

Original article

Study on the methods for the concentration determination of collagens from calf and fish

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Introduction

Collagens are a family of extracellular proteins, and at least 20 type collagens (type I-XX) have currently been described to date [1]. Given the wide range of development prospects of collagens, they are attracting considerable research attention. The determination of collagen concentration is one of the key steps in collagen research. Measurement of collagen concentration usually involves the Biuret method [2], the Lowry method [3], and so on. Currently, the Kjeldahl method is still used as the most recognized measurement method. Other methods, such as the Coomassie brilliant blue method [4], Biuret method, Sirius red method [5], etc. do not possess a specific uniform determination of the conditions [6]. Determination of the concentration of collagen using the Biuret method is usually lower than its true value, due to the collagen's triple helix structure. To obtain more accurate measurement results, the conditions for the determination of collagen concentration by the Biuret method were studied. The precision and accuracy of the modified Biuret and Lowry methods for collagen determination was also investigated and compared.

Materials and methods

Materials

Calf tendon collagen; fish collagen.

Methods

Extraction of Collagen [7]; Biuret method [2]; SDS-PAGE (polyacrylamide gel electrophoresis) [8]; Lowry method [3].

Results and discussion

Optimal conditions for collagen determined by Biuret method

(1) Calf tendon collagen

The samples were heated at 40, 60, and 80°C for 5 min. The Abs was measured and compared with the unheated

ones. As shown in Fig 1, the maximum Abs was achieved when the heating temperature was 40°C, demonstrating an increasing rate of 57%. Therefore, 40°C was the most suitable treatment temperature for calf tendon collagen.

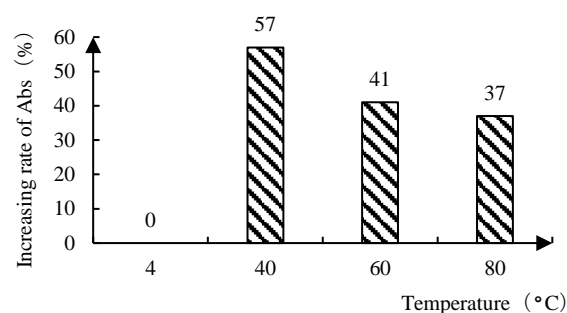


Fig. 1. Increasing rate of samples' Abs treated at different temperatures for 5min.

The samples were heated at 40°C for 5, 15 and 30 min. The Abs was determined and compared with the unheated ones. When the heating time increased from 5 to 30 min, the measured value decreased (Fig. 2). The

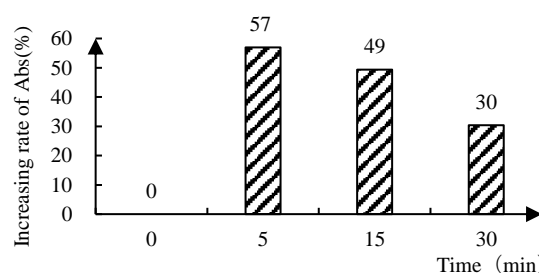


Fig. 2. Increasing rate of samples' Abs treated at 40°C for different periods.

increasing rate of the Abs reached maximum value after 5 min of treatment. The results of SDS-PAGE indicated that the triple helix structure was already destroyed after heating at 60°C for 5 min (Fig. 3). Collagen degradation was gradually increased. When the heating temperature reached 80°C, collagen was degraded into small peptides and the Abs was further reduced. Therefore the optimum

conditions for the determination of calf tendon collagen were heated at 40°C for 5 min.

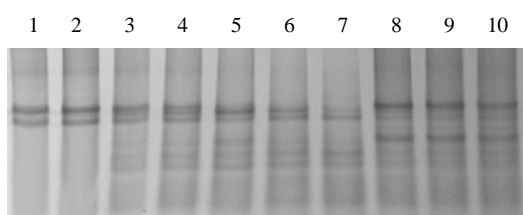


Fig. 3. SDS-PAGE pattern of collagen samples treated at different temperatures. Line 1 4°C. Line 2-4 40°C 5min, 15min and 30min. Line 5-7 60°C 5min, 15min and 30min. Line 8-10 80°C 5min, 15min and 30min.

(2) Fish collagen

The collagen from skin of fish (*Ictalurus Punetaus*) were heated at 30, 35 and 40°C for 2, 5, 15, 30 and 45 min. The change of Abs values were determined by the Biuret method. As shown in Fig. 4, the sample heated at 30°C for 2 min exhibited the highest absorbance. As the heating time was prolonged, the absorbance remained stable. Moreover, at 35°C and 40°C, the same phenomenon occurred. In general, the curve trend was stable, indicating that the temperature and heating time changes affected the determination of fish collagen slightly.

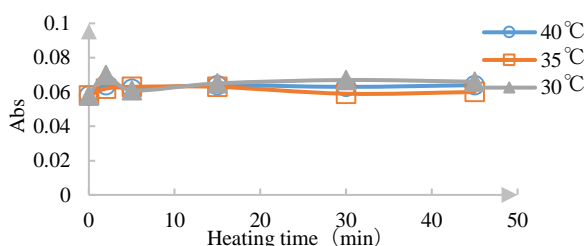


Fig. 4. Abs of samples after different treatment.

Comparison of Lowry Method and Modified Biuret Method

(1) Calf tendon collagen

Freeze-dried collagen was dissolved in 0.5 M Acetic Acid to prepare the solution. The precision of the seven groups were determined by the Biuret and Lowry methods, respectively.

Table 1 shows that the variation coefficient of the Lowry method was 2%, while that of the Biuret method was 17%, which was 8.5 times that of the Lowry method, indicating that the Lowry method was more precise in the determination of calf tendon collagen.

Table 1. Comparison of Precision	
Method	Coefficient of variation
Biuret	17%
Lowry	2%

The Biuret and Lowry methods measured the two groups in parallel. The calculated concentration was compared with the actual concentration, and the relative

errors of the two methods were obtained.

Table 2 indicates that the relative error of the Lowry method was -3% and the relative error of the Biuret method was -41%. The Lowry method for the determination of calf tendon collagen was highly accurate compared with the improved Biuret method. Therefore, the Lowry method was more suitable for the measurement of calf tendon collagen.

Table 2. Comparison of Accuracy	
Method	Relative error
Biuret	-41%
Lowry	-3%

(2) Fish collagen

For fish collagen, the same experiments were performed, demonstrating that the coefficient of variation of the Lowry method was slightly smaller than that of the Biuret method, which were 4% and 7%, respectively. Furthermore, the relative errors of the two methods were both -6%. Therefore, both methods were applicable for the determination of fish skin collagen. However, because the Biuret method was easier to operate than the Lowry method, the determination of fish collagen concentration by the Biuret method was more suitable.

Conclusions

In this study, determination of concentration of the calf tendon collagen were studied. The results showed that heating samples at 40°C for 5min was the optimum conditions for the determination by the Biuret method. The Lowry method was precise and accurate than the Biuret method in the determination of calf tendon collagen. However, there was little difference in accuracy between the Lowry and the modified biuret methods for the determination of collagen from fish skin. Therefore, the modified biuret method was more applicable for fish collagen due to its simplicity of operation.

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