Response Surface Optimization of Conditions for Gel Preparation of Perccottus Glenii Myofibril Proteins

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Abstract. In order to improve the quality of Perccottus gleniis myofibril protein gel, the index of hardness and springiness, the single factor experiment and response surface methodology were used to optimize the conditions of heating temperature, PH value and heating time in the process. Results showed that the optimal conditions for strengthening gelation properties of P. glenii myofibril were 84\degree C, pH6.5, 49 min, at the same time, the hardness and springiness values were 0.856 and 76.825, in this condition Proven test to obtain hardness and elasticity were 76.798 and 0.857, which were close to the predicted value. Results showed that the model could could be used in the well on process optimization.

Introduction

Old fish (Perccottus glenii) is one of the main fresh-water fish species in China [1], located in the northern part of Korea and Heilongjiang River, Liaohe River, etc. The study found that the fish meat extract [2] could increase the hypoxia tolerance and strengthen the immune system, it is favored by consumers for its tender meat, delicious, thorn less and unique flavor for its tender meat, delicious, thornless and unique flavor, favored by consumers, thus promoting and promote the development of P. glenii farming industry. In terms of the development and market prospects of glenii, the functional properties of the muscle proteins understanding will be the first step in product development.

Muscle Protein is the main component of myofibril protein, accounting for 50% to 60% protein content. The quality and characteristics of the meat has a very important influence on their functional properties of myofibrils such as solubility, emulsification, gelation properties, water retention and dispersion and other meat processing quality [3]. Myofibril protein changes directly affect the sensory properties of protein (meat springiness, juicy, taste, etc.) [4]. Because freshwater fish gel processing capability is poor than seawater fish, which influence the myofibril protein gel formation, and it is crucial that increase product quality for improve gel properties.

At present, domestic and foreign scholars have more study on muscle protein gel properties, Chen and Jian et al [5, 6] study of pork and silver carp myofibril protein gel properties, respectively, found that gel WHC was positively correlated with gel hardness and temperature, the solution of pH value, extraction time significant affected; Researches in recent years, the influence of ultra-high pressure and rinsing process on gel properties report by Yan and Lu et al [7]; but about P. glenii gel properties of heating conditions have not been reported.

In this experiment, fresh P. glenii materials, discussion the impact of heating temperature, pH value, heating time on myofibril protein gel texture characteristics of hardness and elasticity. According to Box-Behnken center design principles and the actual production, the use of RSM
optimized of myofibril protein thermal gel preparation conditions. Aims to improve *P. glenii* muscle protein gel performance, in order to high-quality products provide a theoretical basis.

**Materials and Methods**

**Experiment Material**

Percottus glenii (Jilin Agricultural University to provide farmers, Changchun, China), sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, EDTA, magnesium chloride, sodium hydroxide, hydrochloric acid are all of analytical grade.

**Instruments and Equipments**


**Method**

**Myofibril Protein Extraction.** Myofibril protein was extracted according to the method of Liu [8] with some modifications, fish muscle was obtained by fresh *P. glenii* was peeled, bone, offal, then adding 4 volumes previously cooled at 4 °C of 50mM phosphate buffer solution (0.1M NaCl, 1mM EDTA, 2mM MgCl2, pH7.0) and homogenized for 60s using IKA dispersing machine at 8000 r, muscle homogenates was centrifugation at 8000g for 15 min 4 °C, washed 3 times, and then precipitated with 4 volumes of 0.1M NaCl solution washed three times under the same conditions as above, the obtained myofibril protein was storage at 4 °C, run out of within 36 hours. Concentration of myofibril proteins was measured using the Lowry method [9], using bovine serum albumin as the standard.

**Preparation of Myofibril Protein Gels.** Reference method of Xiao[10], the protein concentration was adjusted to 40 mg/mL with 50mM Na2HPO4 buffer, placed in 50mL beaker in a water bath heated from 25 °C heated to gels, then immediately cooled in the ice water, storage at 4 °C.

**Textural Properties of Myofibril Protein Gels.** TA. XT. Plus type texture analyzer probe select P/0.5, parameter was set before puncturing probe operation rate of 1.0 mm/s, puncture run rate of 1.0 mm/s, return rate of 1.00 mm/s, compression distance 3.0 mm, trigger type: auto, residence time of 5 s, trigger force 5.0 g, search and seize data rate of 200 pps.

**Experimental Design**

**Single-factor Design.**

<table>
<thead>
<tr>
<th>Level</th>
<th>Temperature (°C)</th>
<th>PH</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>5.0</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>5.5</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>6.0</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>6.5</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>7.0</td>
<td>50</td>
</tr>
</tbody>
</table>

**Response Surface Assay.** The content of the heating temperature, buffer PH and heating time were selected with 3 variable factor (A, B and C). The central composite design was evaluated in response to sensory value (x ± n, n = 3). The data and the regression were fitted.
Table 2. Factors and levels of Box-Behnken.

<table>
<thead>
<tr>
<th>Level</th>
<th>Factor</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>A</td>
<td>70</td>
<td>6.0</td>
<td>30</td>
</tr>
<tr>
<td>0</td>
<td>B</td>
<td>80</td>
<td>6.5</td>
<td>40</td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>90</td>
<td>7.0</td>
<td>50</td>
</tr>
</tbody>
</table>

Statistical Analysis

Design Expert 8.0 software and Origin 8.0 were used to analyze.

Results and Analysis

The Results of Single Factor Test

As shown in Fig. 1. The hardness of gels decreased with the increasing of temperature at first again on the rise, the springiness was downward after the first rise, hardness and springiness reached the highest value at 90 °C and 80 °C respectively. The study was same as Li [4], he also found that 80 °C of springiness characteristics was obvious better than 70 °C, 90 °C, This is mainly as the temperature rises, myofibril protein denaturation gradually, to 80 °C has been basically completed denatured, and then continue to increase the heating temperature, it causes deterioration of the gel.

Fig. 2. Effect of protein gel hardness and elasticity of the pH value. With the increasing of pH value, after protein gel hardness increased and then decreased along first, at pH 6.0 the maximum value, leveling off at 6.5 to 7.0. For springiness, the effect of pH on the elasticity was relatively small, flexible change after the first decreases and then increases, but the overall trend was more gradual change, little change overall. This may change with the PH-protein amino acid side chains of the charge distribution, thus related of affecting electrostatic interactions between proteins [11].

Fig. 3. The hardness and springiness with the increase of heating time increases, this experiment results was similar to Li Yanan [12]. Hardness and springiness reached the highest value at 50min.

Results of Response Surface

Response Surface Optimization Test Data.

Table 3. Box-Behnken Design and Results.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Springiness</th>
<th>Hardness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.851</td>
<td>72.844</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.849</td>
<td>72.53</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
<td>-1</td>
<td>0.84</td>
<td>70.090</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.849</td>
<td>72.754</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.855</td>
<td>76.426</td>
</tr>
<tr>
<td>6</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>0.832</td>
<td>69.129</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.85</td>
<td>72.981</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>-1</td>
<td>-1</td>
<td>0.831</td>
<td>70.999</td>
</tr>
</tbody>
</table>
The data were analyzed by the Design-Expert 8.0.5. Experimental design and results of response surface were observed in Tab. 3. By applying multiple regression analysis on the experimental data, the response variable and the test variables were showed as the following second-order polynomial equation:

\[ Y_1 = 72.84 + 1.53A - 0.27B + 2.65C + 0.43AB + 0.5AC + 0.021BC - 1.98A^2 - 0.65B^2 + 1.26C^2 \]

\[ Y_2 = 0.85 + 6.25 \times 10^{-3} + 3.50 \times 10^{-3}B + 6.50 \times 10^{-3}C + 1.75 \times 10^{-3}AB + 1.75 \times 10^{-3}AC - 2.50 \times 10^{-3}BC - 6.125 \times 10^{-3}A^2 - 4.625 \times 10^{-3}B^2 - 2.625 \times 10^{-3}C^2 \]

Where \( Y_1 \) and \( Y_2 \) is Springiness and Hardness. A, B and C corresponding to the heating temperature, buffer pH, heating time, respectively. It is possible to obtain Eq. which showed the relationship between quantity of springiness, hardness and dependent variables.

Table 4. Regression Analysis of Variance for the Springiness.

<table>
<thead>
<tr>
<th>sources of variance</th>
<th>sum of square</th>
<th>DOF</th>
<th>mean square</th>
<th>F value</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>model</td>
<td>1.097E-003</td>
<td>9</td>
<td>1.198E-004</td>
<td>66.53</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>A</td>
<td>3.125E-004</td>
<td>1</td>
<td>3.125E-004</td>
<td>168.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B</td>
<td>9.800E-005</td>
<td>1</td>
<td>9.800E-005</td>
<td>52.77</td>
<td>0.0002</td>
</tr>
<tr>
<td>C</td>
<td>3.380E-004</td>
<td>1</td>
<td>3.380E-004</td>
<td>182.00</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AB</td>
<td>1.225E-005</td>
<td>1</td>
<td>1.225E-005</td>
<td>6.60</td>
<td>0.0371</td>
</tr>
<tr>
<td>AC</td>
<td>1.225E-005</td>
<td>1</td>
<td>1.225E-005</td>
<td>6.60</td>
<td>0.0371</td>
</tr>
<tr>
<td>BC</td>
<td>2.500E-007</td>
<td>1</td>
<td>2.500E-007</td>
<td>0.13</td>
<td>0.7245</td>
</tr>
<tr>
<td>A^2</td>
<td>1.580E-004</td>
<td>1</td>
<td>1.580E-004</td>
<td>85.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B^2</td>
<td>9.007E-005</td>
<td>1</td>
<td>9.007E-005</td>
<td>48.50</td>
<td>0.0002</td>
</tr>
<tr>
<td>C^2</td>
<td>2.901E-005</td>
<td>1</td>
<td>2.901E-005</td>
<td>15.62</td>
<td>0.0055</td>
</tr>
<tr>
<td>residuals</td>
<td>1.300E-005</td>
<td>7</td>
<td>1.857E-006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lack of fit items</td>
<td>9.000E-006</td>
<td>3</td>
<td>3.000E-006</td>
<td>3.00</td>
<td>0.1581</td>
</tr>
<tr>
<td>errors</td>
<td>4.000E-006</td>
<td>4</td>
<td>1.000E-006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sum</td>
<td>1.092E-003</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ R^2 = 0.9881, \text{R}^2_{\text{Adj}} = 0.9728 \]

Notes: **.P<0.01, very significant difference; *.P<0.05, Significant difference, the same as below.

Table 5. Regression Analysis of Variance for the Hardness.

<table>
<thead>
<tr>
<th>sources of variance</th>
<th>sum of square</th>
<th>DOF</th>
<th>mean square</th>
<th>F value</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>model</td>
<td>101.18</td>
<td>9</td>
<td>11.24</td>
<td>76.13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>A</td>
<td>18.62</td>
<td>1</td>
<td>18.62</td>
<td>126.10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B</td>
<td>0.60</td>
<td>1</td>
<td>0.60</td>
<td>4.09</td>
<td>0.0830</td>
</tr>
<tr>
<td>C</td>
<td>56.12</td>
<td>1</td>
<td>56.12</td>
<td>380.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AB</td>
<td>0.74</td>
<td>1</td>
<td>0.74</td>
<td>5.03</td>
<td>0.0599</td>
</tr>
<tr>
<td>AC</td>
<td>1.00</td>
<td>1</td>
<td>1.00</td>
<td>6.77</td>
<td>0.0353</td>
</tr>
<tr>
<td>BC</td>
<td>1.764E-003</td>
<td>1</td>
<td>1.764E-003</td>
<td>0.012</td>
<td>0.9160</td>
</tr>
<tr>
<td>A^2</td>
<td>16.47</td>
<td>1</td>
<td>16.47</td>
<td>111.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B^2</td>
<td>1.76</td>
<td>1</td>
<td>1.76</td>
<td>11.92</td>
<td>0.0107</td>
</tr>
<tr>
<td>C^2</td>
<td>6.67</td>
<td>1</td>
<td>6.67</td>
<td>45.18</td>
<td>0.0003</td>
</tr>
<tr>
<td>residuals</td>
<td>1.03</td>
<td>7</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lack of fit items</td>
<td>0.84</td>
<td>3</td>
<td>0.28</td>
<td>5.82</td>
<td>0.0609</td>
</tr>
<tr>
<td>errors</td>
<td>0.19</td>
<td>4</td>
<td>0.048</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sum</td>
<td>102.21</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ R^2 = 0.9899, \text{R}^2_{\text{Adj}} = 0.9769 \]
**Response Surface Analysis of Variance.** Results of analysis of variance (Tab. 4 and 5), which was used to test the adequacy and significance of the response surface quadratic model. As can be known, the model indicated that the regression model was highly significant (p < 0.0001) \(^{[10]}\). F-value for the lack of fit was insignificant (P > 0.05) confirming the validity of the model. Generally, the hardness and springiness coefficient of determination (R\(^2\)) was 0.98999, 0.9881 respectively, the value of the adjusted determination coefficient (R\(^2\)adj = 0.9769, 0.9728 respectively) also confirmed that the model was highly significant. Therefore, the model can be used to analyze and predict the experimental values. Regression equations for each variable in response to the impact of significant value from the F test, items A, B, C, and quadratic A\(^2\), B\(^2\) and C\(^2\) showed a highly significant. The relationship between the various factors as: A (Temperature) > B (PH) > C (Time).

**Response Surface Optimization Figure.** Through optimization, when the heating temperature was 83.96 °C, pH 6.46, the heating time 49.20min, the gel hardness and springiness of the maximum. Bai et al [13] studies have shown that the longer time, higher temperature, the greater the degree of polymerization of proteins, the harder of formation gel. When the pH of the isoelectric point, due to the electrostatic repulsion is reduced and exposed hydrophobic groups, causing irregular protein aggregation, resulting in a low gel hardness \(^{[14]}\). The test results also showed that under appropriate heating temperature, pH and heating time, in favor of *P. gleniis* myofibril proteins form a good gel.
Conclusions

By Design-Expert analysis, consideration significance of various factors influence the response value, combined with the actual operation. The P.glenii gels effect optimum conditions set temperature 84°C, pH 6.5, time 49 min, under this condition, preparation of gel hardness and springiness was 76.798g, 0.857, respectively, close to the predicted value and have better level. Indicate selection appropriate of model, in this condition extracted myofibril proteins has better gel properties.

References


