Correlation Between Physicochemical Characteristics and Binding Capacities of Chitosan Products

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ABSTRACT: Physicochemical characteristics (molecular weight, nitrogen, ash, degree of deacetylation, bulk density, viscosity, and residual amino acids) of 6 commercial chitosans differed with products. Significant correlations were observed between molecular weight and viscosity and between nitrogen and degree of deacetylation. Binding capacities of water, fat, and three different dyes also varied depending on the products. Results of correlations between binding capacities and physicochemical characteristics indicated that both water and fat binding capacities were significantly negatively correlated with bulk density. In addition, fat binding capacity showed a significant correlation with viscosity. Significant negative correlations were observed between dye binding capacities of three red, yellow, and blue dyes and ash.

Key Words: Chitosan, physicochemical characteristics, binding capacities, correlations

Introduction

DURING THE PAST FEW DECADES, CHITOSAN HAS BEEN RECEIVING increased attention for its commercial applications in biomedical, food, and various chemical industries (Muzzarelli 1977; Knorr 1984). In studies on functional properties of chitinous polymers, chitosan has been documented to possess several distinctive properties for use in water and fat uptake, emulsification (Knorr 1982), dye binding (Knorr 1983), and gelation (Vorlop and Klein 1981). This natural, nontoxic, biopolymer chitosan is now widely produced commercially from crab and shrimp waste shells.

Earlier studies (Wu and Bough 1978; Brine and Austin 1981a; Shimahara and others 1984) demonstrated that physicochemical characteristics of chitosan affect its functional properties that differ with preparation methods. More recent studies (Ahn and Lee 1992; Byun and others 1992; Lee and others 1995) have revealed notable variability in the dye, water, and fat binding capacities of various chitins, chitosans, and their derivatives prepared in the laboratory. More recently, Cho and others (1998) also reported differences between products in physicochemical characteristics and functional properties of chitosan. Therefore, it is mandatory that physicochemical characteristics and functional properties of chitosan products should be constantly monitored to insure uniform product quality and to effectively utilize these products for particular applications.

Recently in Korea, chitosan has attracted considerable interest in view of varied proposed novel uses, especially in so-called health foods. As of 1998, the potential size of the chitosan market in Korea was estimated to be around 600 tons yearly. About 10 plants produce chitosan and its related products on a commercial scale to meet such demand. However, no information is available on the yearly production volume (Anonymous 1998). Few attempts have been made to critically evaluate the physicochemical characteristics and functional properties of domestic commercial chitosan products. Information on the relationships between physicochemical characteristics and functional properties is needed to improve functional properties of chitosan products by providing proper product for the particular intended usage.

The objectives of our study were to evaluate physicochemical characteristics and binding capacities of commercially available Korean chitosan products and to determine functional correlations.

Materials and Methods

Materials

Six commercially available chitosans (designated 1-6) were obtained from Biotech (Seoul), Keumho Chemical Co. (Seoul), and Kitto Life (Seoul) in Korea. All chitosans were acid-soluble, white-colored powder forms, and prepared from crab (*Chionoecetes opilio*) leg shell.

To obtain a uniform size product, these samples were sifted with a 60-mesh (0.250 mm) sieve using a portable sieve shaker (JISICO, Seoul, Korea), placed in opaque plastic bottles, and stored at ambient temperature. Chitosan powder of < 0.250 mm size was used throughout this research to obtain reproducible and consistent results. Prior to binding studies, all samples were dried at 105 °C for 2 h.

The dyes used for evaluation of binding capacity were FD&C Red No. 40 (disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid), FD&C Yellow No. 4 (1-[2-methylphenyl)azo]-2-naphthylamine), and FD&C Blue No. 1 (N-ethyl-N-[4-[[4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl] (2-sulfophenyl) methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfobenzenemethanaminium hydroxide inner salt, disodium salt) (designated red, yellow, and blue dye, respectively). Commercially available refined soybean oil was used for the fat binding study.

Preparation of dye solution

Dye solution was prepared by dissolving dye in deionized water at a concentration of 500 mg/L. For the standard curve determination, the maximum absorbance of the aqueous dye solutions containing 2.5 – 20 mg of dye/L was measured with a spectrophotometer (Shimadzu UV-160A, Shimadzu Co., Tokyo, Japan) using deionized water as a blank.

Dye binding capacity.

Dyeing of chitosan was achieved by shaking 0.25 g chitosan and 10 mL of aqueous dye solution (containing 5 mg of dye) in horizontally positioned screw-capped test tubes at 25 °C for 1 h using a shaking water bath (80 rpm), followed by procedures described by Cho and others (1998). The amount of dye bound to chitosan was determined by calculating differences in concentra-

tions between the initial dye solution and the combined filtrate. Dye binding capacity (DBC) was expressed as % adsorption.

Water and fat binding capacity

Water binding capacity (WBC) and fat binding capacity (FBC) of chitosan were measured using a modified method of Wang and Kinsella (1976). Water or fat absorption was initially carried out by weighing a centrifuge tube containing 0.5 g sample, adding 10 mL of water or soybean oil, and mixing on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with shaking for 5 s every 10 min and centrifuged at 3200 rpm for 25 min. After the supernatant was decanted, the tube was weighed again. WBC and FBC were calculated as follows: WBC (%) = [water bound (g)/sample weight (g)] $\times$ 100; FBC (%) = [fat bound (g)/sample weight (g)] $\times$ 100.

Proximate analysis and bulk density (BD)

Nitrogen was determined using an elemental analyzer (EA 1110, CE Instrument, Rodano-Milan, Italy). Ash was calculated by standard methods (AOAC 1990). BD was determined following procedures of Cho and others (1998) and computed as g/mL of the sample.

Degree of deacetylation (DD) and molecular weight (Mw)

The DD was determined by a colloid titration method (Toei and Kohara 1976) using N/400 potassium polyvinyl sulfate (f = 1.006, Wako Pure Chemical Ind., Osaka, Japan).

For the determination of viscosity average Mw of chitosan, chitosan was dissolved in 0.1 M acetic acid-0.2 M NaCl and Automated Solution Viscometer (Relative Viscometer Model Y501, Viscotek Corp., Houston, TX, U.S.A.) was used to measure the intrinsic viscosity [η]. The Mark-Houwink equation [η] = KMw^a was used to calculate the molecular weight. The values of K and a of 1.81×10^{-3} cm^3/g and 0.93 respectively were used.

Viscosity

Viscosity was measured with a Brookfield viscometer, Model RVT (Brookfield Engineering Laboratories, Inc., Stoughton, Mass., U.S.A.). Chitosan solution was prepared in 1% acetic acid at a 1% concentration. Measurements were made in duplicate using a No. 2 spindle at 5 rpm on solutions at 25 °C with values reported in centipoise units.

Amino acid analysis

Chitosan samples (0.1 g) were hydrolyzed with 6 N HCl for 24 h at 110 °C in sealed tubes. The hydrolysates were filtered, evaporated to near dryness, and dissolved in 20 mL of sodium citrate buffer of pH 2.2. An aliquot was filtered through a cartridge filter (0.2 μm, Sartorius) and analyzed by an amino acid analyzer (Biochrom 20, Pharmacia Biotech Ltd., Cambridge, England).

Statistical analysis

All experiments were carried out in duplicate, and average values or means $\pm$ standard deviations are reported. Mean separation and significance for correlation were analyzed using the SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, U.S.A.) software package.

Results and Discussion

Characteristics of chitosan products

The physicochemical characteristics of 6 chitosan products were determined, with results shown in Table 1. The Mw differed with products ranging from 0.22 to 1.67 $\times$ 10^6 Da. These Mws are considered to be representative ranges generally useful in various commercial applications. The nitrogen content ranged from 7.24 to 7.90%. Ash content was below 1.0%, indicating the effectiveness of the method used for removal of calcium carbonate. DD was mostly high, over 87%, except for chitosan 4 with 79.2%. Chitosan 2 showed a DD of about 100%. BD was in the range of 0.18 – 0.33 g/mL, indicating up to 1.8 times difference in porosity. Viscosity differed significantly ($P < 0.05$) with products.

Table 1—Characteristics[1] of chitosan products

Chitosan product	Mw[2] (× 10^6 Da)	N (%)	Ash (%)	DD[3] (%)	BD[4] (g/mL)	Viscosity[5] (cP)
1	1.19^e	7.40 ± 0.14^a	0.5 ± 0.1^{ab}	87.6 ± 0.2^b	0.24^c	134 ± 3^d
2	0.75^b	7.90 ± 0.07^c	0.3 ± 0.1^a	100 ± 0.5^e	0.21^b	40 ± 0^b
3	1.67^f	7.67 ± 0.03^b	0.6 ± 0.1^{ab}	98.9 ± 0.6^e	0.18^a	360 ± 0^f
4	1.11^d	7.24 ± 0.02^a	0.7 ± 0.3^{bc}	79.2 ± 0.2^a	0.24^c	144 ± 0^e
5	0.22^a	7.67 ± 0.03^b	1.0 ± 0.0^c	92.0 ± 1.2^c	0.31^d	26 ± 3^a
6	0.78^c	7.70 ± 0.09^b	0.3 ± 0.0^a	96.6 ± 0.2^d	0.33^e	52 ± 6^c

[1]Mean ± standard deviation or average of duplicate determinations, on a dry basis.
[a–f]Means with different superscripts within a column indicate significant differences ($P < 0.05$).
[2]Mw = Viscosity average molecular weight. Standard deviation of each sample was less than 0.02 × 10^6.
[3]DD = Degree of deacetylation.
[4]BD = Bulk density. Standard deviation of each sample was less than 0.005.
[5]Viscosity was measured with 1% chitosan solution in 1% acetic acid.

The above results demonstrate that physicochemical characteristics of chitosan differ with products. Such dissimilarities in chemical and physical properties of chitosans are considered due to different preparation methods used for intended usage of the product. The characteristics of chitosan products presented in this study are comparable to those reported by Van Ornum (1992), Li and others (1992), and Cho and others (1998) for commercial chitosan products. According to a literature review (No and Meyers 1995), the average Mw of chitosan was 0.12 – 1.5 $\times$ 10^6, with the nitrogen content ranging from 7.06 to 7.97%, and ash content less than 1%. The DD ranged from 56 to 99%. The viscosity, all dissolved in acetic acid, varied considerably from 60 to 5110 cP, depending on the species and preparation methods.

Amino acid composition

Table 2 reveals residual amino acids in chitosan products. All chitosans contained less than 2.0 mg/g of residual amino acids, the relatively predominant one being lysine. These residues are considerably lower than those (44.6 – 47.4 mg/g) reported by Sophanodora and Hutadilok (1995) for black tiger shrimp chitosan. Differences in residual amino acids of chitosans reported in our study and by Sophanodora and Hutadilok (1995) probably are due to different preparation methods applied or to species-related variability (Brine and Austin 1981b).

Correlation among physicochemical characteristics

Results of correlations among physicochemical characteristics of chitosan products are shown in Table 3. Significant correlations were observed between molecular weight and viscosity ($r = 0.903$, $P < 0.05$), and between nitrogen and DD ($r = 0.942$, $P < 0.01$).

These results clearly demonstrate that reduction in molecular weight results in a less viscous chitosan product, as reported by previous workers (Bough and others 1978; Sophanodora and Hutadilok 1995). Therefore, to obtain a more viscous chitosan product, processing conditions that will produce a less degraded product must be employed for deacetylation. A positive correlation between nitrogen and DD suggests the possibility that DD can be accurately estimated by measurement of nitrogen content.

Water and fat binding capacity

Water binding capacity (WBC) and fat binding capacity (FBC) of chitosans were measured with results shown in Table 4. WBC

Table 2—Amino acid composition[1] of chitosan products (Unit:mg/g)

Composition	Chitosan product 1	2	3	4	5	6
Asp	0.015	0.003	0.013	0.040	0.016	0.005
Thr	0.007	0.002	0.008	0.024	0.005	0.002
Ser	0.003	0.006	0.021	0.029	0.022	0.010
Glu	0.021	0.005	0.021	0.057	0.026	0.010
Gly	0.039	0.081	0.080	0.047	0.312	0.145
Ala	0.013	0.006	0.014	0.041	0.016	0.002
Cys	0.004	0.004	0.004	0.002	ND	0.002
Val	0.081	0.080	0.081	0.082	0.080	0.083
Met	0.028	0.019	0.026	0.051	0.027	0.017
Ile	0.008	0.003	0.007	0.018	0.003	0.009
Leu	0.022	0.052	0.033	0.047	0.016	0.032
Tyr	ND	ND	0.037	0.058	ND	ND
Phe	ND	ND	0.009	0.022	ND	0.008
His	0.151	0.086	0.026	0.214	0.138	0.061
Lys	1.301	0.766	0.048	0.258	0.012	0.740
Arg	0.168	0.013	0.018	0.167	0.050	0.160
Total	1.861	1.126	0.446	1.157	0.723	1.286

[1]Average of duplicate determinations.
ND=Not detectable.

Table 3—Correlation (r) among physicochemical characteristics of chitosan products

	Mw (kDa)	N (%)	Ash (%)	DD (%)	BD (g/mL)	Viscosity (cP)	AA[1] (mg/g)
Mw (kDa)	1.000	-0.315	-0.286	0.025	-0.731	0.903*	-0.038
N (%)		1.000	-0.346	0.942**	0.014	-0.216	-0.379
Ash (%)			1.000	-0.431	0.161	0.054	-0.440
DD (%)				1.000	-0.116	0.065	-0.389
BD (g/mL)					1.000	-0.696	0.205
Viscosity (cP)						1.000	-0.408
AA[1] (mg/g)							1.000

[1]AA = Amino acids.
*P < 0.05, **P < 0.01

differed depending on the products, ranging from 355 to 611%. The range of WBC found in our study was somewhat lower than that (458 – 805%) reported by Cho and others (1998) who examined one domestic and four foreign chitosan products. FBC of chitosans also differed considerably with products, ranging from 217 to 403%. Overall, higher FBC values (314 – 535%) compared with our results were observed by Cho and others (1998). According to Knorr (1982), possible explanations for the differences in water uptake between chitinous polymers include differences in the crystallinity of the products, differences in the amount of salt-forming groups, and differences in the protein content of the materials.

Dye binding capacity

DBCs of chitosan products were compared, with results given in Table 4. Marked differences in binding capacity were observed among products. Chitosans showed DBC (as % adsorption) of 21.3 to 100% for red, 7.4 to 99.8% for yellow, and 14.9 to 98.0% for blue, at a concentration of 5 mg dye/0.25 g sample. Considerably high DBC was observed with chitosans having molecular weights in the 0.7×10^6 Da range. On the other hand, chitosan 5 with the lowest molecular weight (0.22×10^6 Da), showed the lowest DBC for the three dyes. Considerable variations in DBC (35.2 – 85.6%) with chitosan products also were observed by Cho and others (1998) with FD&C Red No. 40.

To elucidate whether there is any difference in DBC even among particle sizes (>60 mesh) of chitosan used as a sample, two different particle size ranges (60 – 80 and >100 mesh) of chitosan were investigated to compare their DBC using a concentration of 10 mg red dye/0.25 g chitosan 2. Results showed comparable binding capacities (97.9 ± 0.3% and 97.2 ± 0.4%) at both size ranges of 60 – 80 mesh (0.250 – 0.180 mm) and >100 mesh (<0.150 mm).

Table 4—WBC, FBC, and DBC of chitosan products

Chitosan product	WBC[1] (%)	FBC[1] (%)	DBC (%)[1,2] Red	Yellow	Blue
1	610 ± 0^{c}	306 ± 1^{cd}	95.0 ± 0.6^{d}	76.0 ± 0.2^{c}	87.3 ± 2.2^{c}
2	581 ± 33^{c}	323 ± 16^{d}	100 ± 0.0^{e}	99.8 ± 0.1^{e}	96.9 ± 0.0^{d}
3	611 ± 2^{c}	403 ± 6^{e}	67.4 ± 4.1^{b}	55.5 ± 0.4^{b}	71.0 ± 0.1^{b}
4	535 ± 10^{b}	296 ± 4^{c}	83.1 ± 0.3^{c}	54.2 ± 4.2^{b}	74.7 ± 1.0^{b}
5	372 ± 3^{a}	237 ± 3^{b}	21.3 ± 1.6^{a}	7.4 ± 0.4^{a}	14.9 ± 7.3^{a}
6	355 ± 6^{a}	217 ± 7^{a}	99.8 ± 0.0^{e}	96.7 ± 0.1^{d}	98.0 ± 0.1^{d}

-[1]Mean ± standard deviation of duplicate determinations, on a dry basis. $^{a–e}$Means with different superscripts within a column indicate significant differences (P < 0.05).[2]At a concentration of 5 mg dye/0.25 g sample.

Table 5—Correlation (r) between physicochemical characteristics and binding capacities of chitosan products

	WBC (%)	FBC (%)	DBC (%) Red	Yellow	Blue
Mw (kDa)	0.739	0.802[1]	0.406	0.279	0.466
N (%)	-0.186	0.020	-0.033	0.262	0.055
Ash (%)	-0.239	-0.100	-0.919**	-0.993**	-0.949**
DD (%)	-0.045	0.207	0.053	0.330	0.168
BD (g/mL)	-0.939**	-0.968**	-0.230	-0.167	-0.277
Viscosity (cP)	0.601	0.834*	-0.005	-0.089	0.082
AA (mg/g)	0.128	-0.335	0.625	0.509	0.524

*P < 0.05, **P < 0.01, [1]P = 0.055

From the above results, it is apparent that chitosan with high DBC for one dye exhibits a similar trend for other dyes in the given particle size ranges.

Correlation between binding capacities and physicochemical characteristics

Results of correlations between binding capacities and physicochemical characteristics of chitosan products are seen in Table 5. Both WBC and FBC were significantly correlated negatively with bulk density ($r = -0.939, -0.968$, $P < 0.01$). In addition, FBC showed a trend of correlation with molecular weight ($r = 0.802$, $P = 0.055$), and a significant correlation with viscosity ($r = 0.834$, $P < 0.05$). Significant ($P < 0.01$) negative correlations were observed between DBCs of three red, yellow, and blue dyes and ash ($r = -0.919, -0.993, -0.949$).

A negative correlation between WBC and bulk density of chitosan also was observed by Chang and others (1994). More recently, Cho and others (1998) found that both WBC and FBC were significantly correlated positively with ash ($r = 0.81, 0.80$), and negatively with bulk density ($r = -0.98, -0.95$). However, in our investigation, ash showed no correlation with either WBC ($r = -0.239$) or FBC ($r = -0.100$), but significant correlations with DBC. It is noteworthy that there are no correlations between DBC and molecular weight and between DBC and DD.

Conclusions

THIS STUDY HAS CLEARLY DEMONSTRATED THAT BOTH PHYSICOchemical characteristics and binding capacities of commercially available chitosans differ with individual products. In physicochemical characteristics, significant correlations were observed between molecular weight and viscosity and between nitrogen and degree of deacetylation (DD). In binding capacities, both water binding capacity (WBC) and fat binding capacity (FBC) were significantly correlated with bulk density. Significant correlations were observed between dye binding capacities (DBCs) of three dyes and ash. Thus, to effectively utilize chitosan as a functional ingredient in various commercial applica-

tions, relationships between critical functional properties and characteristics of chitosan products must be constantly monitored. Better understanding of the relationships reported in this paper ultimately will improve functional properties of chitosan by providing proper product quality information for specific applied usage.

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