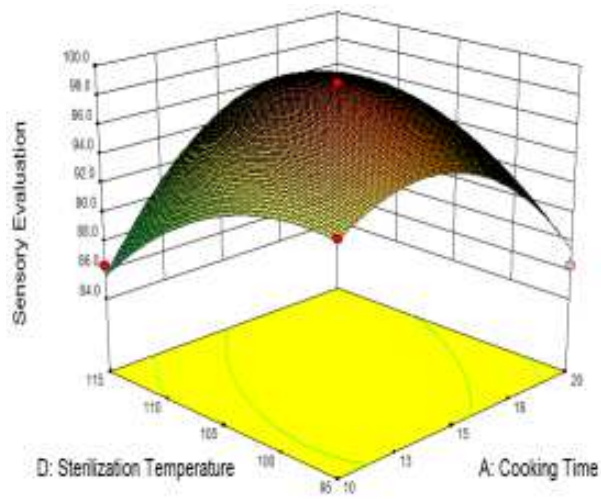
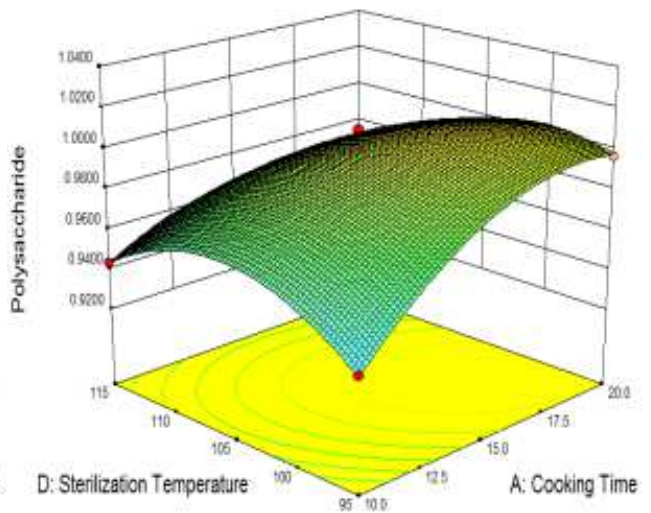
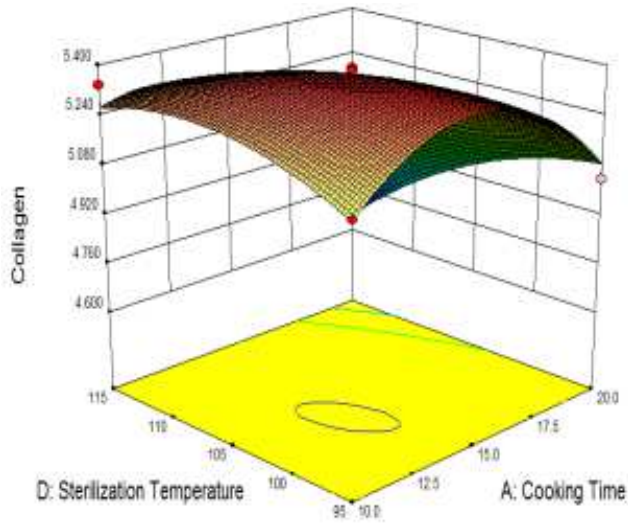


Fig. 1. Effect of different processing conditions on the collagen and polysaccharide contents and the sensory evaluation





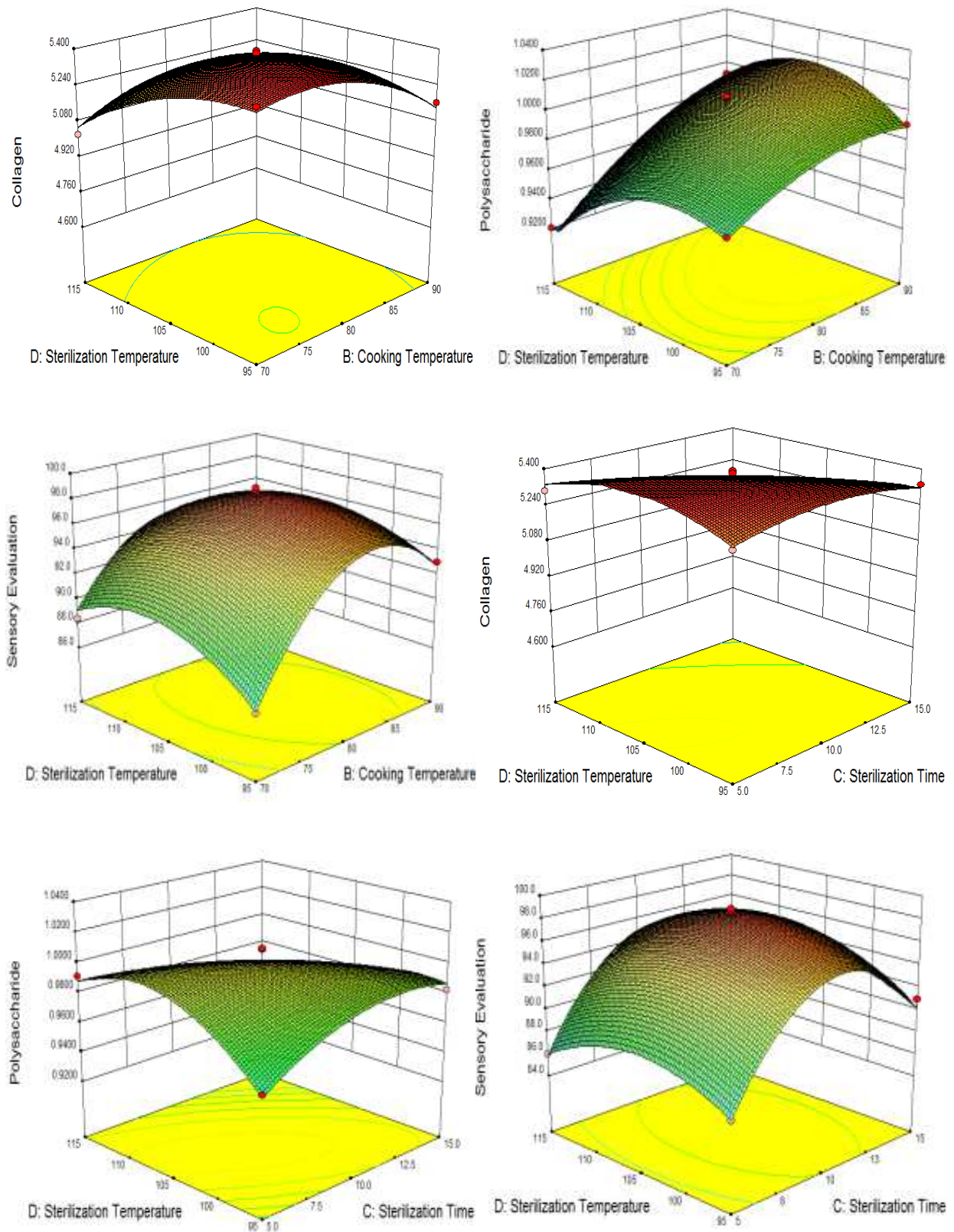


Fig. 2. Response surface plots for the effect of independent variables on response variables

Table 4. Experimental designs and results for response surface design

Experiment number	Cooking time (min)	Boiling temperature (°C)	Sterilization time (min)	Sterilization temperature (°C)	Experimental data		
					Collagen Protein (%)	Polysaccharide (%)	Sensory evaluation
1	15	80	15	115	4.9550	0.9082	91.1
2	15	70	10	95	5.3811	0.9607	86.6
3	15	90	15	105	5.1560	1.0072	92.0
4	15	80	10	105	5.3481	1.0098	97.6
5	15	90	10	95	5.1652	0.9907	93.0
6	15	90	10	115	5.0850	0.9923	89.5
7	15	70	5	105	5.3860	0.9828	88.3
8	15	80	5	95	5.2910	0.9582	86.5
9	15	80	15	95	5.3309	0.9826	91.0
10	20	70	10	105	4.8510	0.9310	89.6
11	10	80	5	105	5.2620	0.9528	82.5
12	20	80	5	105	5.1040	0.9928	84.6
13	20	80	10	115	4.6739	0.9438	94.6
14	20	80	10	95	5.0380	0.9962	86.3
15	15	80	10	105	5.3790	0.9945	98.9
16	15	80	10	105	5.3520	0.9986	97.2
17	15	80	5	115	5.3049	0.9914	86.0
18	10	90	10	105	5.0550	0.9550	90.7
19	15	80	10	105	5.3870	1.0090	98.6
20	10	80	10	115	5.3380	0.9438	86.3
21	10	80	10	95	5.1740	0.9362	93.9
22	15	70	10	115	5.0238	0.9133	88.4
23	15	90	5	105	5.2140	0.9928	80.2
24	20	90	10	105	4.8710	1.0380	90.0
25	15	70	15	105	5.0960	0.9101	80.2
26	20	80	15	105	4.7200	0.9752	88.7
27	10	70	10	105	5.2809	0.9490	81.9
28	15	80	10	105	5.3890	0.9975	97.5
29	10	80	15	105	5.2100	0.9452	88.7

The coefficient of determination (R^2), correction fit (RA_{dj}^2) and Coefficient of Variation (CV) of the regression equation are shown in Table 6. The collagen, polysaccharide and sensory evaluation models could respectively yield the following values: 96.83, 97.36 and 94.70% response changes. The experimental data were highly reliable and the test error was small. The model exhibited good fitting degree and high stability, which provided a good mathematical model to optimize high-quality instant sea cucumber products.

Response Surface Analysis

The data in Table 4 were subjected to quadratic multiple regression fitting by using Design-Expert 8.0.6. The response surface analysis of surface collagen and polysaccharide and the sensory evaluation of instant sea cucumber are shown in Fig. 2. The response images of the two interactions between the two factors indicated that the interaction of the experimental factors was significant. The 3D response surface is the graphical representation of the regression function. These images were obtained by maintaining two of the variables constant, which corresponded to the changes in the collagen and

polysaccharide contents and sensory quality under different conditions. The effects of response surface were significant when the curves were steep but were unremarkable when the curves were flat. The most significant interaction effect was sensory score, followed by polysaccharide and collagen contents (Fig. 2). The quadratic polynomial regression model and response surface analysis results demonstrated that the regression model yielded a stable point, with three response values combined the highest point, under the following conditions: Cooking time of 17 min, cooking temperature of 83°C, sterilization time of 10 min and sterilization temperature of 103°C.

Verification Test

Three sets of repeated tests were conducted under the obtained optimum conditions. The sea cucumber consisted of a collagen content of (5.263±0.020%), a polysaccharide content of (1.021±0.068%) and a sensory score of 97.27±0.85 and these findings are consistent with the respective theoretical predictions of 5.270, 1.0163 and 97.00. Therefore, the regression model can be used to describe the influence of each factor on the response value and reliably predict the quality of instant sea cucumber products.

Table 5. Analysis of variance for response surface regression model

Variance sources	Sum of squares	Freedom	Mean square	F	P
Collagen protein (%)					
Model	1.140	14	0.082	62.1	<0.0001
X ₁ (Cooking time)	0.350	1	0.350	269.62	<0.0001
X ₂ (Boiling temperature)	0.019	1	0.019	14.19	0.0021
X ₃ (Sterilization time)	0.100	1	0.100	75.9	<0.0001
X ₄ (Sterilization temperature)	0.083	1	0.083	63.29	<0.0001
X ₁ X ₂	0.015	1	0.015	11.51	0.0044
X ₁ X ₃	0.028	1	0.028	20.97	0.0004
X ₁ X ₄	0.070	1	0.070	53.04	<0.0001
X ₂ X ₃	0.013	1	0.013	10.24	0.0064
X ₂ X ₄	0.019	1	0.019	14.6	0.0019
X ₃ X ₄	0.038	1	0.038	28.94	<0.0001
X ₁ ²	0.360	1	0.360	276.96	<0.0001
X ₂ ²	0.084	1	0.084	63.59	<0.0001
X ₃ ²	0.020	1	0.020	15.14	0.0016
X ₄ ²	0.051	1	0.051	39.1	<0.0001
Regression value	0.018	14	1.31E-03		
Missing item	0.017	10	1.69E-03	4.4	0.0832
residual	1.53E-03	4	3.84E-04		
Total residuals	1.16	28			
Polysaccharide (%) model					
X ₁ (Cooking time)	3.169 E-003	1	3.169 E-003	107.86	<0.0001
X ₂ (Boiling temperature)	9.026 E-003	1	9.026 E-003	307.22	<0.0001
X ₃ (Sterilization time)	1.687 E-003	1	1.687 E-003	57.44	<0.0001
X ₄ (Sterilization temperature)	1.448 E-003	1	1.448 E-003	49.28	<0.0001
X ₁ X ₂	2.550 E-003	1	2.550 E-003	86.81	<0.0001
X ₁ X ₄	9.000 E-004	1	9.000 E-004	30.64	<0.0001
X ₂ X ₃	1.897 E-003	1	1.897 E-003	64.56	<0.0001
X ₂ X ₄	6.003 E-004	1	6.003 E-004	20.43	0.0005
X ₃ X ₄	2.894 E-003	1	2.894 E-003	98.52	<0.0001
X ₁ ²	2.758 E-003	1	2.758 E-003	93.87	<0.0001
X ₂ ²	1.035 E-003	1	1.035 E-003	35.23	<0.0001
X ₃ ²	1.575 E-003	1	1.575 E-003	53.61	<0.0001
X ₄ ²	4.324 E-003	1	4.324 E-003	147.19	<0.0001
Regression value	4.113 E-004	14	2.938 E-005		
Missing item	2.135 E-004	10	2.135 E-005	0.43	0.8718
residual	1.978 E-004	4	4.946 E-005		
Total residuals	0.031	28			
Sensory score model					
X ₁ (Cooking time)	8.07	1	8.07	5.4	0.0356
X ₂ (Boiling temperature)	35.09	1	35.09	23.5	0.0003
X ₃ (Sterilization time)	46.18	1	46.18	30.93	<0.0001
X ₁ X ₂	17.98	1	17.98	12.04	0.0038
X ₁ X ₄	62.33	1	62.33	41.75	<0.0001
X ₂ X ₃	98.8	1	98.8	66.18	<0.0001
X ₂ X ₄	7.18	1	7.18	4.18	0.0457
X ₁ ²	142.99	1	142.99	95.77	<0.0001
X ₂ ²	204.88	1	204.88	137.23	<0.0001
X ₃ ²	314.9	1	314.9	210.92	<0.0001
X ₄ ²	49.77	1	49.77	33.34	<0.0001
Regression value	20.9	14	1.49		
Missing item	18.67	10	1.87	3.34	0.1282
residual	2.24	4	0.56		
Total residuals	788.38	28			

Table 6. Second-order polynomial equations and coefficients of determinations of response variables

Response value	Two regression equation	R^2	R_{Adj}^2	CV (%)
Collagen protein	$Y = 5.37 - 0.17X_1 - 0.039X_2 - 0.091X_3 - 0.083X_4 + 0.062X_1X_2 - 0.083X_1X_3$ $- 0.13X_1X_4 + 0.058X_2X_3 + 0.069X_2X_4 - 0.097X_3X_4 - 0.24X_1^2 - 0.11X_2^2$ $- 0.055X_3^2 - 0.089X_4^2$	0.9842	0.9683	0.70
Polysaccharide	$Y = 1.00 + 0.016X_1 + 0.027X_2 - 0.012X_3 - 0.011X_4 + 0.025X_1X_2$ $- 2.5E - 003X_1X_3 - 0.015X_1X_4 + 0.022X_2X_3 + 0.012X_2X_4 - 0.027X_3X_4$ $- 0.21X_1^2 - 0.013X_2^2 - 0.016X_3^2 - 0.026X_4^2$	0.9868	0.9736	0.56
Sensory evaluation	$Y = 97.97 + 0.082X_1 + 0.171X_2 + 1.96X_3 - 0.12X_4 - 2.12X_1X_2 - 0.50X_1X_3$ $+ 3.95X_1X_4 + 4.97X_2X_3 - 1.34X_2X_4 + 0.18X_3X_4 - 4.70X_1^2 - 5.62X_2^2$ $- 6.97X_3^2 - 2.77X_4^2$	0.9735	0.9470	1.36

Conclusion

In this study, fuzzy mathematics was applied to evaluate the sensory quality of instant sea cucumber. The overall quality of instant sea cucumber was also monitored by measuring its collagen and polysaccharide contents. Our single factor experiments and response surface analysis revealed the following optimal processing conditions: Cooking time, 17 min; cooking temperature, 83°C; sterilization time, 10 min; and sterilization temperature, 103°C. Under these conditions, the protein content, polysaccharide content and sensory score were 5.263±0.020%, 1.021±0.068% and 97.27±0.85, respectively and these findings were consistent with the predicted values. The model could reliably predict the quality of instant sea cucumber products. Worker, processing and equipment requirements were low and the process was simple and easily operated. This study provided a basis for the production of sea cucumber products.

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Author's Contributions

Jianfeng Sun: Involved in sample preparation, study design, performing in the laboratory work and manuscript writing and revising and scientific discussion.

Tianshu Yang and Qian Liu: Involved in study design, manuscript writing and scientific discussion.

Zhaoping Sun: Involved in manuscript writing.

Ethics

The authors declare that they have no conflict of interest. All authors have read and approved the manuscript and no ethical issues involved.

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