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 mucopolysachharide-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 anticogulative activity (Bjornsson et al., 1982; Nishno 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

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Table 1. Proximate composition of chicken keel cartilage

Components	Fresh	Oven dried
	---------- % ----------	
Moisture	82.85±1.42[1]	7.50±0.12
Crude protein	11.78±0.21	60.55±2.24
Crude lipid	0.29±0.72	0.95±0.21
Crude ash	1.21±0.12	6.50±2.54
Total carbohydrate[2]	3.87±0.35	24.20±1.62

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

grounded.

The content of moisture, crude ash, crude fat and crude protein were determined by method of AOAC (AOAC, 1990). The content of sulfated mucopolysaccharide was by determination of glucuronic acid (modified from Bitter and Muir, 1962; Korea Food & Drug Administration Korean food code, 1999) as following. Each sample (0.3 g) that was divided into four treatments with seven replicate in hot water extraction and in enzyme extraction was dissolved in 100 ml. 4 ml of this solution was filled up to 20 ml the filtrated to be a sample solution. 5 ml of sodium borate sulfuric acid solution was placed to each test tube cooled enough with ice water. Sample solution and glucuronolactone standard (Sigma Co.) of 1 ml was carefully added to the each sodium borate sulfuric acid solution and mixed under a cooling condition. It was heated in a water bath at 100°C for 10 min then cooled with ice water. Carbazole solution (Sigma Co.) of 0.2 ml was added to the each test tube and mixed, the heated again for 15 min followed by cooling. The absorbance was measured at 530 nm. The content of chondroitin sulfate was calculated following development of the standard curve (2.593: M.W. of chondroitin sulfate/M.W. of glucuronic acid).

Chondroitin sulfate content (%)
= glucuronic acid (%)×2.593

To separate crude CS from alcalase hydrolysate, 60% ethanol precipitation was performed. Then it allowed standing for 1 h. To confirm the identity of CS, cellulose acetate membrane electrophoresis was performed on samples including 2% sample solutions prepared by hot water extraction and alcalase hydrolysis. The solution was loaded on a cellulose acetate membrane, which was set in the electrophoresis bath filled with buffer. After electrophoresis (0.5 mA, 20 min), the membrane was stained with dyeing solution then destained in 1-3% citric acid followed by drying.

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).

Table 2. Changes in yield and chondroitin sulfate content of water extracts of chicken keel cartilage with heating time[1]

Heating time (min)	Yield	Chondroitin sulfate
	---------- % ----------	
30	34.74±0.64^{c}	27.35±1.76^{ab}
60	36.85±0.90^{bc}	25.83±2.19^{b}
90	37.86±4.02^{ab}	29.50±1.28^{a}
120	40.09±1.45^{a}	28.46±2.06^{a}

[1] Water extracts were prepared at 100°C.
Mean±SD. (n = 7 for each treatment). $^{a-c}$ Mean values within a column with no common letter significantly different among heating time ($p<0.01$).

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 of11.78%, ether extract of 0.29%, ash of 1.21%, and carbohydrate (3.87%). The dried keel cartilage contained 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 mucopolysccharide-protein from core protein, the papain has been commercially used in enzyme. However, Gu et al. (1999) reported the treatment of alcalase had higher sulfated mucopolysaccharide content than those by other proteases. Cha et al. (1995) also reported that alcalase was the most efficient for the hydrolysis of pen shell by-product. Thus, this study was performed to hydrolysis of chicken keel cartilage with alcalase. The changes in yield and chondroitin sulfate content of water extracts of chicken keel cartilage with heating time were shown in Table 3. The highest yield was 75.78% in 120 min extraction ($p<0.01$). CS content of 120 min extraction was significantly higher ($p<0.05$) than any other treatments and it suggested that the optimum condition of alcalase extraction was incubating for 120 min. Park (2000) reported that 4 h heating with 2% alacalse was the most efficient for extraction of CS from shark cartilage. This study used only alcalase for extraction of sulfated mucopolysacchraide in chicken. The further investigations using a various enzymes will be required. Nakano (2000) reported that the change of

Table 3. Changes in yield and chondroitin sulfate content of alcalase hydrolysates of chicken keel cartilage with incubation time[1]

Heating time (min)	Yield	Chondroitin sulfate
	----------%----------	
30	72.53±0.35[c]	25.09±1.94[b]
60	72.78±0.48[c]	23.00±0.71[c]
90	73.66±0.29[b]	22.66±1.30[c]
120	75.78±1.20[a]	26.61±1.64[a]

[1] Chicken keel cartilage powder was hydrolyzed with 2% alcalase at 55°C, pH 8.0.

Mean±SD. (n = 7 for each treatment). [a-c] Mean values within a column with no common letter significantly different among heating time ($p<0.05$).

Table 4. Yield and chondroitin sulfate content of ethanol (60%) precipitate from the alcalase hydrolysate of chicken keel cartilage[1]

Reagent	Yield	Chondroitin sulfate
	----------%----------	
60% Ethanol	21.41±0.29[2]	46.31±2.50

[1] Chicken keel cartilage powder was hydrolyzed with 2% alcalase at 55°C, pH 8.0.

[2] Mean±SD. (n = 7 for each treatment).

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 higher 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 keel cartilage in poultry by-product. The results indicated that a considerable amount of mucopolysacchride-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.

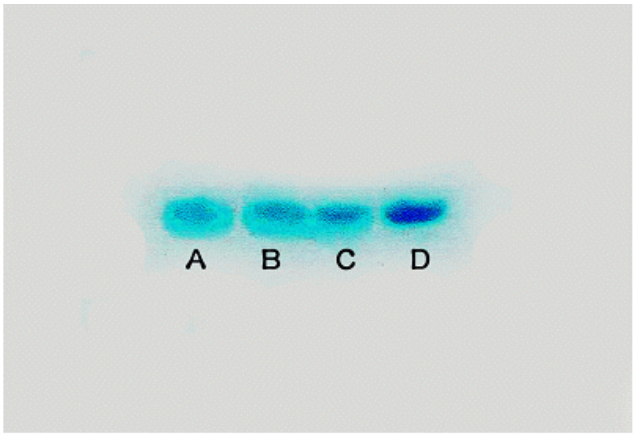

Figure 1. Cellulose acetate membrane electrophoresis of chicken keel cartilage extracts. Chondroitin sulfate A (A), hot water extract (B), alcalase hydrolysate (C), ethanol precipitate (D).

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