Optimization of Collagen Extraction Process from Carp Scale

Xuguang Wang, Zhike Xie, Shuyan Yu, Ming He, Yingxin Wei, Haifang Xiao*
(School of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo 255049, Shandong, China)
Corresponding Author: Haifang Xiao

Abstract: In this experiment, collagen was extracted from the squid scales by water extraction. The content of collagen in the extract was determined by spectrophotometry. The effects of extraction temperature, ratio of material to liquid, pH value and extraction time on the extraction rate of collagen in carp scales were studied. From the single factor test, the better parameters for extracting collagen by water extraction were extraction temperature of 20 ~ 30°C, ratio of material to liquid of 1:20 ~ 1:30 (g/mL), pH of 5 ~ 6 and extraction time of 5h. Based on the above, three factors including extraction temperature, solid-liquid ratio and pH value which had great influence on the extraction rate of collagen were selected to carry out orthogonal test. The optimum process parameters for water extraction of collagen were as follows: extraction temperature of 20 °C, pH value of 6 and the ratio of material to liquid of 1:20 (g/mL).

Keywords: Carp scale of, Collagen, Water extraction

I. Introduction
Collagen is an important functional protein and closely related to cell proliferation, differentiation, exercise, immunity, joint lubrication, wound healing and so on[1-3]. Due to the special functions of collagen, it has been widely used in the fields of medicine, food, daily chemical, biosynthesis and other industries[4-6]. The main raw material sources of collagen products are the skin, bone and tendon of terrestrial mammals such as pig and cattle[7,8]. In recent years, many global mad cow disease, foot-and-mouth disease and other human-animal cross-epide epidemics have resulted in many regulations and restrictions. Products extracted from cow and other mammals can’t be used in food and medicine. As a result, people began to look for collagen extracted from aquatic animals[9-10]. Fish scales are rich in protein and various minerals, of which organic matter accounts for 41% to 55% of the total weight of fish scales, and inorganic matter is 38% to 46%. The most abundant substances in organic matter are proteins, especially collagen and ichthyepilin which account for more than 90% of fish scale organic matter. In general, fish scales are thrown away as waste in the processing of aquatic products. In this experiment, collagen was extracted from carp scales.

II. Materials and Methods

2.1 Experiment material
Carp scales were purchased from Zibo seafood market in Shandong Province of China. Analytical pure AR of Sodium acetate, hydrochloric acid, anhydrous ethanol, calcium hydroxide, phosphate, Coomassie Brilliant Blue G-250 and acetic acid were purchased Laiyangshuangshuang chemical Co., Ltd. Collagen and pepsin was purchased from Hangzhou Brothers Industry Co., Ltd.

2.2 Determination of collagen content
The collagen content was determined by Coomassie Brilliant Blue G-250 staining.

2.2.1 Establish of standard curve
Standard collagen solution of different concentrations (1mL) were mixed with 50mL coomace bright blue G-250 reagent, respectively. After shaken well, the mixture was placed for 2 min. The absorbance was determined by colorimetry at 595nm (maximum absorbance of collagen). The standard curve was shown in Figure 1. As shown in Figure 1, the concentrations of collagen standard solutions and their absorbances were good linearity.
Optimization of Collagen Extraction Process from Carp Scale

2.2.2 Determination of collagen content in extract

The collagen samples were diluted, the absorbances were within the range of the standard curve. The sample solution (1 mL) was mixed with 50 mL of Coomassie Brilliant Blue G-250 reagent and stood for 2 min. Then the absorbances were measured at 595 nm. The collagen concentration in the sample was calculated using the formula (1).

\[ C = n \times (A - 0.1778) \quad (\mu g/mL) \] (1)

The extraction rate (T) of collagen was defined as the ratio of the weight of collagen in the extract to the weight of the raw material, whereby the extraction rate of collagen was calculated according to formula (2):

\[ T = \frac{V}{G} \times \frac{C}{A} = \frac{V}{G} \times (833.333 \times A - 0.1778) \] (2)

Where:
- A - Absorbance of collagen extract / 595 nm;
- V - Volume of collagen extract (mL);
- G - The weight of the scale (g).

1.3 Single factor test

1.3.1 Effect of extraction temperature on extraction rate of collagen

Carp scales (1g) was added in 40 mL 0.5 mol/L acetic acid-sodium acetate solution (1:1 ratio by volume), and extracted for 5 h at 10°C, 20°C, 30°C, 40°C, 50°C, respectively. After filtration, the sample solution was taken after centrifugation, and the extraction rate of collagen was calculated.

1.3.2 Effect of ratio of material to liquid on extraction rate of collagen

Carp scales (1g) was added in a certain 0.5 mol/L acetic acid-sodium acetate solution to make the ratio of material to liquid 1:10, 1:20, 1:30, 1:40, 1:50 (g/mL), respectively, and extracted at 20°C for 5 h. After filtration, the sample solution was taken after centrifugation, and the extraction rate of collagen was calculated.

1.3.3 Effect of pH on extraction rate of collagen

Carp scales (1g) was added in corresponding concentrations of 40 mL of acetic acid-sodium acetate solution to make the pH was 4, 5, 6, 7, and 8, respectively, and extracted at 20°C for 5 h. After filtration, the sample solution was taken after centrifugation, and the extraction rate of collagen was calculated.

1.3.4 Effect of extraction time on extraction rate of collagen

Carp scales (1g) was added in 40 mL 0.5 mol/L acetic acid-sodium acetate solution and extracted at 20°C for 1 h, 2 h, 3 h, 4 h, 5 h, respectively. After filtration, the sample solution was taken after centrifugation, and the extraction rate of collagen was calculated.

1.4 Orthogonal test

On the basis of the single factor test, appropriate factors and levels were selected to carry out orthogonal test to obtain the optimal extraction process parameters of collagen (Table 1).

<table>
<thead>
<tr>
<th>Table 1 Factors and levels table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor level</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

Figure 1 Standard curve of collagen

DOI: 10.9790/2402-1308024448  www.iosrjournals.org
III. Results and Discussion

3.1 Single-factor experiments

3.1.1 Effect of extraction temperature on extraction rate of collagen

![Figure 2](image2.png)

*Figure 2* Effect of extraction temperature on extraction rate of collagen

The effect of extraction temperature on extraction rate of collagen was shown in Figure 2. As shown in Figure 2, as the extraction temperature increased, the extraction rate of collagen from carp scales increased first and then decreased. Therefore, the better extraction temperature of collagen from carp scales was 20 to 30°C.

3.1.2 Effect of the ratio of material to liquid on extraction rate of collagen

![Figure 3](image3.png)

*Figure 3* Effect of the ratio of material to liquid on extraction rate of collagen

The effect of the ratio of material to liquid on extraction rate of collagen was shown in Figure 3. As shown in Figure 3, the extraction rate of collagen from carp scales was increased with the increase of the ratio of material to liquid. The main reason was that the increase of the ratio of material to liquid increased the concentration difference and the diffusion speed. Therefore, the ratio of material to liquid was preferably 1:20 to 1:30.
3.1.3 Effect of pH on extraction rate of collagen

The effect of pH on collagen extraction rate was shown in Figure 4. As shown in Figure 4, the extraction rate of collagen increased first and then decreased with the pH value increase, because collagen has the lowest solubility at the isoelectric point. Therefore, it was preferable to select pH 5-6 in the water extraction of collagen from carp scales.

3.1.4 Effect of extraction time on extraction rate of collagen

The effect of extraction time on collagen extraction rate is shown in Figure 5. As shown in Figure 5, the extraction rate of collagen from carp scales was increased with the extension of extraction time. Therefore, the extraction time was preferably 5h.

3.2 Analysis of orthogonal test and verification test

Table 2 Results and analysis of collagen protein water-extraction orthogonal test

<table>
<thead>
<tr>
<th>Test number</th>
<th>Extration temperature/°C (A)</th>
<th>Material to liquid ratio (g/mL) (B)</th>
<th>pH value (C)</th>
<th>(D)</th>
<th>Extraction rate(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.779</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0.792</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0.815</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0.852</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0.855</td>
</tr>
</tbody>
</table>

Figure 4 Effect of pH on extraction rate of collagen

Figure 5 Effect of extraction time on extraction rate of collagen
The range analysis of the test results in Table 2 showed that the order of the effects of various factors on the extraction rate of collagen from carp scales was extraction temperature > pH value > material to liquid ratio. The optimal level combination of factors was A2B1C3. That was, the optimal extraction conditions were the extraction temperature of 20°C, the ratio of material to liquid to 1:20, and the pH of 6.

IV. Conclusion

In this experiment, water extraction was carried out to get collagen from carp scales. The results of single factor experiments showed that the extraction rate of collagen from carp scales increased first and then decreased with the increase of extraction temperature and pH. With the increase of the ratio of material to liquid and the extension of extraction time, the extraction rate of collagen from carp scales increased. The extraction time was selected to be 5h. Through orthogonal test, the optimal extraction parameters of collagen from carp scales by water extraction method were extraction temperature of 20°C, pH value of 6 and ratio of material to liquid to 1:20.

Acknowledgements

This work was supported by SDUT & Zibo City Integration Development Project(2017ZBXC004).

References