IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 13, Issue 8 Ser. II (August. 2019), PP 44-48
www.iosrjournals.org

Optimization of CollagenExtraction Processfrom CarpScale

Xuguang Wang, ZhikeXie, 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 experimentcollagen 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 extractiontemperature, 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 extractionof collagenwere 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*

Date of Submission: 21-08-2019 Date of Acceptance: 05-09-2019

I. Introduction

Collagen is an important functional proteinand 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'tbe direct usedin 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 andichthylepidin which accountfor 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

Carpscales werepurchased from Zibo seafood market in Shandong Province of China. Analytical pure AR ofSodium acetate, hydrochloric acid, anhydrous ethanol,calcium hydroxide,phosphate, Coomass Brilliant Blue G-250 and acetic acid were purchased Laiyangshuangshuang chemical Co., Ltd.Collagen and pepsin was purchased from Hangzhou Brothers IndustryCo., 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 shakenwell, 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 Figure1, the concentrations of collagen standard solutions and theirabsorbances were good linearity.

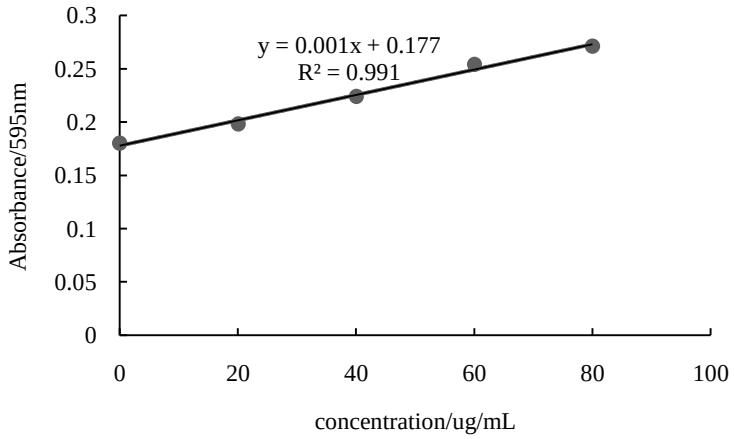

Figure 1standard curve ofcollagen

2.2.2 Determination of collagen content in extract

Tthe collagen sampleswere diluted, the absorbanceswere 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 weremeasuredat 595 nm. The collagen concentration in the sample was calculated using the formula (1).

$$C = n\ (833.333 \times A - 0.1778)\ (\ \mu g/mL\ ) \quad (1)$$

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):

$$T = C \cdot V / G = (833.333 \times A - 0.1778) \cdot V / G \quad (2)$$

Where:

A - Absorbance of collagen extract / 595nm;

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 rateof collagen

Carp scales (1g)was added in 40mL 0.5mol/L acetic acid-sodium acetate solution (1:1 ratio by volume), and extractedfor 5h 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 rateof collagen

Carp scales (1g)was added in a certain 0.5mol/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, andextractedat 20°C for 5h. 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 rateof collagen

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

1.3.4 Effect of extraction time onextraction rateof collagen

Carp scales (1g)was added in40mL 0.5mol/L acetic acid-sodium acetate solutionand extracted at 20°C for 1h, 2h, 3h, 4h, 5h, 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 parametersof collagen (Table 1).

Table 1 Factors and levels table

Factor level	temperature/°C (A)	Material to liquid ratio/ g：mL(B)	pH value (C)
1	10	1:20	4
2	20	1:30	5
3	30	1:40	6

III. Results and Discussion

3.1 Single-factor experiments

3.1.1 Effect of extraction temperature on extraction rateof collagen

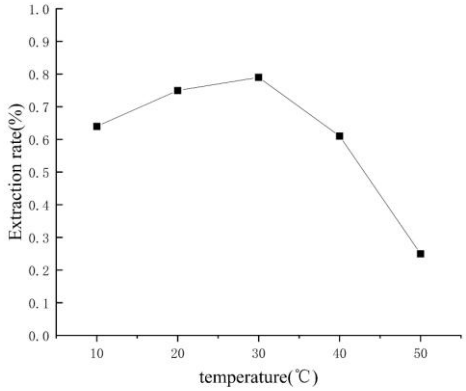

Figure 2 Effect of extraction temperature on extraction rate of collagen

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 scaleswas 20 to 30°C.

3.1.2 Effect of the ratio of material to liquidon extraction rate of collagen

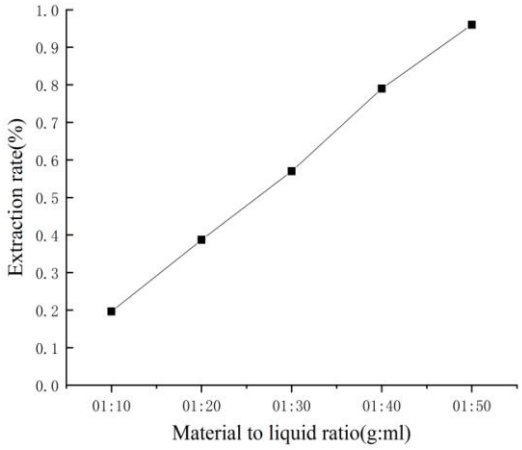

Figure 3 Effect of the ratio of material to liquid onextraction rate of collagen

The effect of the ratio of material to liquid onextraction rate of collagen was shown in Figure 3.As shown in Figure 3, the extraction rate of collagen from carp scales was increasedwith 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 rateof collagen

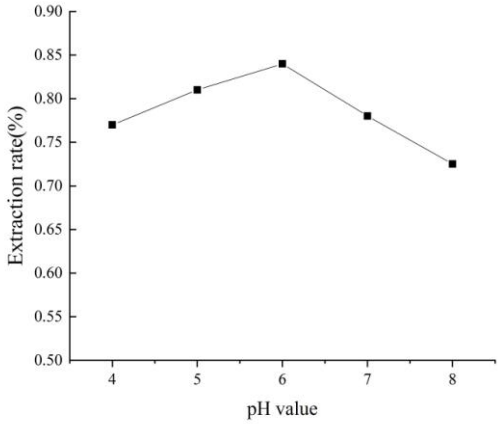

Figure 4 Effect of pH on extraction rate of collagen

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

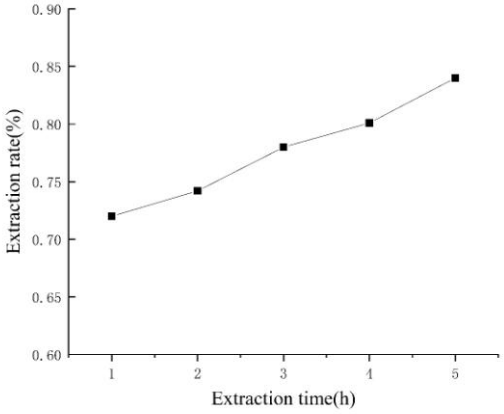

Figure 5 Effect of extraction time on extraction rate of collagen

The effect of extraction time on collagen extraction rate is shown in Figure 5.As shown in Figure 5, the extraction rate of collagen fromcarp 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

Test number	factor				Extraction rate(%)
	Extraction temperature/ °C(A)	Material to liquid ratio(g/mL) (B)	pH value (C)	(D)	
1	1	1	1	1	0.779
2	1	2	2	2	0.792
3	1	3	3	3	0.815
4	2	1	2	3	0.852
5	2	2	3	1	0.855

6	2	3	1	2	0.841
7	3	1	3	2	0.842
8	3	2	1	3	0.799
9	3	3	2	1	0.784
K_1	2.386	2.473	2.419		
K_2	2.548	2.446	2.428		
K_3	2.425	2.44	2.512		
$\bar{K}_1$	0.795	0.824	0.806		
$\bar{K}_2$	0.849	0.815	0.809		
$\bar{K}_3$	0.808	0.813	0.837		
RangeR	0.054	0.011	0.031		

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 wasextraction temperature > pH value > material to liquid ratio.The optimal level combination of factorswas A2B1C3. That was, the optimal extraction conditions were the extraction temperature of 20°C, the ratio of material to liquid of 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 of 1:20.

Acknowledgements

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

References

[1]. Fan, J., Zhuang, Y., & Li, B. (2013). Effects of collagen and collagen hydrolysate from jellyfish umbrella on histological and immunity changes of mice photoaging. Nutrients, 5(1), 223-233.
[2]. Rodkey, William, G., Steadman, Richard, J., Li, &ShuTuna. (1999). Collagen scaffolds: a new method to preserve and restore the severely injured meniscus. Sports Medicine & Arthroscopy Review, 7(1), 63-73.
[3]. Gopinath, D., Ahmed, M. R., Gomathi, K., Chitra, K., Sehgal, P. K., &Jayakumar, R. (2004). Dermal wound healing processes with curcumin incorporated collagen films. Biomaterials, 25(10), 1911-1917.
[4]. Sell, S. A., Mcclure, M. J., Garg, K., Wolfe, P. S., &Bowlin, G. L. (2009). Electrospinning of collagen/biopolymers for regenerative medicine and cardiovascular tissue engineering ☆☆☆. Adv Drug Deliv Rev, 61(12), 1007-1019.
[5]. Asghar, A., &Henrickson, R. L. (1982). Chemical, biochemical, functional, and nutritional characteristics of collagen in food systems. Advances in Food Research, 28(1), 231-372.
[6]. Gelse, K., Pöschl, E., &Aigner, T. (2003). Collagens—structure, function, and biosynthesis. Adv Drug Deliv Rev, 55(12), 1531-1546.
[7]. Hai, L., Wei-Hua, D., Rui, Z., Nian-Hua, D., Chi, C., & Jian-Jian, Q. U., et al. (2006). The optimization of techniques and modeling of the process of collagen extraction from pigskin with pepsin. China Leather, 35(5), 24-27+31.
[8]. Zhao, C. B., Huang, Y. D., & Yan-Hui, L. I. (2004). Extraction of collagen from cattle tendons. Journal of Harbin Institute of Technology, 36(4), 515-519.
[9]. Hayashi, Y., Yamada, S., Yanagi, G. K., Koyama, Z., & Ikeda, T. (2012). Chitosan and fish collagen as biomaterials for regenerative medicine. Adv Food Nutr Res, 65(66), 107-120.
[10]. Ren, S., Jing, L. I., & Guan, H. (2010). The antioxidant effects of complexes of tilapia fish skin collagen and different marme oligosaccharides. Journal of Ocean University of China, 9(4), 399-407.

Haifang Xiao. " Optimization of CollagenExtraction Processfrom CarpScale." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 13.8 (2019): 44-48.