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Evaluation of chondroitin sulfate in shark cartilage powder as a dietary supplement: Raw materials and finished products

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Abstract

We evaluated the content and biochemical properties of chondroitin sulfate (CS) in shark cartilage powders being used as nutraceutical supplements. The quantities of CS in shark cartilage products (SCPs) and finished products containing shark cartilage powder were determined by analyzing unsaturated disaccharides after treatment with chondroitinase ABC, and the results were compared with the specifications on the product labels. This method was validated and good linearity ($r \geqslant 0.999$) was obtained. The recovery ranged from 95.27% to 102.39% with precision from 2.27% to 3.95%. Furthermore, the average molecular weights (MW) and the origins of CS in SCPs and finished products were evaluated by agarose gel-electrophoresis and assessment of disaccharide compositional patterns, respectively. Quantitative and compositional analysis of disaccharides after enzymatic depolymerization showed that the amount of CS in the samples of SCPs ranged from 0% to 28.92 ± 0.03%. All of the SCP samples except for SCP D had ΔDi-2,6diS and had more ΔDi-6S than ΔDi-4S, indicating that they originated from shark cartilage. In the finished products, the amount of CS ranged from 0.58 ± 0.01% to 21.30 ± 0.08%. With the exception of SCP D and two finished products (F and D), which contained CS with lower MWs, the average MW of CS in the SCPs and finished products was approximately 40 kDa or higher than that of MW standard (40 kDa) of CS. These analyses contribute to the evaluation of the quantity and quality of CS in SCPs and finished products containing CS, which is necessary for the manufacture of high-grade nutraceuticals.

Keywords: Evaluation; Shark cartilage; Chondroitin sulfate; Raw materials; Nutraceutical; Validation

1. Introduction

Cartilage is a type of connective tissue comprised of several types of collagens, other proteins, and glycosaminoglycans (Fontenele, Araujo, de Alencar, & Viana, 1997). Shark cartilage is widely used as a dietary supplement because it has been shown to have anti-angiogenic and anti-tumor activities in animals and humans (Berbari et al., 1999; Gonzalez et al., 2001; Lee & Langer, 1983; Norrby, Jakobsson, & Sorbo, 1990). Furthermore, shark cartilage has been reported to have therapeutic efficacy in the treatment of osteoarthritis, rheumatoid arthritis, progressive systemic sclerosis and neurovascular glaucoma (Fontenele et al., 1997; Sculti, 1994).

An essential component of cartilage is chondroitin sulfate (CS), which is composed of an alternating sequence of sulfated and/or unsulfated D-glucuronic acid (GlcA) and *N*-acetyl-D-galactosamine (GalNAc) residues linked through alternating $\beta(1 \rightarrow 3)$ and $\beta(1 \rightarrow 4)$ bonds (Fig. 1). CS plays an important role in the elasticity and function of articular cartilage and is mainly attached covalently to core proteins in the form of proteoglycans (Hardingham & Bayliss, 1990). Because of its efficacy in the treatment of degenerative arthritis and its bioavailability

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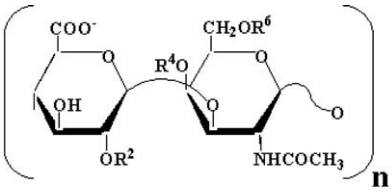

	R^2	R^4	R^6
△ Di-0S	H	H	H
△ Di-6S	H	H	SO_3^-
△ Di-4S	H	SO_3^-	H
△ Di-2,6 di S	SO_3^-	H	SO_3^-
△ Di-4,6 di S	H	SO_3^-	SO_3^-
△ Di-2,4 di S	SO_3^-	SO_3^-	H
△ Di-2,4,6 tri S	SO_3^-	SO_3^-	SO_3^-

Fig. 1. Structure of CS disaccharides and compositional properties.

in animals and humans (Campo et al., 2003; Cho et al., 2004; Imanari et al., 1999; Morreale et al., 1996; Omata, Itokazu, Inoue, & Segawa, 2000; Uebelhart, Thonar, Delmas, Chantraine, & Vignon, 1998; Volpi, 2002), CS can serve as an active biomarker of the anti-arthritic activity of shark cartilage powder.

Since the therapeutic bioactivities of shark cartilage powder and CS have become well known, the market of nutraceuticals containing shark cartilage powder and CS has dramatically increased. However, the quality of many nutraceutical raw materials remains in question. The limited supply of shark cartilage makes it expensive. The price of bovine and porcine cartilage powders is cheaper than that of shark cartilage powder and all of them contain CS. It is possible that some suppliers may have added other materials or cartilage powders obtained from other sources into nutraceuticals instead of SCP to cut the cost of production. It is therefore very important to identify the sources of nutraceutical raw materials and finished products, and to differentiate shark cartilage powders from bovine and/or porcine cartilage powders. The compositional analysis of CS disaccharides after enzymatic depolymerization can offer valuable information on the CS sources, because the component ratio of the disaccharides in samples of CS is dependent on the species from which it is derived (Mucci, Schenetti, & Volpi, 2000; Sim et al., 2005; Volpi, 2004).

In our previous research, we reported a quantitative analytical method to determine the content of CS in raw materials and various pharmaceutical preparations (Sim et al., 2005). We also identified several low-quality CS raw materials (pharmaceutical grade) and found that the purity of these pharmaceutical preparations was inconsistent with the specifications claimed on the product labels. In this study, we have focused on the quantification and biochemical properties of CS in SCPs and finished products including disaccharide composition, sources, and average molecular weights (MW).

2. Materials and methods

2.1. Materials

SCPs and finished products containing shark cartilage powder were obtained from the Korea Food Research Institute, and several local suppliers. Two authentic CSs, originating from bovine trachea and shark cartilage, were purchased from New Zealand Pharmaceuticals (Palmerston North, New Zealand) and Seikagaku Co. (Tokyo, Japan), respectively. Another form of authentic CS was purified from porcine placenta according to the method previously described (Kim et al., 1998). Authentic shark (*Squatina oculata*) cartilage powder was provided from the Korea Food Research Institute. Maxazyme NNP (180,000 PC/g), a commercial protease, purified from *Bacillus subtilis* was kindly donated from BISION Biochem (Sungnam, Korea). Two CSs (MW 15 and 40 kDa) used as MW standards were obtained from Seikagaku. Chondroitinase ABC from *Proteus vulgaris*, unsaturated CS disaccharides (ΔUA-[1 → 3]-GalNAc: ΔDi-0S, ΔUA-[1 → 3]-GalNAc-6S: ΔDi-6S, ΔUA-[1 → 3]-GalNAc-4S: ΔDi-4S, ΔUA-2S-[1 → 3]-GalNAc-4S: ΔDi-2,4diS, and ΔUA-2S-[1 → 3]-GalNAc-6S: ΔDi-2,6diS), boric acid, sodium chloride, sodium acetate, Tris[hydroxymethyl]aminomethane (Trizma® base), ethylenediamine tetra acetic acid (EDTA), and azure A were purchased from Sigma (St. Louis, MO, USA). Agarose was obtained from Cambrex Bio Science (Rockland, ME, USA). Other reagents were of the best grade available.

2.2. Sample treatment

Eight kinds of SCPs as nutraceutical raw materials were used in our experiments. Among these, half of SCPs (A, B, D, and E) were shark cartilage water extracts, and the others (C, F, G, and H) were raw materials of shark cartilage powder. To remove non-soluble ingredients in SCP C, F, G, and H, each sample (200 mg) was treated with 2 ml of Maxazyme NNP solution (2% v/v, pH 7.0) (Jo et al., 2004) with continuous inversion at 50 °C for 5 h on a mechanical rotary mixer (Roto-Torque, Model 7637) from Cole-Parmer Instrument Co. (Chicago, IL, USA). Sample solutions were boiled for 10 min and centrifuged at 2500*g* for 30 min. Each aqueous fraction was accurately transferred to a new tube, lyophilized, and weighed to make the water-soluble extracts. In case of shark cartilage powders in finished products, seven kinds (from A to G) were all raw materials of shark cartilage powder. Thus, the pretreatment of shark cartilage powders in hard capsules by using Maxazyme NNP was performed and the authentic shark cartilage powders from *Squatina oculata* was also analyzed under equal conditions just like the pretreatment of SCP for nutraceutical raw materials.

Stock solutions (30 mg/ml) of all the water-soluble shark cartilage powders in SCPs and finished products

were prepared. Authentic CSs (originating from bovine, porcine, or shark) were dissolved with water to make authentic CS solutions (5 mg/ml).

2.3. Agarose gel-electrophoresis

To characterize the content, average MW, and homogeneity of CS in SCPs and finished products, agarose gel-electrophoresis was performed using 1% gels in TBE buffer (45 mM Tris–borate, 1 mM EDTA). 10 μl of MW standards of CSs (10 mg/ml), and water-soluble samples (30 mg/ml) of SCPs and finished products were mixed with 10 μl of 60% sucrose solution, respectively. These preparations were loaded onto the gels and then a constant voltage (100 V) was applied for 1.5 h. The gels were stained with 0.5% azure A (in 1% acetic acid) solution for 10 min, and visualized after destaining with water–methanol–acetic acid (60:30:10, v/v/v).

2.4. Enzymatic depolymerization by chondroitinase ABC

To determine the content, disaccharide composition, and source of CS in SCPs and finished products, the enzymatic depolymerization by chondroitinase ABC was carried out as previously described (Sim et al., 2005). Briefly, 100 μl of authentic CS solutions (5 mg/ml) and water-soluble shark cartilage powder samples (15 mg/ml) in SCPs and finished products were mixed with 850 μl of Tris–acetate buffer (50 mM Trizma® base and 60 mM sodium acetate, pH 8.0), respectively. The samples were depolymerized with 50 μl of chondroitinase ABC (1 mU/μl) overnight at 37 °C. After heating for 5 min and filtering on 0.45 μm filters (Millipore, Bedford, MA, USA), the depolymerization mixtures (100 μl) were analyzed by strong anion-exchange high performance liquid chromatography (SAX-HPLC).

2.5. Disaccharide analysis of CS in SCPs and finished products

HPLC analysis was performed on the ÄKTA purifier system (Amersham Pharmacia, Uppsala, Sweden) equipped with a P-900 pump, a UV detector, and a fraction collector. UNICORN software version 3.1 (Amersham Pharmacia) was used to control the apparatus and to collect the data. Disaccharide analysis was performed with a Hypersil SAX column (4.6 × 250 mm, 5 μm) from Thermo Hypersil-Keystone (Bellefonte, PA, USA) as previously reported with minor modification (Cho et al., 2003). 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 and the system was operated at ambient temperature.

2.6. Determination of content and source of CS in SCPs and finished products

The SAX-HPLC was performed to analyze CS disaccharides derived from SCPs and finished products. Peaks of the disaccharides were confirmed with each authentic CS disaccharide (ΔDi-0S, ΔDi-6S, ΔDi-4S, ΔDi-2,4diS and ΔDi-2,6diS). The amount of CS in samples injected was determined by comparing the total peak area of the disaccharides and that of the authentic CS derived from bovine trachea. Final CS contents (%) in SCPs and finished products were calculated by using following equations.

$$\text{CS (\%) in water-soluble SCPs} = \frac{\text{PA of samples (150 μg)}}{\text{PA of authentic CS (50 μg)} \times 3} \times 100 \quad (1)$$

$$\text{CS (\%) in raw materials of shark cartilage powder} = (1) \times \frac{\text{A}}{200\ \text{mg}} \quad (2)$$

$$\text{CS (\%) in finished products} = (2) \times \frac{100}{\text{B}} \quad (3)$$

PA represents the peak area of disaccharides derived from CS in samples. A represents the amount (mg) of water-soluble fraction extracted from raw materials of shark cartilage powder (200 mg) and B represents the labeled percent of CS in finished products.

The source of CS in SCPs and finished products was determined by the ratio of disaccharides for CS source and the values were compared with those of three authentic CS samples.

$$\text{The ratio of disaccharides for CS source} = \frac{\text{PA of } \Delta\text{Di-4S}}{\text{PA of } (\Delta\text{Di-6S} + \Delta\text{Di-2,4diS} + \Delta\text{Di-2,6diS})}$$

2.7. Proximate composition analysis of SCPs and finished products

Moisture content was measured by sample weight-loss after oven-drying at 105 °C until a constant weight was obtained (AOAC, 1990). Crude protein content was calculated by converting the nitrogen content determined by Kjeldahl's method (nitrogen content ×6.25) (AOAC, 1990) in a Tecator 2020 digestor and Kjeltec 1035 auto-analyzer (Tecator, Sweden). Fat content was obtained by using Soxhlet system (AOAC, 1990). Ash content was determined from the weight of samples after burning overnight at 550 °C. Carbohydrate content was calculated according to the following formula: Carbohydrate (%) = 100 − (moisture % + protein % + fat % + ash %).

3. Results

3.1. Agarose gel-electrophoresis

After removing the non-soluble ingredients in some SCPs (C, F, G, and H), the eight kinds of water-soluble samples

were analyzed by agarose gel-electrophoresis using MW standard samples (CSs with MW 15 and 40 kDa, respectively). Bands of SCP F, G, and H (lanes h, i, and j, respectively) were more faint and there was no band of SCP C (lane e) (Fig. 2a). Most of SCPs had CS with approximately average MW 40 kDa. While the average MW of SCP A (lane c) was very high (more than 40 kDa), SCP D (lane f) had low MW CS (≈15 kDa) (Fig. 2a). In case of CS in finished products (without insoluble ingredients), the average MW of the finished product F (lane p) and G (lane q) was less than MW 40 kDa. The band density of finished product A (lane k) and C (lane m) was very low (Fig. 2b).

3.2. Quantification of CS in SCPs and finished products

CS in SCPs and finished products were depolymerized to completion using excess chondroitinase ABC, which specifically degrades all linkages found within both chondroitin (and dermatan) sulfate without acting on other polysaccharides. CS disaccharides derived by enzymatic depolymerization were quantitatively analyzed by SAX-HPLC. The CS content (%) in SCPs versus the authentic CS ranged from 0% to 28.92 ± 0.03%. Only two SCPs (A and B) exhibited more than 20% purity, and the other SCPs had small amounts of CS (D and E <20% and F, G, H <5%). No CS was detected in SCP C using a SAX-HPLC (Table 1) or agarose-gel electrophoresis. In finished products, the contents of CS were less than those of SCPs. Only finished product F (21.30 ± 0.08%) and D (5.98 ± 0.05%) contained more than 5% CS. There was small amounts of CS in the other finished products (A, B, C, E and G) and the CS content of authentic shark cartilage powder from *Squatina oculata* was 13.37 ± 0.09% (Table 2).

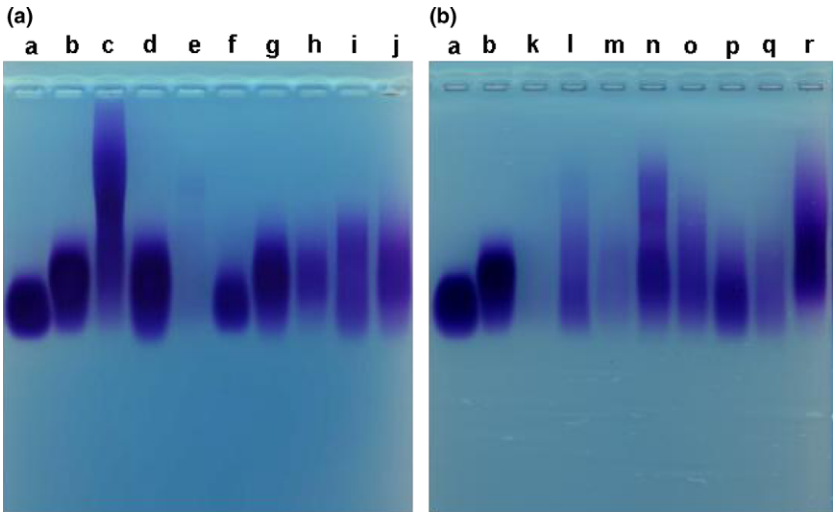

Fig. 2. Agarose gel-electrophoresis: (a) water-soluble fraction in SCPs; (b) water-soluble fraction in finished products. Lane a, Seikagaku CS (MW 15 kDa); lane b, Seikagaku CS (MW 40 kDa); lane c, SCP A; lane d, SCP B; lane e, SCP C; lane f, SCP D; lane g, SCP E; lane h, SCP F; lane i, SCP G; lane j, SCP H; lane k, finished product A; lane l, finished product B; lane m, finished product C; lane n, finished product D; lane o, finished product E; lane p, finished product F; lane q, finished product G; lane r, authentic shark cartilage powder from *Squatina oculata*. The authentic CSs (100 μg) and water-soluble fractions (300 μg) from SCPs and finished products were loaded onto 1% agarose gel.

Table 1
Characteristics of CS in SCPs on the basis of CS content (%), ΔDi-4S/(ΔDi-6S + ΔDi-diSs) and source

SCPs	Type	Extraction yield (%)	CS (%) in SCWE	Final CS (%)	ΔDi-4S/ (ΔDi-6S + ΔDi-diSs)	Source
A	SCWE[a]		28.92 ± 0.03	28.92 ± 0.03	0.49	SC[c]
B	SCWE		26.36 ± 0.12	26.36 ± 0.12	0.54	SC
C	RM[b]	35.9 ± 0.29	0	0	X	SC
D	SCWE		12.85 ± 0.02	12.85 ± 0.02	1.53	BT[d]
E	SCWE		18.36 ± 0.13	18.36 ± 0.13	0.56	SC
F	RM	17.05 ± 0.16	5.06 ± 0.17	0.86 ± 0.03	0.26	SC
G	RM	32.75 ± 0.23	7.12 ± 0.04	2.33 ± 0.01	0.32	SC
H	RM	28.15 ± 0.31	9.73 ± 0.06	2.74 ± 0.02	0.41	SC

[a] SCWE, shark cartilage water extract.
[b] RM, raw materials of shark cartilage powder containing non-soluble components.
[c] SC, shark cartilage.
[d] BT, bovine trachea. Values are expressed as means ± SD ($n = 3$).

Table 2
Characteristics of CS in finished products on the basis of CS content (%), ΔDi-4S/(ΔDi-6S + ΔDi-diSs) and source

Finished products	SC (%)	Extraction yield (%)	CS (%) in SCWE	Final CS (%)	ΔDi-4S/ (ΔDi-6S + ΔDi-diSs)	Source
A	100	57.15 ± 0.45	1.08 ± 0.01	0.62 ± 0.01	0.14	SC[b]
B	64.83	33.05 ± 0.28	7.21 ± 0.07	3.67 ± 0.04	0.15	SC
C	100	35.15 ± 0.21	1.64 ± 0.04	0.58 ± 0.01	0.14	SC
D	100	40.5 ± 0.47	14.80 ± 0.13	5.98 ± 0.05	0.34	SC
E	100	26.7 ± 0.29	8.12 ± 0.06	2.17 ± 0.02	0.22	SC
F	60	88.15 ± 0.56	14.50 ± 0.05	21.30 ± 0.08	0.37	SC
G	100	31.7 ± 0.27	3.89 ± 0.12	1.23 ± 0.04	0.13	SC
ASC[a]	100	47.15 ± 0.93	28.35 ± 0.19	13.37 ± 0.09	0.46	SC

[a] ASC, authentic shark cartilage from *Squatina oculata*.
[b] SC, shark cartilage. Values are expressed as means ± SD ($n = 3$).

3.3. Determination of CS source in SCPs and finished products

To assess the source of SCPs and finished products, the compositional analysis of CS disaccharides was performed by a SAX-HPLC and the ratio of disaccharides for CS source [ΔDi-4S/(ΔDi-6S + ΔDi-2,4diS + ΔDi-2,6diS)] was calculated. The peak areas of CS disaccharides derived from SCPs and finished products was calculated as previously described (Section 2.6). The results showed that CSs in SCPs have the typical disaccharide pattern of CS obtained from shark cartilage with the exception of SCP D; they have large quantities of ΔDi-6S and ΔDi-2,6diS. The source of CS in SCP D was shown to be the cartilage of land animals (probably bovine) based on the ratio of disaccharides for CS source and absence of ΔDi-2,6diS (Table 1 and Fig. 3). Its ratio of disaccharides for CS source was similar to that of authentic CS derived from bovine. In the case of CS in finished products, they all have the same disaccharide pattern of CS obtained from shark

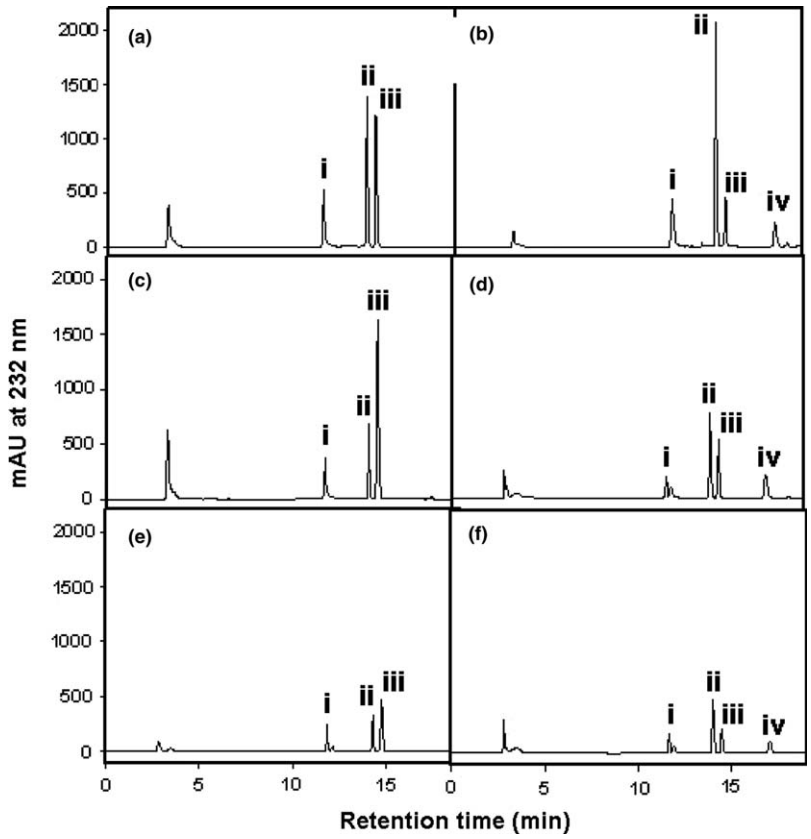

Fig. 3. SAX-HPLC analysis of CS disaccharides produced by enzymatic depolymerization: (a) authentic CS derived from bovine trachea; (b) authentic CS derived from shark cartilage; (c) authentic CS derived from porcine placenta; (d) authentic shark cartilage powder derived from *Squatina oculata*; (e) SCP D; (f) finished product D. (i) ΔDi-0S (ΔUA-[1 → 3]-GalNAc); (ii) Δ Di-6S (ΔUA-[1 → 3]-GalNAc-6S); (iii) Δ Di-4S (ΔUA-[1 → 3]-GalNAc-4S); (iv) ΔDi-2,6diS (ΔUA-2S-[1 → 3]-GalNAc-6S). The authentic CSs (500 μg/ml) and the other samples (1.5 mg/ml) were depolymerized by chondroitinase ABC as described in Section 2.4.

cartilage. The ratio of disaccharides for CS source indicate that CSs can be distinguished according to the difference of their disaccharide patterns and the results of authentic CSs from bovine trachea, shark cartilage, and porcine placenta were 1.13, 0.19, and 3.07, respectively.

3.4. Validation of the method

To assess the linearity of this method, various concentrations (from 0 to 2 mg/ml) of authentic CS (originating from bovine) solutions were depolymerized by chondroitinase ABC and analyzed by a SAX-HPLC as previously described in Sections 2.4 and 2.5. An eight point calibration curve (from 5 to 1000 μg/ml) was obtained and the linear equation of regression analysis was calculated between the concentrations (μg/ml) of the authentic CS and the total peak areas of CS disaccharides. The calibration curve had a correlation coefficient higher than 0.999 (Table 3).

The limit of detection (LOD), defined as a signal-to-noise (S/N) ratio >3, was 1.7 μg/ml of authentic CS solution. The limit of quantification (LOQ), defined as the lowest analyte concentration yielding a S/N ratio >10, was 5 μg/ml (Table 3).

The accuracy was estimated from the recovery experiments by analyzing a blank sample (a water-soluble fraction from authentic shark cartilage powder) spiked with authentic CS at three concentrations (200, 300, and 500 μg/ml). The sample solutions were depolymerized by chondroitinase ABC, analyzed by SAX-HPLC, and quantified based on the calibration curve. The average recoveries of authentic CS at 200, 300, and 500 μg/ml were 102.39%, 96.62%, and 95.27%, respectively (Table 4).

Table 3
Linearity and sensitivity of the method

Concentration range (μg/ml)	Correlation coefficient	LOD[a] (μg/ml)	LOQ[b] (μg/ml)
5–1000	0.9998	1.7	5

[a] LOD, limit of detection.
[b] LOQ, limit of quantification.

Table 4
Recovery of CS from a water-soluble fraction obtained from authentic shark cartilage powder

Sample	Concentration added (μg/ml)	Recovery (%)	RSD[a] (%)
SCWE[b] (1.5 mg/ml) of authentic SCP	200	102.39	3.95
	300	96.62	2.65
	500	95.27	2.27

[a] RSD, relative standard deviation ($n = 3$).
[b] SCWE, shark cartilage water extract.

The precision was determined by measuring the total peak area of CS disaccharides in spiked blanks at the three concentrations. The relative standard deviation (RSD) for triplicate determinations at 200, 300, and 500 μg/ml were 3.95%, 2.65%, and 2.27%, respectively (Table 4).

3.5. Proximate composition analysis of SCPs and finished products

Proximate compositions of several SCPs and finished products were determined in terms of moisture, protein, fat, ash, and carbohydrate (Table 5). SCPs were mainly comprised of significant amount of proteins (SCP C and D, $52.37 \pm 0.26\%$ and $63.92 \pm 0.16\%$, respectively) or carbohydrate (SCP B, $69.57 \pm 0.72\%$). The finished products F and G had protein content more than $59.89 \pm 0.30\%$, while the finished products B (Table 5), C, D, and E contained ash between $45.33 \pm 0.19\%$ and $48.05 \pm 0.28\%$ (data not shown). In case of the finished product F containing shark cartilage powder, glucosamine, and several vitamins, the content (%) and source of CS were consistent with the specifications claimed on the product labels, although it had much protein amount. It is possible that other components having nitrogen molecule such as glucosamine and vitamins can result in overestimating protein content according to this method. Although we cannot analyze the proximate compositions of all SCPs and finished products due to the shortage of the sample amount, there is considerable difference among the compositional patterns of

Table 5
Proximate composition analysis of SCPs and finished products

Samples	Content (%)				
	Moisture	Protein	Fat	Ash	Carbohydrate
SCP B	4.49 ± 0.08	18.29 ± 0.12	4.49 ± 0.62	3.16 ± 0.06	69.57 ± 0.72
SCP C	6.08 ± 0.09	52.37 ± 0.26	0.91 ± 0.05	10.57 ± 0.46	30.07 ± 0.68
SCP D	6.77 ± 0.16	63.92 ± 0.16	1.27 ± 0.08	8.51 ± 0.07	19.53 ± 0.15
FP[a] B	9.77 ± 0.10	32.50 ± 1.78	4.43 ± 0.17	48.05 ± 0.28	5.25 ± 1.77
FP F	4.95 ± 0.18	60.88 ± 0.18	1.07 ± 0.11	11.70 ± 0.11	21.40 ± 0.15
FP G	2.59 ± 0.22	59.89 ± 0.30	0.76 ± 0.01	17.93 ± 0.06	18.83 ± 0.45
ASC[b]	3.82 ± 0.22	25.03 ± 1.31	0.50 ± 0.06	41.31 ± 0.11	29.34 ± 1.37

[a] FP, finished product.
[b] ASC, authentic shark cartilage from *Squatina oculata*. Values are expressed as means ± SD ($n = 2$).

SCPs and finished products compared to that of the authentic shark cartilage powder from *Squatina oculata*. The authentic shark cartilage powder was composed of $3.82 \pm 0.22\%$ moisture, $25.03 \pm 1.31\%$ crude proteins, $0.50 \pm 0.06\%$ lipids, $41.31 \pm 0.11\%$ ashes, and $29.34 \pm 1.37\%$ carbohydrates (Table 5).

4. Discussion

We performed the quantification and characterization of CS in SCPs and finished products based on its disaccharide composition, source, and average MW. Because the commercial cartilage powders (obtained from bovine, porcine and shark) have CS, the characterization of CS can be an important parameter for the quality evaluation of cartilage powders. The SAX-HPLC method was validated and confirmed to be applicable in the quantification of CS in SCPs and finished products. The contents of CS in SCPs and finished products were 0–$28.92 \pm 0.03\%$ and 0.58 ± 0.01–$21.30 \pm 0.08\%$, respectively. The contents of CS in shark cartilage water extracts (SCP A, B, D, and E) were generally higher than those in raw materials of shark cartilage powder (SCP C, F, G, and H). Two SCPs (A and B) showed that CS contents are similar to that of a water-soluble fraction obtained from the authentic shark cartilage powder (*Squatina oculata*). Raw materials of shark cartilage powder showed the discordance between their CS contents and their specifications. Except for the finished product F ($21.30 \pm 0.08\%$), there was a small amount of CS in other finished products compared with the content of CS in authentic shark cartilage powder (*Squatina oculata*, $13.37 \pm 0.09\%$). The source of SCPs and finished products was estimated by the disaccharide patterns of CS in SCPs and finished products after enzymatic depolymerization. The ratio of disaccharides for CS source showed that all SCPs and finished products have the typical disaccharide pattern of CS obtained from shark cartilage with the exception of SCP D (probably bovine). The average MWs of CS in SCPs and finished products were approximately more than 40 kDa except for SCP D (15 kDa).

Proximate composition analysis of SCPs and finished products showed that there is significant difference among the contents of ash, protein, and carbohydrate. Although the composition of shark cartilage may be dependent on the species of shark, some samples (SCP C and D and finished product G) contained much protein amount representing over $52.37 \pm 0.26\%$, which is twice higher than that of an authentic shark cartilage powder representing $25.03 \pm 1.31\%$. It is not clear why the composition of the same sort of samples from shark cartilage is quite different.

Based on these analytical results, the quality of SCPs and finished products is poor and the strict regulation for quality control should be required to guarantee the manufacture of high quality products.

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