Study on Extraction of Mucopolysaccharide-protein Containing Chondroitin Sulfate from Chicken Keel Cartilage

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ABSTRACT: The objective of this study was to investigate technical methods for extraction of mucopolysaccharide-protein containing chondroitin sulfate from keel cartilage of chickens. The chemical composition of chicken keel cartilage was determined. For the preparation of mucopolysaccharide-protein from lyophilized chicken keel cartilage, hot water extraction and alcalase hydrolysis methods were examined. Results showed that the optimum condition of hot water extraction was incubation for 120 min with a yield of 40.09% and chondroitin sulfate content of 28.46%. For alcalase hydrolysis, the most effective condition was 2% alcalase in 10 volumes of distilled water for 120 min. The yield of hydrolysate was 75.87%, and chondroitin sulfate content was 26.61%. For further separation of chondroitin sulfate from the alcalase hydrolysate, which has a higher yield than that of hot water, 60% ethanol precipitation was performed. The yield of the ethanol precipitate was 21.41% and its chondroitin sulfate content was 46.31%. The hot water extract, alcalase hydrolysate and ethanol precipitate showed similar electrophoretic migration with standard chondroitin sulfate (chondroitin sulfate A), using cellulose acetate membrane electrophoresis. These results indicated that a significant amount of mucopolysaccharide-protein containing chondroitin sulfate could be acquired form chicken keel cartilage. Therefore, keel cartilage in chicken may provide an inexpensive source of chondroitin sulfate for commercial purposes. (Asian-Aust. J. Anim. Sci. 2006, Vol 19, No. 4 : 601-604)

Key Words: Mucopolysaccharide, Chondroitin Sulfate, Alcalase Hydrolysis, Ethanol Precipitation, Cellulose Acetate Membrane Electrophoresis

INTRODUCTION

Chondroitin sulfate (CS) is one of the sulfated mucopolysaccharides (MPS), which are largely responsible for the high elasticity and resilience of tissue. CS which is comprised of repeating disaccharide units of D-glucuronic acid, N-acetyl-D-galactosamine and the sulfate group, attached covalently to core protein in a form of proteoglycan (Nakano, 2000). CS has been reported to play biologically important roles in a human body, which revealed include control of pericellular ions (Tanaka, 1978), protection of connective tissues (Bayliss, 1999), management of osteoarthritis (Deal and Moskowitz, 1999; Hauslmann, 2001), prevention of cornea (Mac-Rae et al., 1983), and anticoagulative activity (Bjornsson et al., 1982; Nishio and Nagumo, 1991). Since the discovery of these various functional activities, CS has been a favorable functional material used in medicine, cosmetics, as well as in functional food. CS, including sulfated MPS, has been found to exist in various organs and tissues of animal bodies, such as trachea and bones of bovine, skin and cartilage of squid. It was also isolated from the body wall of sea cucumber and shark cartilage. However, it is too expensive to manufacture chondroitin sulfate at a large scale for commercial purpose.

Recently, efforts have been made to extract CS from sea animals such as sea cucumber, skate cartilage and shark cartilage. Luo et al. (2002) reported that keel cartilage of chicken contained a quantity of CS. There are forty seven million broiler chickens are raised in Korea among which approximately 5% are used for production of poultry meat fillets and then keel bones are discarded (Agricultural statistics, the ministry of agriculture and forestry, 2002).

This study, therefore, was conducted to investigate economical techniques for extraction of mucopolysaccharides containing CS from keel cartilage in poultry by-product.

MATERIAL AND METHODS

Chicken keel bones were obtained from local poultry meat processing plant. They were washed thoroughly with running tap water and stored at -20°C until analysis. Before processing, cartilage was oven dried at 50°C for 24 h and ground with at 60 mesh. To prepare hot water extracts each sample was extracted with 10 volumes (v/w) of distilled water at 100°C every 30 min intervals for 2 h. After centrifugation (1,610×g, 30 min), supernatants were dried at 50°C, and then ground. Preparing for alcalase hydrolysates each samples were hydrolyzed by 2% Alcalase (Novo Nordish-Denmark, 55°C, pH 8.0) at its optimal condition, with 10 volumes of buffer solution every 30 min intervals for 2 h, and then centrifuged (HA-1,000-3, Hanil industrial co.) at 1,610×g for 30 min at room temperature. The resulting supernatants were dried at 50°C and then...
The proximate composition of chicken keel cartilage is presented in Table 1. The analysis revealed that the moisture content of the fresh keel cartilage was 82.85%, while the carbohydrate content was 24.20%. The dried keel cartilage contained moisture of 75.00%, crude protein of 60.55%, crude lipid of 6.50%, and crude ash of 6.50%.

<table>
<thead>
<tr>
<th>Components</th>
<th>Fresh</th>
<th>Oven dried</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>82.85±1.42 ±0.12</td>
<td>75.00±0.12</td>
</tr>
<tr>
<td>Crude protein</td>
<td>11.78±0.21</td>
<td>60.55±2.24</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>0.29±0.72</td>
<td>0.95±0.21</td>
</tr>
<tr>
<td>Crude ash</td>
<td>1.21±0.12</td>
<td>6.50±2.54</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>3.87±0.35</td>
<td>24.20±1.62</td>
</tr>
</tbody>
</table>

1 Values are mean±SD. 2 100-(moisture+crude protein+crude lipid+crude ash).

The chemical composition of chicken keel cartilage was determined and shown in Table 1. The proximate analysis of the wet keel cartilage resulted in moisture of 82.85%, CP of 60.55%, carbohydrate of 24.50%, and ash of 6.50%.

Table 2 showed the CS content and yield of water extracts of chicken keel cartilage according to heating time. The result showed that the yield of 120 min extraction was significantly higher (p<0.01) than any other treatments. CS contents had higher value at 90 or 120 min of heating time as compared to that of 60 min treatment. Thus, the optimum condition of hot water extraction was incubating for 120 minutes in terms of the yield. Nakano et al. (2001) reported that it can be acquired considerable mucopolysaccharide-proteins containing CS by using autolysis. Therefore, the further investigations including changes of incubation time, temperature and pH will be required. In general, for separation of mucopolysaccharide-protein from core protein, the papain has been commercially used in enzyme.

RESULTS AND DISCUSSION

The chemical composition of chicken keel cartilage was determined and shown in Table 1. The proximate analysis of the wet keel cartilage resulted in moisture of 82.85%, CP of 60.55%, carbohydrate of 24.50%, and ash of 6.50%.

The main effects between treated groups were subjected to ANOVA using the general linear models procedure of SAS (2002), and significant differences were determined using Duncan’s multiple range test at the level of p<0.05 (Duncan, 1955).
pH play an important role in separating CS from tissue and thus, the more investigation including optimum pH will be needed.

For further separation of CS from the hydrolysate by alcalase which has relatively high yield than those of hot water, 60% ethanol precipitation was performed. The yield of the ethanol precipitate was 21.41% and its CS content was 46.31% as shown in Table 4. Lou et al. (2002) reported that the final product extracted from chicken keel cartilage was 308.4 mg/g and its CS content was 75.5%, but the final product of this study was 162.4 mg/g, and its CS content was 46.31%. The total yield and CS content of this study relatively lower than those of previous study may be partly attributed to the differences of species and extraction procedure.

Figure 1 showed that cellulose acetate membrane electrophoresis of chicken keel cartilage extracts. Alcalase hydrolysate, hot water extract and ethanol precipitate showed similar electrophoretic migration with standard chondroitin sulfate A and it means all of these extracts contained CS.

CS is used particularly in the treatment of osteoarthritis. Most CS had been prepared from shark cartilage or bovine tracheal cartilage. Thus, this study was conducted to investigate to economical techniques for extraction of mucopolysaccharides containing CS from chicken keel cartilage. Alcalase hydrolysate, hot water extract and ethanol precipitate was performed. The results indicated that a considerable amount of mucopolysaccharide-protein containing CS could be acquired from chicken keel cartilage. Therefore, keel cartilage in chicken may contribute to inexpensive source of CS for commercial purposes.

ACKNOWLEDGMENT

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