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Effect of Freeze-Thaw Pretreatment on Thermal Drying Process and Physicochemical Properties of Chitosan

Wei Zhang,[1] Wenshui Xia[2]

[1]College of Food Science and Engineering, Wuhan Polytechnic University, Wuhan 430023, Hubei, People's Republic of China
[2]State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi 214122, Jiangsu, People's Republic of China
Correspondence to: W. Zhang (E-mail: zhangwei_food@163.com)

ABSTRACT: The effect of freeze-thaw pretreatment on the thermal drying process and physicochemical properties of chitosan was investigated in this study. Results showed that the freeze-thaw treatment changed the form of chitosan paste and reduced 75.6–77.7% of the water content. The freeze-thaw treatment decreased the drying time of chitosan from 16–19 h to 2.75–4 h and the dried product was loosely packed powder. After freeze-thaw treatment, the molecular weight of chitosan was unchanged during the thermal drying. The heat-induced browning effect of chitosan during drying was greatly alleviated by the pretreatment. Furthermore, the pretreatment increased the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity of dried product by 40.4–59.8%. The molecular weight, color, and DPPH radical-scavenging activity of the pretreated dried chitosan product were close to those of freeze-dried product. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 41017.

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INTRODUCTION

Chitosan is a cationic aminopolysaccharide obtained by partial deacetylation of chitin, which originates from shells of crustaceans.[1,2] Due to its unique polycationic nature, chitosan has been proposed for applications in food, pharmaceutical, and chemical industries.[3–5]

Drying is an important operation in chitosan production, which guarantees the moisture for stable storage.[6] Industrially, chitosan in form of paste is dried in tray dryers and spouted bed.[7] However, during drying processes of these methods, the color of chitosan will get dark and the molecular weight will increase due to the heat-induced Maillard reaction.[8] In laboratory, chitosan can be dried with freeze drier,[9,10] but this method is not economic for industrial application.

Freeze-thaw is an ordinary phenomenon during storage and consumption of frozen food materials and is found able to change the physicochemical state of materials and decrease the water content.[11,12] The freeze-thaw treatment is also reported to have pronounced influence on macromolecular gel.[13–16] Charoenrein et al.[17] indicated that the freezing treatment increased the molecular interactions between starch chains and the following thawing treatment expelled the unbounded water from starch gel.

In this study, the effect of freeze-thaw pretreatment on thermal drying process of chitosan was investigated. The physicochemical properties as particle size, molecular weight, deacetylation degree (DD), color, and antioxidative activity of pretreated dried products were studied.

MATERIALS AND METHODS

Materials

Chitosan with viscosity molecular weight (Mv) of 450 kDa and DD of 85.3% were obtained from Nantong Shuanglin biotechnology Co. (Jiangsu, China). Cellulase was supplied by Golden-Shell Biochemical Co. (Zhejiang, China). All other chemicals and regents were of analytical grade unless otherwise stated.

The Preparation of Chitosan Paste

Chitosan (Mv = 450 kDa, DD = 85.3%) was completely dissolved in acetic acid-sodium acetate buffer (pH 4.6) to make a solution of 2% (w/v). A cellulase solution was added to chitosan solution to initiate reaction. During reaction, the solution was stirred constantly and the temperature was controlled at 50°C. After the reaction, the solution was adjusted to pH 7.0 with NaOH solution and then centrifuged at 3000× *g* for 15 min to collect the paste. The paste was washed with deionized water and then recovered with centrifugation (3000× *g*, 15 min). The processes of washing and centrifugation were repeated for three

Table I. The Water Content of Chitosan Paste Before and After Freeze-Thaw Treatment

	Mv[a] (kDa)	X_0 (g water/g dry solid)	X_0' (g water/g dry solid)	WLR (%)
CH-1	379.4	22.1 ± 1.4*	4.9 ± 0.2**	77.7
CH-2	175.3	21.5 ± 1.0*	5.1 ± 0.2**	76.2
CH-3	90.3	20.5 ± 1.1*	5.0 ± 0.3**	75.6
CH-4	67.0	21.9 ± 1.3*	5.1 ± 0.1**	76.8
CH-5	54.5	22.7 ± 1.4*	5.4 ± 0.2**	77.1

Values were expressed as means ± standard deviation ($n = 3$). Values with different symbols (*, **) within the same row were significantly different at $P < 0.05$.
[a] Viscosity average molecular weight (Mv) of chitosan product from freeze drying.

times. The obtained paste products for reaction times of 0, 0.5, 1, 1.5, and 2 h were coded as CH-1, CH-2, CH-3, CH-4, and CH-5, respectively.

The Freeze-Thaw Pretreatment of Chitosan Paste

The chitosan paste was frozen at −18°C in a refrigerator. When totally frozen up, the sample was thawed at room temperature, and then centrifuged at 3000 × *g* for 15 min to separate the water.

Water lose rate (WLR) of chitosan was calculated as follows,

$$\mathrm{WLR} = \frac{X_0 - X_0'}{X_0}$$

Where X_0 is the water content (dry basis) of original chitosan paste, and X_0' is the water content (dry basis) of chitosan after freeze-thaw treatment.

Thermal Drying

Thermal drying process was performed with an oven (ENGZI 101-1-BS, Shanghai Yuejin Machine Factory, China). The samples were spread in a single layer on a stainless steel tray for drying. Moisture content (X) determined on dry weight basis were recorded during different time interval.

Freeze Drying

The paste was firstly frozen in a refrigerator at −80°C and then lyophilized in LG-3 freeze dryer (Ningbo Biochemical Instrument Plant, China) at −40°C.

Determination of Molecular Weight and DD

The viscosity average molecular weight (Mv) and DD of chitosan were determined using Ubbelohde viscometer and potentiometric titration, respectively, as described in our previous report.[18]

Scanning Electron Microscopy

Morphological characterization of dried chitosan powder was performed using a scanning electron microscope (Quanta-200 SEM, FEI, Netherlands). The sample was coated with spraying gold powder to make it conductive.

Color Analysis

The color of chitosan powder was measured using Minolta Chromameter CR-400 (Japan). The results were displayed as CIE Lab tristimulus values ($L^*a^*b^*$ color space). L^* is the lightness variable; a^* and b^* are the chromatic coordinates. Three measurements were performed at random positions.

Determination of Particle Size

Particle size of chitosan powder was determined using the laser scattering method with a Mastersizer 2000 laser diffractometer (Malvern Instrument Co., Southborough, UK) equipped with a He-Ne laser (wavelength 632.8 nm). Before measurement, the chitosan sample was dispersed in alcohol.

DPPH Radical Scavenging Assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity of chitosan was determined as described by Kim & Thomas.[19] Two milliliter of 10 mg/mL chitosan solution (2 g/L acetic acid) was mixed with 2 mL 2×10^{-4} mol/L DPPH ethanol solution. The mixture was then incubated for 30 min in the dark at room temperature and was measured at 517 nm with ethanol as blank. The scavenging ratio of sample on DPPH radical can be calculated as follows,

$$\text{Scavenging ratio } (\%) = [A_c - (A_s - A_0)] / A_c \times 100$$

Where, A_s was the absorbance of mixed solution, A_0 was the absorbance of mixed solution with DPPH ethanol solution replaced by ethanol, and A_c was the absorbance of mixed solution with chitosan solution replaced by acetic acid.

Statistical Analysis

The test data were statistically analyzed using DPS 7.05 for windows (Zhejiang University, Hangzhou, China). Duncan's *t*-test was used to compare means at the 5% significance level.

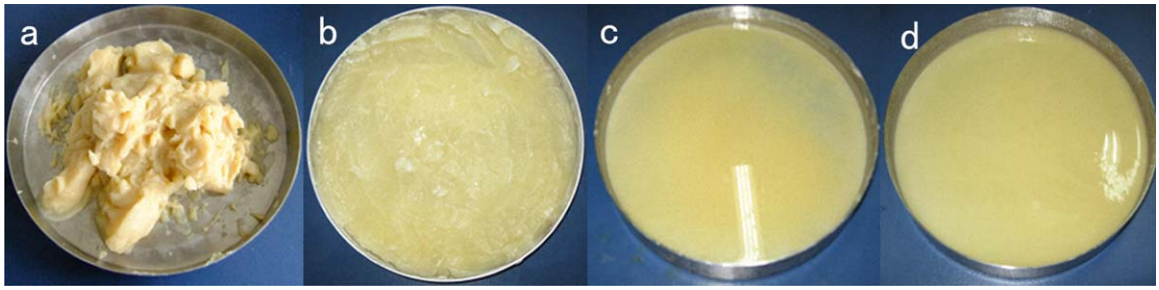

Figure 1. The state change of precipitated chitosan paste (CH-3) during freeze-thaw pretreatment. (a) original paste; (b) frozen paste; (c) thawed chitosan; (d) thawed chitosan after centrifugation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

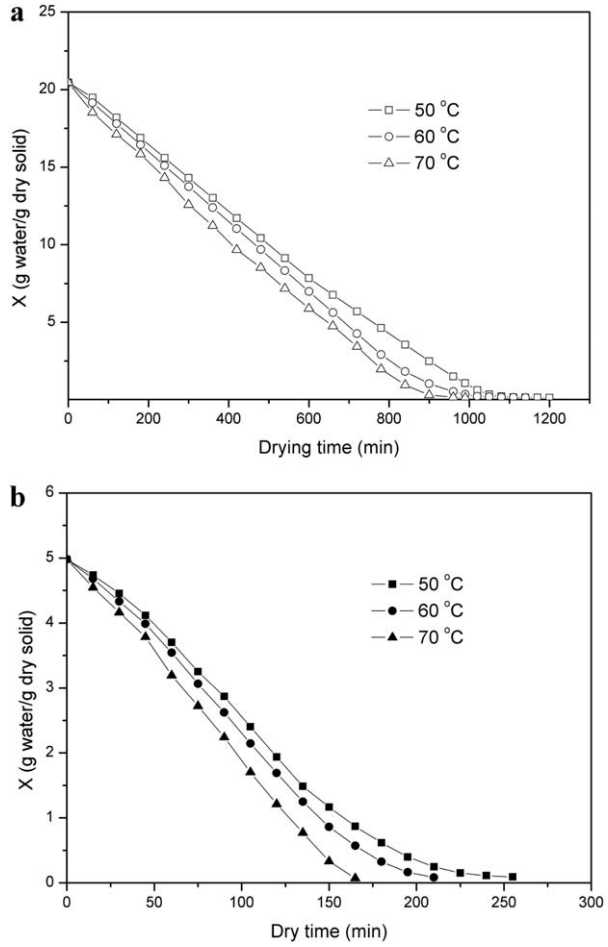

Figure 2. The moisture content of chitosan (CH-3) during drying. (a) direct drying; (b) drying with freeze-thaw pretreatment.

RESULTS AND DISCUSSION

The Effect of Freeze-Thaw Treatment on the Moisture and State of Chitosan

The moistures of chitosan before and after freeze-thaw treatment are shown in Table I. The original chitosan paste contained large amount of water and dry basis moistures of the five samples were in a range of 20.5–22.7 g water/g dry solid, corresponding to wet basis moistures of 95.3–95.8%. The freeze-thaw treatment greatly decreased the water content of chitosan paste and the paste lost 75.6–77.7% water after the pretreatment.

The state change of chitosan during freeze-thaw process is shown in Figure 1. The original precipitated chitosan was in the form of paste. After freeze-thaw treatment, paste lost its viscosity, and formed particles. This phenomenon might be attributed to the rearrangement of chains during the formation of big ice crystals. The rearrangement of macromolecules often occurred during freeze-thaw treatment. After the treatment, starch gel also lost some water.[17,20]

The Effect of Freeze-Thaw Treatment on the Drying Process of Chitosan

Figure 2 presents the moisture content changes of chitosan samples during thermal drying. For original paste, the drying process took about 16–19 h as the drying temperature was at 50–70°C. The paste formed large blocks during the drying process [Figure 3(a)]. This phenomenon might be due to the high viscosity of the paste. Compared with original paste, the drying process of freeze-thaw treated sample was greatly shortened and the drying time was decreased to about 2.75–4 h. Moreover, the pretreated dried product was loosely packed powder [Figure 3(b,c)]. This product did not need intensive milling, which was different from the product from direct drying.

The Effect of Freeze-Thaw Treatment on the Properties of Dried Products

Particle Size. The particle size of chitosan products are shown in Table II. The volume mean diameter ($D[4,3]$) and specific surface mean diameter ($D[3,2]$) of freeze-dried powder was 335.3 and 216.5 μm, respectively. The products from direct drying were in the form of large blocks, which had been discussed above. After freeze-thaw pretreatment, the dried product was packed powder. The particle size of pretreated dried powders was close to freeze-dried product, with $D[4,3]$ of 331.4–342.3 μm and $D[3,2]$ of 208.6–223.4 μm.

Molecular Weight and DD. The viscosity average molecular weights (Mv) and DDs of chitosan products from thermal drying with and without freeze-thaw pretreatment are shown in

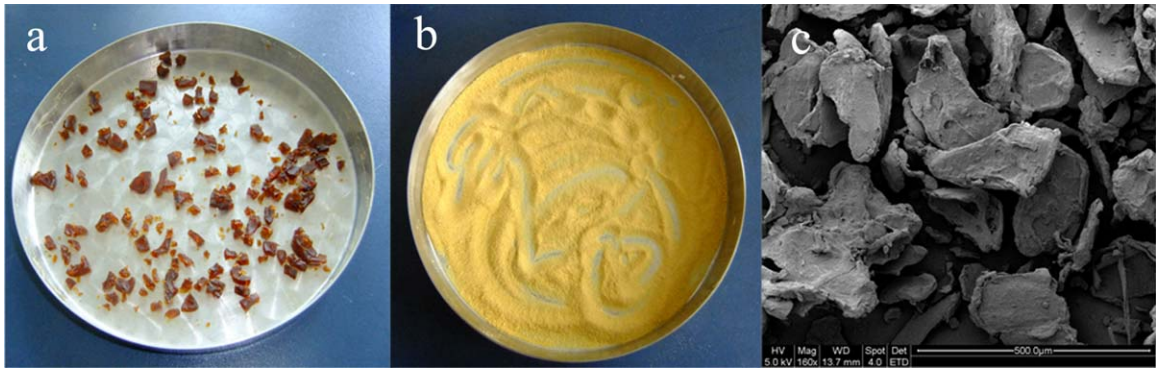

Figure 3. The images of dried chitosan products (CH-3, drying temperature = 60°C). (a) optical image of product from direct drying; (b) optical image of product from drying with freeze-thaw pretreatment; (c) SEM image of product from drying with freeze-thaw pretreatment. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. The Particle Size of Chitosan Products (CH-3) from Different Drying Methods

		Direct thermal drying			Thermal drying after freeze-thaw treatment		
	Freeze drying	50°C	60°C	70°C	50°C	60°C	70°C
D[4,3] (μm)	335.3 ± 14.3	-	-	-	342.3 ± 9.2[a]	328.6 ± 13.2[a]	331.4 ± 8.5[a]
D[3,2] (μm)	216.5 ± 12.6	-	-	-	223.4 ± 9.0[a]	208.6 ± 8.7[a]	215.5 ± 6.3[a]

Values were expressed as means ± standard deviation ($n = 3$).
[a] The difference between thermally dried product and freeze-dried product was not significant at $P < 0.05$.
[b] The difference between thermally dried product and freeze-dried product was significant at $P < 0.05$.

Table III. The Viscosity Average Molecular Weights (Mv) and Deacetylation Degree (DD) of Chitosan Product (CH-3) from Different Drying Methods

		Direct thermal drying			Thermal drying after freeze-thaw treatment		
	Freeze drying	50°C	60°C	70°C	50°C	60°C	70 C
Mv (kDa)	90.3 ± 6.0	115.5 ± 8.8*,b	123.5 ± 9.3*,b	118.6 ± 7.1*,b	89.6 ± 4.7**,a	91.3 ± 6.2**,a	91.5 ± 3.8**,a
DD (%)	85.4 ± 0.4	85.2 ± 0.4*,a	85.2 ± 0.2*,a	85.1 ± 0.5*,a	85.1 ± 0.3*,a	85.2 ± 0.3*,a	85.2 ± 0.4*,a

Values were expressed as means ± standard deviation ($n = 3$). Values with different symbols (*, **) for thermally dried products at same temperatures are significantly different at $P < 0.05$.
[a] The difference between thermally dried product and freeze-dried product was not significant at $P < 0.05$.
[b] The difference between thermally dried product and freeze-dried product was significant at $P < 0.05$.

Table III. It can be observed that in all thermal drying experiments, DD of chitosan was equal to the freeze-dried product. Similar result was obtained by Youn et al.[21] in sun drying of chitosan at different times. In this case, DD was not affected. At all three drying temperatures (50, 60, and 70°C), the Mv of products from direct drying were significantly higher than freeze-dried products. The thermal drying-induced change in molecular weight had been previously reported by Dotto et al.,[22,23] who found that molecular weight of chitosan increased when chitosan paste was treated with sprouted bed drying and tray drying. These researchers indicated that during drying process of chitosan paste, polymerization of chitosan molecules occurred due to bonding of chains. The Mv difference of chitosan from freeze drying and thermal drying with freeze-thaw pretreatment was not significant. The results indicated that the freeze-thaw pretreatment could inhibit the molecular weight increase of chitosan during thermal drying.

Color. The color of chitosan was of great significance to its application. As Table IV shows, compared with freeze drying, the six products from thermal drying showed significantly decreased L^* value and increased a^* and b^* values. This suggested that browning occurred during both direct drying process and the drying with pretreatment. Srinivasa et al.[8] indicated that the heat-induced browning of chitosan was attributed to Maillard reaction, due to the existence of free amino and carbonyl groups in the molecules. Besides, oxygen was found able to oxidize the Amadori compounds from Maillard reaction,[24] which might also contribute to the browning effect of chitosan during drying. It can be observed from Table IV that there were significant differences between the products from direct drying and the pretreated dried products in color values, and the color values of latter products were close to freeze-dried product. This result suggested that the freeze-thaw pretreatment greatly alleviated the heat-induced browning effect of chitosan.

Antioxidative Activity. Antioxidative activity is one of the well-known functionalities of chitosan.[25,26] Here in this study, DPPH radical model was used to compare the antioxidative activity of

Table IV. The Color Values of Chitosan Products (CH-3) from Different Drying Methods

		Direct thermal drying[a]			Thermal drying after freeze-thaw treatment		
	Freeze drying	50°C	60°C	70°C	50 °C	60 °C	70 °C
L*	85.34 ± 0.82	72.64 ± 0.64**,b	74.33 ± 0.47**,b	75.45 ± 0.53**,b	83.26 ± 0.58*,b	82.15 ± 0.63*,b	81.67 ± 0.49*,b
a*	−0.58 ± 0.06	7.21 ± 0.17*,b	6.84 ± 0.24*,b	7.02 ± 0.15*,b	0.38 ± 0.09**,b	0.34 ± 0.06**,b	0.25 ± 0.01**,b
b*	11.84 ± 0.26	26.63 ± 0.46*,b	25.22 ± 0.32*,b	22.31 ± 0.28*,b	13.07 ± 0.45*,b	12.49 ± 0.38*,b	15.82 ± 0.34bb

Values were expressed as means ± standard deviation ($n = 3$). Values with different symbols (*, **) for thermally dried product at same temperatures were significantly different at $P < 0.05$.
[a] Product from direct thermal drying was powdered to determine color values.
[b] The difference between thermally dried product and freeze-dried product was significant at $P < 0.05$.
[c] The difference between thermally dried product and freeze-dried product was not significant at $P < 0.05$.

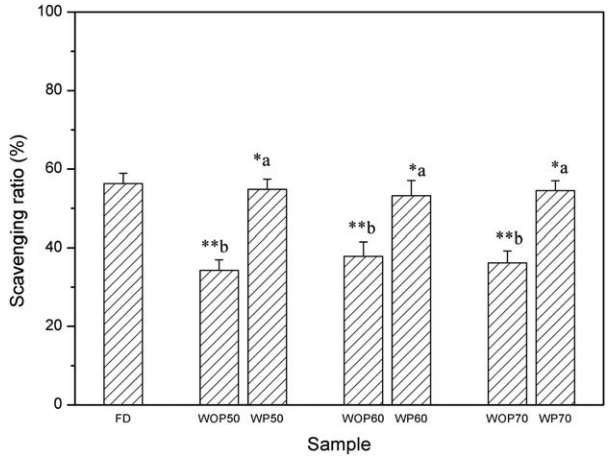

Figure 4. The DPPH radical-scavenging ratios of dried chitosan products (CH-3). FD: freeze drying; WOP-X: direct drying at *X*°C without pretreatment; WP-X: drying at *X*°C with pretreatment. Values with different symbols (*, **) for thermally dried products at same temperatures were significantly different at $P < 0.05$. a: The difference between thermally dried product and freeze-dried product was not significant at $P < 0.05$; b: The difference between thermally dried product and freeze-dried product was significant at $P < 0.05$.

chitosan samples, with results shown in Figure 4. At all three drying temperatures, the DPPH radical-scavenging ratios of products from direct drying were significantly lower than freeze-dried products and pretreated dried products, which might be due to the decrease of reducing end induced by Maillard reaction. Compared with the untreated sample, the DPPH radical-scavenging ratio of pretreated dried product at 50°C, 60°C, and 70°C were increased by 59.8, 40.4, and 50.6%, respectively. There was no significant difference between freeze-dried products and pretreated dried products in scavenging ratio. This suggested that the freeze-thaw pretreatment could well preserve the antioxidative activity.

CONCLUSIONS

The drying process and properties of chitosan were greatly changed by freeze-thaw pretreatment. After freeze-thaw treatment, chitosan paste lost 75.6–77.7% water and the drying time was reduced from 16–19 h to 2.75–4 h. The final product from drying with pretreatment was in the form of powder. After pretreatment, the molecular weight of chitosan was unchanged during the thermal drying. The browning effect of chitosan was greatly alleviated by the pretreatment. Besides, the pretreatment increased the DPPH radical-scavenging activity of dried product by 40.4–59.8%. The molecular weight, color, and DPPH radical-scavenging activity of pretreated dried chitosan product were close to those of freeze-dried product. These findings suggest the freeze-thaw treatment can be used to produce high quality of chitosan.

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