

Asian Journal of Food and Agro-Industry

ISSN 1906-3040

Available online at www.ajofai.info

Research Article

Type and content of chondroitin sulphate and collagen in poultry tracheas

Tusanee Jaroenviriyapap and Manee Vittayanont*

Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Haad Yai, Songkhla, 90112 Thailand.

*Author to whom correspondence should be addressed, email: manee.v@psu.ac.th

This paper was originally presented at Food Innovation Asia, Bangkok, Thailand, August 2009.
Received 13 June 2009, Accepted 4 February 2010

Abstract

Tracheas of chicken, duck and ostrich are wastes from poultry processing industry. To evaluate potential of these wastes to be used as a source of chondroitin sulphate (CS) and collagen, their proximate composition, cartilage proportion, CS and hydroxyproline contents were analyzed. Percentage of cartilage in studied materials ranged from 49.20 to 73.05. Total proteins and hydroxyproline contents were 66.19-84.38% and 4.04-7.49% (dry basis), respectively. The peptide patterns obtained by SDS-PAGE showed that pepsin-solubilized collagen from all samples comprised of type I and type II with different proportion. The CS content determined by HPLC was found to be 0.574 - 6.357% (dry basis) with chondroitin-4-sulfate as the majority. All sample had only trace amount of di-sulphated CS. Ostrich trachea possessed the highest content of CS and collagen but it is still low available in Thailand. Duck trachea and larynx had high proportion of cartilage and type II collagen, and contained high content of CS next to ostrich trachea indicating a high potential as a source of CS and type II collagen.

Keywords: poultry, cartilage, waste recovery, Type II collagen, CS, Thailand

Introduction

Thailand is one of the major world exporters of poultry meat products. Each day, tons of viscera are produced from poultry industry as by-product which is discarded or used as animal feed. Trachea is a part of this waste containing connective tissue, especially cartilage as a main

composition. Cartilage is composed of type II collagen, other proteins, and glycosaminoglycans, mainly chondroitin sulfate (CS) [1]. CS comprised of an alternating sequence of sulfated and/or unsulfated D-glucuronic acid (GlcA) and N-acetyl-D-galactosamine(GalNAc) residues linked through alternating $\beta(1-3)$ and $\beta(1-4)$ bonds. Type II collagen is characteristic of cartilaginous tissues. Both CS and type II collagen are available in nutraceutical market especially for healthy joint concern.

There were studies showing the effectiveness of oral collagen type II preparations in humans for maintenance of healthy joints. A randomized controlled trial found that oral administration of chicken collagen type II for 3 months led to a significant decrease in swollen and tender joints [2]. CS has been widely used as a treatment for osteoarthritis because of its efficacy in the treatment of degenerative arthritis and its bioavailability in animals and humans [3-4]. Since the therapeutic bioactivities of CS have become well known, the market of nutraceuticals containing CS has dramatically increased. However, the availability and quality of these nutraceutical raw materials remains in question. The limited supply of shark cartilage makes it very expensive, while bovine and porcine cartilages are in serious concern about mad cow disease and Islamic prohibition, respectively. This study was aimed to evaluate the potential of poultry tracheas (chicken, duck and also ostrich) to be used as raw material for the production of nutraceuticals containing both CS and type II collagen.

Materials and Methods

Materials

Tracheas of chicken and duck were obtained from Charoen Pokphand Food. Ostrich trachea was donated by Oasis farm. Papain (EC 3.4.22.2), Chondroitinase ABC from *Proteus vulgaris*, and unsaturated CS disaccharides (Δ UA-[1 \rightarrow 3]-GalNAc: Δ Di-0S, Δ UA-[1 \rightarrow 3]-GalNAc-6S: Δ Di-6S, Δ UA-[1 \rightarrow 3]-GalNAc-4S: Δ Di-4S) were purchased from Sigma (St. Louis, MO, USA).

Proximate analysis

Proximate composition (moisture, crude protein, fat and ash contents) of chicken trachea, duck trachea, ostrich trachea and duck larynx were analyzed following the AOAC method [5]. Hydroxyproline content was determined using method modified from Kolar [6]. Larynx was cut from trachea of male duck and used as a separated sample. Muscle meat ligaments and tendon were removed manually from all raw materials and the remaining cartilage was weighed to determine the weight percentage of the cartilage in each source.

Extraction and analysis of chondroitin sulphate (CS)

Raw material was soaked in hot water (70-75°C) for 2 min to remove meat and connective tissue. The remaining cartilage was chopped and CS was extracted by method reported by Ganjanagoonchorn *et al* [7].

To determine the content and type of CS, the enzymatic depolymerization by chondroitinase ABC was performed [8]. Briefly, 100 μ l of CS solutions (5 mg/ml) were mixed with 850 μ l of Tris-acetate buffer. Then the samples were depolymerized with 50 μ l of chondroitinase ABC (1 mU/ μ l) overnight at 37°C. After heating for 5 min and filtering on 0.2 μ m filters, disaccharide composition in the depolymerized mixtures (100 μ l) were analyzed with a

Hypersil SAX column (4.6 x 250 mm, 5 μ m) from Thermo Hypersil-Keystone (Bellefonte, PA, USA). After injecting the samples, the column was washed with water (pH 3.5) for 4.155 min corresponding to one column volume (CV). Then, a linear gradient of 0–1.0 M NaCl (pH 3.5) for 41.55 min (10 CV) was used and the profile was monitored at 232 nm. The flow rate was 1.0 ml/min.

Collagen extraction and electrophoretic characterization of collagen

Poultry tracheas were chopped and defatted with chloroform: methanol (2:1) at 20°C for 1hr. Collagen was then hydrolyzed by 0.5% pepsin (in 0.5 M acetic acid) at 4°C for 24hrs. The extract was filtered and the obtained viscous solution was centrifuged at 12,000 rpm for 1hr at 4°C. The collagen in supernatants was salted out by adding NaCl to a final concentration of 2.5 M and centrifugation at 12,000 rpm for 1hr. The obtained collagen pellet was re-dissolved in 0.5 M acetic acid, dialyzed against 0.1 M acetic acid, distilled water and lyophilized. The residue from pepsin hydrolysis was treated with 0.1MNaOH for 3hrs before re-extracted with 0.5% pepsin and the collagen separation was then repeated 2 more times using the same procedure formerly mentioned.

The peptide pattern of trachea collagens was investigated by SDS-PAGE using 5% resolving gel. The collagen samples and molecular weight standard sample (type I and type II collagen) were loaded into the wells of Mini-Protein III unit (Bio-Rad Laboratories, Hercules, CA) and the electrophoresis was conducted at constant current of 25 mA. The gel was stained with Coomassie Brilliant Blue R250 solution (0.25%) and de-stained with acetic acid : methanol : water (10:40:50 v/v/v) for 10-15min.

Results and Discussion

Cartilage proportion and proximate composition of poultry tracheas

Since cartilage is mainly composed of chondroitin sulfate (CS) and type II collagen, the high proportion of cartilage could indicate the high potential of the raw material to be used as source of both substances. The results showed that all trachea samples contained over 50% of cartilage excepted for chicken trachea (Table 1). Duck trachea comprised highest cartilage proportion of $73.05 \pm 3.31\%$. Proximate analysis results (Table 2) indicate that protein is the major component of poultry trachea (66.19 ± 0.96 - 84.38 ± 1.59) with the highest content in ostrich trachea followed by chicken trachea, duck larynx and duck trachea. The HyP content of raw material ranged from 4.04 ± 0.11 - $7.49 \pm 0.37\%$ (Table 2). The amount of HyP correlates to collagen content but not specific only to type II collagen. Therefore, highest cartilage proportion of duck trachea may be an indicator for its high content of type II collagen.

Table 1. Percentage of cartilage in poultry tracheas.

Source	Cartilage(%wt)
Chicken trachea	49.20 ± 2.06
Duck trachea	73.05 ± 3.31
Ostrich trachea	60.43 ± 3.12
Duck larynx	57.01 ± 1.71

Values are expressed as means \pm S.D. ($n = 3$).

Table 2. Proximate composition and hydroxyproline content of poultry tracheas.

Source	Composition (%)*				
	Protein	Fat	Ash	Carbo-hydrate	HyP
Duck trachea	66.19±0.96	9.27±0.05	11.48±0.03	13.06	4.11±0.07
Chicken trachea	79.19±3.96	3.03±0.71	2.70±0.06	15.08	4.95±0.03
Ostrich trachea	84.38±1.59	3.99±0.10	3.54±0.15	8.09	7.49 ±0.37
Duck larynx	74.65±0.62	0.80±0.12	7.65±0.68	16.90	4.04 ±0.11

*Based on dry weight of raw material. Values are expressed as means ± S.D. ($n = 3$).

Types and content of CS of poultry tracheas

The chondroitin sulphate (CS) content (%) of all trachea samples ranged from 0.572±0.027 to 6.357±0.545% dry basis with the highest value in ostrich trachea (Table 3). The 16.8% CS content of chicken keel cartilage has been reported [9]. CS content of other cartilage sources that were previously reported are as follows: crocodile sternum cartilage 11.55%, crocodile trachea cartilage 9.51%, shark fin cartilage 9.6%, crocodile rib cartilage 5.56% and ray cartilage 5.27% [7]. The differences in CS content involve the source of cartilage: species and locations. Moreover, differences in methods and reference materials used in analysis could bring about variation in obtained data [7, 12].

Types of CS were identified by SAX-HPLC. The chromatograms of the CS samples extracted from various sources of cartilage are shown in Fig. 1b-e in comparison with those of standard chondroitin-0-sulfate (Δ Di-0S), chondroitin-4-sulfate (Δ Di-4S) and chondroitin-6-sulfate (Δ Di-6S) (Fig 1a). The chromatograms show that poultry tracheas contained more Δ Di-4S than Δ Di-6S and Δ Di-0S, respectively. Chondroitin 4-sulfate and chondroitin 6-sulfate are the most abundant mucopolysaccharides in the body and occur both in skeletal and soft connective tissue. The sulfation pattern of chondroitin disaccharides from normal human cartilage varies with age, topography of the joint surface, and the zone of cartilage examined. Sim, *et al.* [8] reported that CS from land animal source had Δ Di-4S/ Δ Di-6S+ Δ Di-diSs ratio higher than 1, while CS from shark cartilage which is a marine animal had Δ Di-4S/ Δ Di-6S+ Δ Di-diSs ratio less than 1. This ratio is quite unique characteristic of CS from land and marine animal source. Since we found that CS from poultry tracheas in this study contain only trace amount of Δ Di-diSs, therefore the ratio of Δ Di-4S/ Δ Di-6S was calculated and used instead. Results in Table 3 showed that the Δ Di-4S/ Δ Di-6S ratio (1.719 -5.478) of poultry trachea samples were higher than 1 which is the character of CS from land animal source.

Table 3. Chondroitin sulphate content in poultry tracheas.

Sample	Content (%)*				Δ Di-4S/ Δ Di-6S
	Δ Di-0S	Δ Di-6S	Δ Di-4S	Total CS	
Duck trachea	0.051±0.001	2.111±0.013	3.109±0.002	3.476±0.154	2.370
Duck larynx	0.322±0.003	1.511±0.204	2.935±0.392	2.718±0.189	1.943
Chicken trachea	0.041±0.001	0.157±0.008	0.859±0.045	0.572±0.027	5.478
Ostrich trachea	0.468±0.002	0.157±0.008	3.630±0.348	6.357±0.545	1.719

*Based on dry weight of raw material. Values expressed as means ± S.D. ($n = 3$).

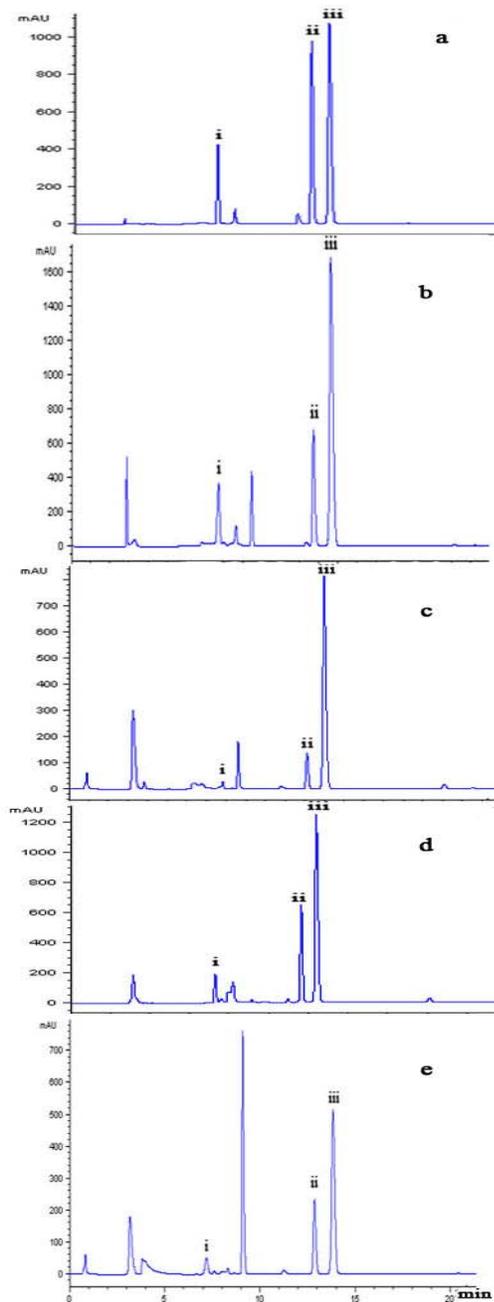


Figure 1. Chromatogram of disaccharides produced by enzymatic depolymerization of chondroitin sulphate from (b) duck trachea, (c) chicken trachea, (d) ostrich trachea and (e) duck larynx compared to that of (a) standard CS where (i) Δ Di-0S (ii) Δ Di-6S (iii) Δ Di-4S.

Electrophoretic characterization of collagen

The peptide pattern of pepsin-solubilized collagen (PSC) from the trachea of duck, chicken and duck larynx were examined by SDS-PAGE (Fig. 3). The first extracted PSC from the duck trachea (lane2) exhibited two distinct α_1 and α_2 chains which is similar to those of standard type I collagen (lane1). The third extracted PSC (lane3), however, had one major band which is similar to $\alpha_1(\text{II})$ of standard type II collagen (lane8). The electrophoretogram of duck larynx PSC both first and third extracts (lane4 and 5) showed the peptide pattern of type II collagen

while peptide patterns of first and third extracted PSC from chicken trachea (lane 6 and 7) were almost identical with two distinct α_1 and α_2 bands of type I collagen. The two small bands between β -component and α_1 (II) in lane 3, 4 and 5 were similar to peptide pattern of type XI collagen in previous reports [10-11]. Type XI is an integral component of the cartilage fibrillar network along with type II and IX collagen [10]. It was also found to be a minor component of pectoral fin of skate [11]. Our results indicate that duck trachea contained mainly type I and II collagen. Duck larynx was composed mostly of type II collagen while type I was the major collagen in chicken trachea. Type XI collagen was found as a minor collagen in all trachea sample with the highest proportion in duck larynx and lowest proportion in chicken trachea.

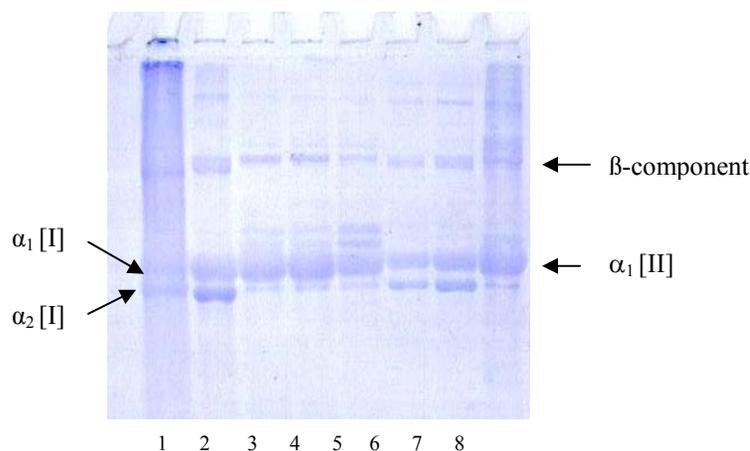


Figure 2. SDS-PAGE patterns of pepsin-solubilized collagens from poultry trachea.

Lane 1: standard type I collagen, lane 2: duck trachea (first extract), lane 3: duck trachea (third extract), lane 4: duck larynx (first extract), lane 5, duck larynx (third extract), lane 6, chicken trachea (first extract), lane 7: chicken trachea (third extract) and lane 8: standard type II collagen.

Conclusions

This study has shown that among three species of Thai economic poultry; chicken, duck and ostrich, the trachea from ostrich contained the highest content of chondroitin sulfate and collagen. Poultry tracheas were composed of chondroitin-4-sulphate and chondroitin-6-sulphate as the majority. Type I and II collagen were found as a major component of duck trachea and larynx along with small proportion of type XI collagen. In Thailand duck trachea and larynx are higher in availability than ostrich. Therefore they could be potential sources of CS and type II collagen for the production of nutraceutical products containing CS and type II collagen.

Acknowledgments

This research work was a part of the development of halal food for export project supported by the National Economic and Social Development Board of Thailand. We thank the Charoen Pokphand Foods Public Company Limited (CPF) and Oasis Farm for kindly donating commercial gelatin samples used in this work.

References

1. Fontenele, J. B., Araujo, G. B., de Alencar, J. W., and Viana, G. S. (1997). The analgesic and anti-inflammatory effects of shark cartilage are due to a peptide molecule and are nitric oxide (NO) system dependent. **Biological and Pharmaceutical Bulletin**, 20, 1151–1154.
2. Trentham, DE., Dynesius-Trentham RA, Orav, EJ., Combitchi, D., Lorenzo, C., Sewell, KL., Hafler, DA., and Weiner, HL. (1993). Effects of oral administration of type II collagen on rheumatoid arthritis. **Science**. 261: 1727-30.
3. Hungerford, D. S. and Jones, L. C. (2003). Glucosamine and Chondroitin sulfate are effective in the management of osteoarthritis. **Journal of Arthroplasty**. 18 (3 suppl1): 5-9.
4. Ronca, F. and Palmieri, L. (1998). Anti-inflammatory activity of chondroitin sulfate, **Osteoarthritis Cartilage**, 6:14–21.
5. A.O.A.C. (2000). Official method of analysis 16th ed. Association of Official Analysis Chemist, Washington, DC, USA.
6. Kolar, K. (1990). Colorimetric determination of hydroxyproline as measure of collagen content in meat and meat products: NMKL collaborative study. *Journal Association of Official Analytical Chemists*, 73: 54-57.
7. Garnjanagoonchorn, W., Wongekalak, A. and Engkagul, A. (2006). Determination of chondroitin sulfate from different sources of cartilage. **Chemical Engineering and Processing**. 46: 465-471.
8. Sim, J-S., Im, A. R., Cho, S.M., Jang, H. J., Jo, J. H. and Kim, Y. S. (2007). Evaluation of chondroitin sulfate in shark cartilage powder as a dietary supplement: Raw materials and finished product. **Food Chemistry**. 101: 532-539.
9. Lou, X. M., Fosmire, G. J., and Leach, R.M. (2002). Chicken keel cartilage as a source of chondroitin sulfate. **Poultry Science**. 81: 1086-1089.
10. Mendler, M., Eich-Bender S. G., Vaughan, L., Winterhalter, K. H. and Bruckner, P. (1989). Cartilage Contains Mixed Fibrils of Collagen Types II, IX, and XI. **The Journal of Cell Biology**, 108: 191-197.
11. Mizuta, S., Hwang, J., & Yoshinaka, R. (2002). Molecular species of collagen from wing muscle of skate (*Raja kenoi*). **Food Chemistry**, 76:53–58.
12. Walker, M. (2006). Analysis of Chondroitin in Supplements. Statutory Analysis Government Chemist Programme Ad Hoc Project. Reference Number: LGC/GC/2007/011.