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Optimization of Processing Technology and Quality Characteristics of Dual-Protein Composite Quinoa Fish Cake

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Abstract
To address the poor nutrition and limited functionality of traditional fish cakes, a dual-protein quinoa fish cake was developed using grass carp surimi, quinoa flour, soy protein isolate (SPI), whey protein (WP), and TG enzyme. Single-factor and response surface experiments were conducted to optimize SPI, WP, TG enzyme, and quinoa flour levels based on sensory and water retention scores. Results showed that SPI had the greatest impact on quality, followed by WP, TG enzyme, and quinoa flour. The optimal formula was: SPI 4.3%, WP 0.3%, TG enzyme 0.4%, quinoa flour 8.1%, yielding a composite score of 93.1 (close to the predicted value). The optimized fish cake exhibited good elasticity, water retention, sensory quality, quinoa flavor, and high nutritional value. These findings provide a reference for using quinoa in surimi products and developing healthy fish cakes.

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Keywords: Quinoa fish cake, dual-protein formulation, soy protein isolate (SPI), whey protein, transglutaminase (TG) enzyme

1. Introduction

Quinoa is a kind of “fully nutritious food” recommended by the Food and Agriculture Organization of the United Nations. It is rich in high-quality protein, dietary fiber, vitamins and a variety of plant active ingredients. It has high nutritional and functional value. In recent years, large-scale cultivation has been formed in our country[1].At present, quinoa has been widely used in biscuits, bread, energy foods and fermented products[2-3], but its development and research in surimi products is still relatively small.As an important direction in the processing of aquatic products, surimi products have good nutritional value and market prospects. Their gel properties, water retention and texture quality are important factors affecting product quality[4-9].In recent years, research on the application of vegetable protein in surimi products has been increasing. Soy protein, pea protein and whole grain powder can not only improve the nutritional structure of the product, but also improve the quality and health properties of the product[10-14].In the context of “Healthy China 2030” and “Dual Protein Project”[15], this research is based on grass carp surimi, quinoa flour is combined with soy protein isolate and whey protein to develop a new type of complex fish cake with more comprehensive nutrition, and explore its effects on the gel properties, water retention and sensory quality of surimi, with a view to providing theoretical basis and practical reference for the deep processing and utilization of quinoa and the development of healthy surimi products.

2. Materials and methods

2.1. Experimental materials and equipment

2.2.1. Main raw materials and reagents

Table 1: List of Raw materials and Accessories

Materials and Reagents	Level	Manufacturer
Fresh Grass Carp	Food Grade	Jingmen Agricultural Wholesale Market, Hubei
Soy Protein Isolate	Food Grade	Zhengzhou Zhongyan Food Trading Co., Ltd., Henan
Whey Protein	Food Grade	Zhengzhou Zhongyan Food Trading Co., Ltd., Henan
Glutamine Transaminase (TGase)	Food Grade	Zhengzhou Zhongyan Food Trading Co., Ltd., Henan
Quinoa Flour	Food Grade	Qinghai Sanjiang Wotu Food Development Co., Ltd.
Sweet Potato Starch	Food Grade	Beijing Gusong Economic and Trade Co., Ltd.
Table Salt	Food Grade	Jingmen Agricultural Wholesale Market, Hubei
Lard	Food Grade	Jingmen Agricultural Wholesale Market, Hubei
Sucrose	Food Grade	Jingmen Agricultural Wholesale Market, Hubei
Cooking Wine	Food Grade	Jingmen Agricultural Wholesale Market, Hubei
Eggs	Food Grade	Jingmen Agricultural Wholesale Market, Hubei
Ginger-Scallion Water	Food Grade	Jingmen Agricultural Wholesale Market, Hubei

2.1.2. Instruments and equipment

Table 2: List of Instruments and equipment

Name	Brand/Model	Manufacturer
Mixer	QSJ-S03G8	Bear Electric Appliance Co., Ltd.
Centrifuge	L535R	Hunan Xiangyi Laboratory Instrument Development Co., Ltd.
Electronic Analytical Balance	FA224C	Shanghai Lichen Bangxi Instrument Technology Co., Ltd.
Digital Electronic Scale	EK4150	SENSSUN
Induction Cooker	WK2102	Tianjin Taisite Instrument Co., Ltd.
Freezer	BD-138WGH90WF	Haier
Texture Analyzer	TA-XT2i	Shanghai Tengba Instrument Technology Co., Ltd.
Colorimeter	WR-10	FRU

2.2. Experimental method

2.2.1. Processing technology of double-protein compound quinoa fish cake

Frozen surimi→thawing→ air ring → seasoning ring (adding formula ingredients and seasonings) → mold forming → high temperature heating → release → cooling→ preservation, packaging → refrigeration

2.2.2. Sensory evaluation method

Refer to the sensory characteristic evaluation system of surimi products.The higher the score of indicators such as smell, color, and overall acceptability of fish cakes, the better the quality of fish cakes [16].The table of sensory evaluation criteria is shown in Table 2-3.

Table 3: Sensory Evaluation Criteria Table

Dimension	Sensory Quality	Scoring Standard
Color	Uniformly clean white with consistent color, glossy and oily surface	4-5
	Slight color difference with dimmer surface gloss	2-3
	Obvious mottling with dry, dull surface	1-2
Flavor	Harmonious quinoa aroma and fish umami, balanced saltiness with pleasant aftertaste	4-5
	Single aroma, overly salty or bland with plain aftertaste	2-3
	Obvious off-flavors, overly salty or tasteless with poor aftertaste	1-2
Texture	Good elasticity, fine and tender texture	4-5
	Slightly hard or soft with minor rough mouthfeel	2-3
	No elasticity, loose and dry-hard texture	1-2
Quinoa Harmony	Natural integration of quinoa powder and fish paste, no separation or powdery feel	4-5
	Weak or overly strong quinoa flavor with slight powdery feel	2-3
	Separated flavors, obvious powdery feel or agglomeration	1-2

2.2.3. Determination of water retention

Refer to Han Bing, Xu Yingnan and other methods and slightly modify them [17].

2.2.4. Formula single-factor experimental design

The basic formulation of fish cake consisted of 10% sweet potato starch (based on surimi mass), 2% salt, 4% sucrose, 1% cooking wine, 8% egg white, 5% oil, and 30% onion–ginger water (prepared by soaking 30 g onion and 20 g ginger in 500 g boiling water for 10 min).Soy protein isolate, whey protein, TG enzyme, and quinoa flour were selected as independent variables for single-factor experiments. Sensory score and water-holding capacity were used as evaluation indices to determine the appropriate addition levels, and the comprehensive score was preliminarily analyzed, as shown in Table 2-4.

Table 4: Single-factor test factor level design

Factor	Level					
	1	2	3	4	5	6
Soy protein isolate (A)/%	0.0	2.0	4.0	6.0	8.0	10.0
Whey protein (B)/%	0.0	0.1	0.2	0.3	0.4	0.5
TG enzyme (C)/%	0.0	0.2	0.4	0.6	0.8	1.0
Quinoa flour (D)/%	0.0	4.0	8.0	12.0	16.0	20.0

2.2.5. Experimental design of response surface optimization

Using the Box-Behnken model, based on the results of the single-factor test, the four factors of soy protein isolate (A), whey protein (B), TG enzyme (C), and quinoa flour (D) were used as the investigation variables. Each factor took three levels and was encoded with -1, 0, and +1. The sensory score and the comprehensive score of water retention[18] (Based on the single-factor experiment, a simple mathematical model was constructed to obtain a comprehensive score) As the response value, and the experimental design was carried out with Design-Expert 8.0.6, as shown in Table 2-5.

Table 5: Response surface design factor Level Table

Factor	Level		
	-1	0	+1
Soy protein isolate (A)/%	2.0	4.0	6.0
Whey protein (B)/%	0.2	0.3	0.4
TG enzyme (C)/%	0.2	0.4	0.6
Quinoa flour (D)/%	4.0	8.0	12.0

3. Results and discussion

3.1. Single-factor test results and analysis

3.1.1. Effect of the amount of soy protein isolate added on product quality

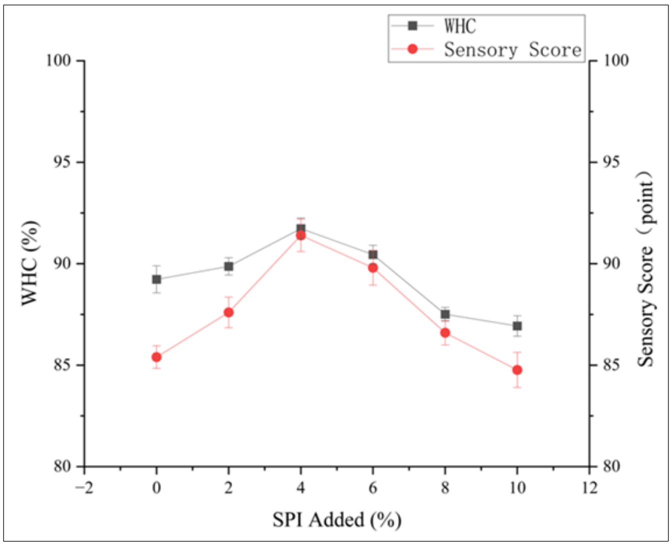

Fig 1: The effect of the addition of soy protein isolate on the sensory score and water retention of fish cakes

The sensory score and water retention of fish cake products changed with the addition of soy protein isolate, as shown in Figure 3-1. Soy protein isolate had a significant effect on the quality of fish cakes. With the addition of soy protein isolate, a gel structure was formed during heating, which improved the gel properties and enhanced the water retention of fish cakes. However, excessive addition caused the water retention to decrease slightly, possibly because high levels of soy protein isolate weakened the gel-forming ability of proteins and reduced gel strength.The sensory score showed a trend similar to water retention. As the amount of soy protein isolate increased, the sensory score gradually improved, but excessive addition led to a decline in sensory quality. This may be due to reduced water retention, resulting in lower moisture content and poorer elasticity of the fish cakes, which negatively affected texture and taste. In addition, excessive soy protein isolate may also affect product color. Therefore, 4% soy protein isolate was selected as the optimal addition level.

3.1.2. The effect of whey protein addition on product quality

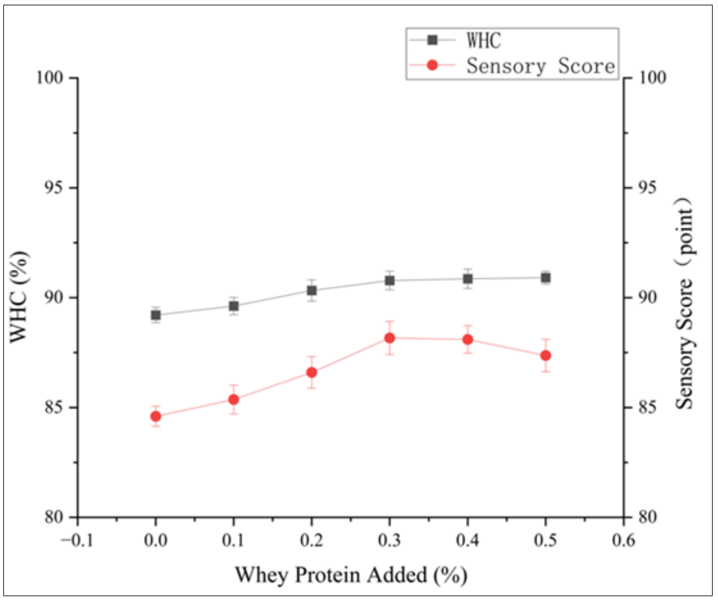

Fig 2: The effect of whey protein addition on the sensory score and water retention of fish cakes

The sensory score and water retention of fish cake products with different whey protein additions are shown in Figure 3-2. As the amount of whey protein increased, water retention showed a gradual upward trend. This may be because whey protein interacts with surimi proteins to form a denser gel network, thereby improving water retention. In addition, whey protein itself has water-binding and water-holding properties, which may also contribute to this increase. However, the overall effect was relatively limited compared with soy protein isolate. Whey protein addition improved gel strength, water retention, and overall juiciness of the fish cakes[18]. However, excessive addition negatively affected flavor, leading to a decline in sensory scores. Therefore, 0.3% whey protein was selected as the optimal level.

3.1.3. Effect of TG enzyme addition on product quality

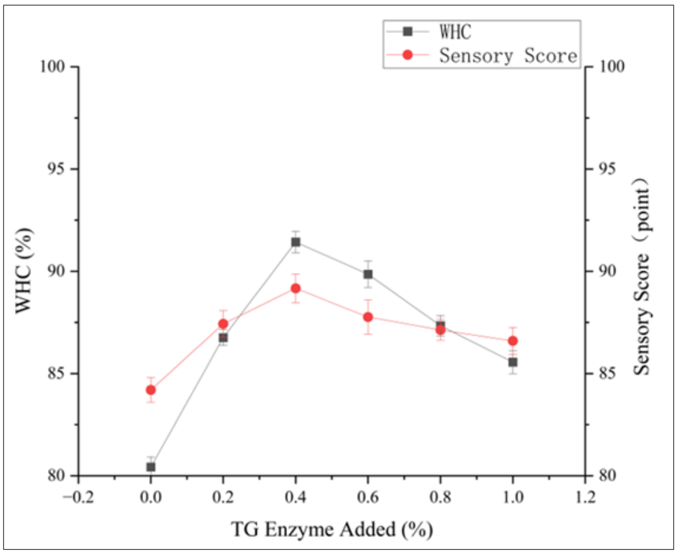

Fig 3: The effect of the amount of TG enzyme added on the sensory score and water retention of fish cakes

The sensory score and water retention of fish cake products with different TG enzyme additions are shown in Figure 3-3. TG enzyme had a significant effect on water retention. Without TG enzyme, the water retentionwas relatively low. As the addition level increased, water retention first increased and then decreased. This is because TG enzyme promotes cross-linking between surimi proteins and added proteins (soy protein isolate and whey protein), forming a more stable gel network and thus improving water-holding capacity. However, when the addition exceeded 0.4%, water retention began to decline, possibly due to excessive cross-linking reducing available charged groups and weakening gel properties[19].TG enzyme also improved the elasticity and hardness of fish cakes, leading to higher sensory scores. However, excessive addition negatively affected gel structure, resulting in reduced water retention and sensory quality[18]. Therefore, 0.4% TG enzyme was selected as the optimal level.

3.3.4. The effect of the amount of quinoa flour added on product quality

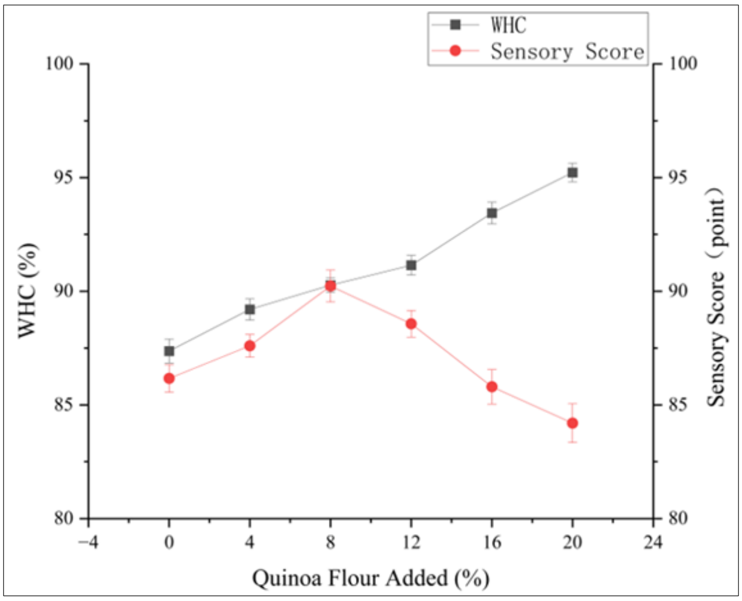

Fig 4: The effect of the amount of quinoa flour added on the sensory score and water retention of fish cakes

The sensory score and water retention of fish cake products with different quinoa flour additions are shown in Figure 3-4. As the amount of quinoa flour increased, water retention gradually increased. This may be because quinoa flour can absorb and retain water, and its proteins can also interact with fish cake proteins during heating to form a gel network, thereby improving water-holding capacity[20].The addition of quinoa flour also imparted a characteristic aroma, which improved the sensory score. However, excessive addition led to moisture loss, resulting in a dry and hard texture, while also masking the original flavor of the fish cake and causing sensory quality to decline. Therefore, 8% quinoa flour was selected as the optimal level.

3.3.5. Construction of comprehensive score

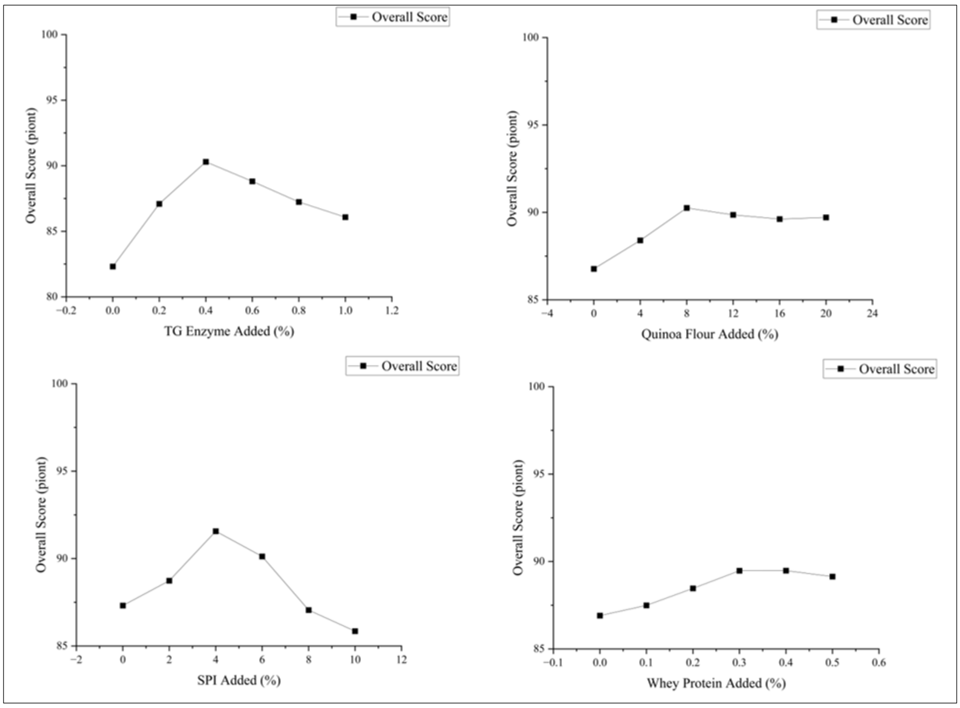

Fig 5: Comprehensive score Summary Chart

From the single-factor test, it can be understood that the changes in water retention and sensory scores are roughly the same or opposite trend, so the weights of the two indicators are set to the same, and the calculation formula is (sensory score + water retention value)/2. The resulting comprehensive score chart is shown in Figure 3-5. The results obtained are exactly the same as the single-factor analysis, so it can be used as the response value for response surface optimization.

3.2. Response surface optimization test results and analysis

According to the design principle of the Box-Benhnken experiment, the comprehensive score is used as the response variable, and the response surface optimization analysis of the four factors is carried out, as shown in Table 2-5.Using a four-factor three-level Box-Behnken design, there are a total of 29 test points (including 5 center point repetitions), as shown in Table 3-1.

Table 1: Response surface design table

Experiment number	A/%	B/%	C/%	D/%	Sensory score	Water-holding	Comprehensive score
1	2	0.2	0.4	8	86.6	88.42	87.5
2	6	0.2	0.4	8	87.4	90.43	88.9
3	2	0.4	0.4	8	86.2	90.05	88.1
4	6	0.4	0.4	8	90.2	91.06	90.6
5	4	0.3	0.2	4	88.6	87.61	88.1
6	4	0.3	0.6	4	89.6	90.03	89.8
7	4	0.3	0.2	12	83.4	95.01	89.2
8	4	0.3	0.6	12	85.4	93.46	89.4
9	2	0.3	0.4	4	86.8	88.62	87.7
10	6	0.3	0.4	4	89.8	90.85	90.3
11	2	0.3	0.4	12	87.8	89.88	88.8
12	6	0.3	0.4	12	85.2	93.69	89.4
13	4	0.2	0.2	8	88.4	88.61	88.5
14	4	0.4	0.2	8	89.4	90.40	89.9
15	4	0.2	0.6	8	86.6	91.27	88.9
16	4	0.4	0.6	8	88.6	90.83	89.7
17	2	0.3	0.2	8	87.0	89.21	88.1
18	6	0.3	0.2	8	89.2	90.26	89.7
19	2	0.3	0.6	8	88.8	89.45	89.1
20	6	0.3	0.6	8	86.2	92.03	89.1
21	4	0.2	0.4	4	87.6	89.07	88.3
22	4	0.4	0.4	4	88.2	89.40	88.8
23	4	0.2	0.4	12	87.8	88.63	88.2
24	4	0.4	0.4	12	86.4	92.87	89.6
25	4	0.3	0.4	8	93.2	94.03	93.6
26	4	0.3	0.4	8	93.4	93.21	93.3
27	4	0.3	0.4	8	92.8	94.02	93.4
28	4	0.3	0.4	8	93.4	93.27	93.3
29	4	0.3	0.4	8	92.4	93.88	93.1

The results of the variance analysis of the response surface were obtained using Design Expert8.0.6 software, as shown in Table 3-2.

Table 2: Response surface analysis of variance results

Source of Variance	**Sum of Squares**	**df**	**Mean Square**	**F**	**P**	**Significance**
Model	92.12	14	6.58	55.18	< 0.0001	Significant
A	6.31	1	6.31	52.89	< 0.0001	***
B	3.41	1	3.41	28.62	0.0001	**
C	0.52	1	0.52	4.37	0.0554	Not significant
D	0.21	1	0.21	1.79	0.2024	Not significant
AB	0.30	1	0.30	2.54	0.1335	Not significant
AC	0.64	1	0.64	5.37	0.0362	*
AD	1.00	1	1.00	8.39	0.0117	*
BC	0.090	1	0.090	0.75	0.3996	Not significant
BD	0.20	1	0.20	1.70	0.2136	Not significant
CD	0.56	1	0.56	4.72	0.0475	*
A^2	32.69	1	32.69	274.15	< 0.0001	***
B^2	33.79	1	33.79	283.38	< 0.0001	***
C^2	25.17	1	25.17	211.10	< 0.0001	***
D^2	31.61	1	31.61	265.07	< 0.0001	***
Residual	1.67	14	0.12			
Lack of Fit	1.54	10	0.15	4.66	0.0757	Not significant
Pure Error	0.13	4	0.033			
Total	93.79	28				

3.2.1. Establishment of regression equation

Use Design Expert8.0.6 software to process the experimental data in Table 3-1 to obtain the quadratic multiple regression equation: $Y = + 93.34 + 0.72*A + 0.53*B + 0.21*C + 0.13*D + 0.28*AB - 0.40*AC - 0.50*AD - 0.15*BC + 0.22*BD - 0.38*CD - 2.24A^2 - 2.28B^2 - 1.97C^2 - 2.21C^2$.As can be seen from Table 3-2, the model F is 92.12 and $P \leq 0.0001$, indicating that the model is very significant; the distortion term F is 4.66, and P is 0.0757>0.05, indicating that the distortion term is not significant; the decision coefficient $R2$ is 0.9822, and the correction decision coefficient R_{adj2} is 0.9644.The above analysis shows that this model has a high degree of fit and can fully fit the experimental data, so the model can predict the experimental results.According to the F value, it can be seen that the significant influence of factors is: the amount of soy protein isolate added > the amount of whey protein added> the amount of TG enzyme added> the amount of quinoa flour added.

3.2.2. Analysis of the interaction of two factors

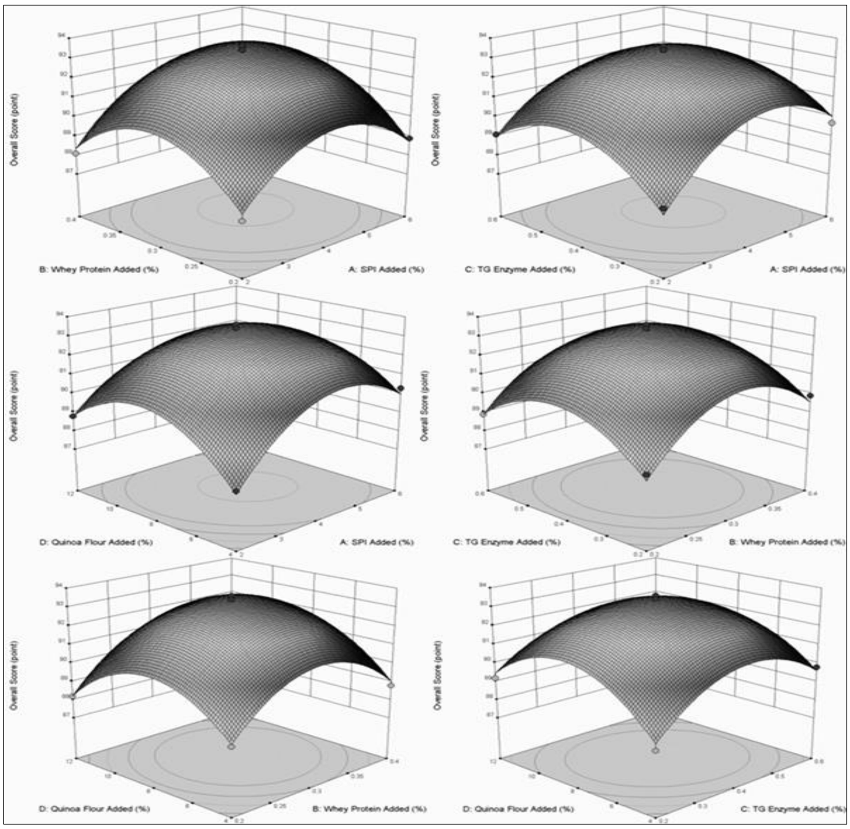

Fig 6: Three-dimensional analysis diagram of the response surface of the interaction of various factors

Using Design-Expert 8.0.6, response surface plots and contour plots were generated for Figure 3-6. The response surfaces showed a downward-opening pattern, indicating that the fish cake score initially increased and then decreased with increasing factor levels, and that an optimum existed for each interaction. A steeper surface suggests a stronger factor effect, while more elliptical contour lines indicate a more significant interaction; by contrast, circular contours indicate a weaker interaction. In Figure 3-6, the contour plots for soy protein isolate (A) and TG enzyme (C), A and quinoa flour (D), and C and D are the most elliptical, showing significant interactions on fish cake score ($P < 0.05$), which agrees with Table 3-2. Among these, the response surface for A and D is the steepest, indicating the most pronounced change in response and the strongest interaction. Excessive A may bind too much water, thicken the protein hydration layer, hinder cross-linking, and reduce water retention [19]. The significant interactions involving D suggest that quinoa flour contributes a distinctive flavor, while its combination with A and C improves gel structure and fish cake quality. Overall, increasing the level of each factor first enhanced and then reduced the fish cake score, confirming the presence of an optimal addition level.

3.2.3. Respond to interview test optimization results

Using Design-Expert 8.0.6, the optimal formulation for the dual-protein composite quinoa fish cake was determined to be 4.329% soy protein isolate, 0.313% whey protein, 0.406% TG enzyme, and 8.064% quinoa flour, with a predicted comprehensive score of 93.438. For practical production, the formulation was adjusted to 4.3% soy protein isolate, 0.3% whey protein, 0.4% TG enzyme, and 8.1% quinoa flour. Three validation experiments yielded an average comprehensive score of 93.1, which was close to the predicted value, indicating good agreement between the model prediction and the experimental results. These findings suggest that the optimized process parameters obtained by response surface methodology are reliable.

4. Conclusion

As a traditional food in China, fish cake is nutritious, but the market is characterized by product diversity with relatively uniform taste. In this study, response surface methodology was used to optimize the formulation of a dual-protein composite quinoa fish cake based on single-factor experiments. The optimal formulation was determined as follows: soy protein isolate 4.3%, whey protein 0.3%, TG enzyme 0.4%, and quinoa flour 8.1%, yielding a comprehensive score of 93.1. The optimized product showed improved flavor and nutritional value, providing additional options for the fish cake market. However, due to limitations in experimental conditions and design, certain deviations in the results may exist. This study therefore provides only a preliminary investigation of the interactions among ingredients in composite quinoa fish cake and offers a reference for further in-depth research.

5. Funding

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