New route of chitosan extraction from blue crabs and shrimp shells as flocculants on soybean solutes

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Abstract The chitosan extracted from blue crabs and shrimp shells using calcium oxide (deproteinization) followed by deacetylation which eliminated the demineralization step to reduce the chemical usage and environmental protection. The extracted chitosan examined the flocculation to soybean solutes. The optical density (OD), solid%, and purity% (carbohydrates/soluble solids) after flocculation were measured. The OD was significantly decreased from 0.76 to 0.16 with blue crabs and 0.06 with shrimp shells chitosan-acetate dosing (0.5 g/L). The removal of about 68 and 66% solids was achieved by the addition of 0.5 g/L chitosan-acetate. The purity% was reached about 80% with blue crabs, and 78% with shrimp shells chitosan-acetate. The results of this study verified that the calcium oxide treatment should remove protein and increase the chitin extraction yield on blue crab and shrimp shells. This new route of chitosan extraction should be a useful method for making flocculants in the soybean solutes.

Keywords Chitosan · Flocculants · Calcium oxide · Blue crabs · Shrimp shell

Introduction

A tremendous amount of crab and shrimp shells are discarded through seafood market and company in recent years due to increase in the population growth and seafood consumption. Continuous production of these renewable biomaterials without developing of utilizing technology has resulted in environmental problems. The shrimp and crab waste contains significant amount of chitin, pigment, protein, and fats. These bioactive compounds have a great potential application to medical, therapies, cosmetics, paper, pulp, and textile industries, biotechnology, and food industries [16, 22, 29].

Chitosan is a linear polymer of D-glucosamine and N-acetyl-D-glucosamine which can be produced by the deacetylation of chitin [20]. Chitosan has been reported as a variety of physical and biological properties resulting in different applications such as cosmetics, pharmaceuticals, biotechnology, agriculture, food processing and nutrition. The coagulation and flocculation in the area of water and wastewater treatment are the particular application of chitosan-based materials due to their low-cost and environmentally friendly behavior [4, 11, 13, 17, 19, 27, 28]. Chitosan possesses several intrinsic characteristics which make to suit as an effective coagulant and flocculant agent for the removal of suspended solids such as high cationic charge density, long polymer chains, and bridging of aggregates. [12, 25, 30]. Especially, there are non-toxic, non-corrosive and safe to handle materials [3, 10, 15]. However, chitosan is efficient within a limited pH range and only soluble in dilute organic acids which are potential limits the application of chitosan in flocculation process [9].

The commercial chitosan extraction from crustacean shell (exoskeleton) has been conducted by chemical or

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biological treatment for last decades. Chemical chitin extraction and purification method has been considered as energy consuming and hazardous to the environment due to the strong acid and base chemical treatment involved. Biological chitosan production method using microbial fermentation has not yet reached the desired yield compared to the chemical method [2]. Lime (calcium oxide) is used in various industry to increase ionic strength to extract protein due to their cheap prices. However, few studies were conducted to chitosan production using lime from shellfish waste. Thus the present study was conducted to examine the chitosan extraction from crab and shrimp shells with a lime treatment to use as a flocculants on soybean solutes which reduce the chemical usage, low energy consumption, and environmental protection.

Materials and methods

Materials

The shrimp used in this study were obtained from a local HEB store (College Station, TX, USA). The shrimp shells were separated from the head and body. The blue crabs were captured by our research group from Galveston bay (Galveston, TX, USA) and separated the meat after mild boiling in the oven (for 10 min at 190 °C). The shrimp and blue crab shells were dried at the oven (120 °C for 2 day). They were ground using a commercial grinder, then sieved with 20 mesh screen. The triturated and sieved shrimp (34.2% protein, 15.8% chitin, 36.2% ash, and 0.3% lipid of the dry weight) and blue crab shells (33.4% protein, 13.6% chitin, 46.2% ash, and 1.93% lipid of the dry weight) were used in all experiments. Calcium oxide (lime, CaO), sodium hydroxide (NaOH), and hydrogen chloride (HCl), acetic acid (> 99%) were purchased from Fisher Scientific (Hampton, NH, USA). Crystal Clear Rapiclear® (commercial flocculants agent) was purchased from Loch New Water Gardens, LLC (Greenwood, SC, USA).

Chitosan extraction

Figure 1 shows that the overall route of chitosan extraction method from shrimp and crab shells using lime (calcium oxide) treatment. Two percent (w/v) of calcium hydroxide solutions were prepared and used for treating shells for 2 h boiling, and then supernatants (liquids) and wet cake solids were separated through centrifugation at 4000 rpm for 15 min. To completely remove the calcium oxide, the wet cake was washed with deionized water several times. The wet solids were treated with 6 M NaOH with 2 h boiling followed by overnight agitation at room temperature. Chitosan was extracted followed by centrifugation

Fig. 1 The new route of chitosan extraction method from blue crab and shrimp shell using lime treatments

(4000 rpm for 15 min), washing, and adjust pH 9 using 1 M HCl solution. The extracted chitosan was centrifuged by 4000 rpm for 15 min and dried at 60 °C for 1 day. The degree of deacetylation was analyzed by first derivative ultraviolet (UV) spectrophotometry [24].

Chitosan flocculants testing with soybean solutes

The extracted chitosan (1.0 g) dissolved in 100 mL of 1.0% acetic acid solution which formed chitosan-acetate and conducted flocculants test. Whole soybeans were obtained from Clarkson Grain Company INC. (Cerro Gerdo, IL, USA). The whole soybeans were ground with a commercial coffee grinder and sieved with 20 mesh screen. The soybean flour was mixed with deionized water (1:10 ratio) and stirred for 1 h at room temperature. These soybean solutes were used for flocculation test. The samples were collected after inoculated different dosing rate of chitosan-acetate with 10 min stirring followed by centrifuged at 4000 rpm for 15 min, and measure the pH, optical density (OD), brix%, solid%, protein and carbohydrate purity%, and mass transfer%.

Analysis of chitosan and flocculants testing

The optical density (OD) was measured by spectrophotometer at 660 nm (Beckman Coulter DU 520, Brea, CA, USA). Brix was measured by refractometer (Cole-Parmer, Vernon Hills, IL, USA). Total solids, moisture, protein, carbohydrate, ash and mineral contents were determined according to the standard methods of the Association of Official Analytical Chemists [1]. Protein contents were measured by using high temperature combustion process (Soil Testing Laboratory at Texas A&M University, College Station, TX, USA). Total solids and moisture contents were determined by weight difference after drying in oven at 120 °C for 2 day. Ash content was determined by using a furnace at 550 °C for 12 h. Mineral contents were analyzed in triplicate by inductively coupled plasma (ICP) emission spectroscopy after a nitric acid digestion [14].

Statistical analysis

All treatments in this study were conducted in triplicate and a 95% confidence level significance was applied for data analysis. Measurements were analyzed by an analysis of variance (ANOVA) using GLM (General Linear Model) procedure in SAS 9.1 software (SAS Institute INC., Cary, NC, USA). The statistical significant difference between the averages in treatments was accessed by Duncan's multiple range tests. Differences were considered significant for *P* values lower than 0.05.

Results and discussion

Chitosan extraction process development

Conventional methods suggest that extraction and purification of chitin from crustacean shell involves deproteinization and demineralization by strong bases and acids treatment followed by deacetylation [5, 7, 8, 18, 21]. Generally, about 4.0% NaOH is used for deproteinization and 4.0% HCl for demineralization to remove calcium carbonate and protein. However, according to the Percot and Viton Domard [26], strong acids negatively effect on molecular weight and intrinsic properties of the purified chitin. In the deproteinization step which is treated with 4.0% NaOH, the protein is isolated from the solid component of the shrimp and crab shells. With the centrifugation or filtration, the protein hydrolysate is easily removed from protein slurry. Shrimp shells contain about 7.0% crude proteins (wet basis) [26]. This protein slurry can be further purified and/or dried in order to produce protein powder. The most shrimp and crab flavor are contained in the protein slurry. The chitin and calcium

carbonate are major components of solid portion, which also including the pigment. However, this conventional deproteinization process is expensive and non-environmental friendly process [32]. Domard [8] suggested that solid sodium chloride treatment (20 g NaCl/g shells) followed by demineralization and deproteinization allowed a good preservation of the chitin structure. In this study, we used lime (calcium oxide) for deproteinization, instead of sodium hydroxide (Fig. 1). Lime is commercially much cheaper than NaOH [6]. Calcium ions in lime provide strong binding between negatively charged functional groups in protein molecules. Therefore, they can effectively remove protein particles which are commonly observed in NaOH treatment conditions. [31].

In the demineralization step, generally the calcium carbonate is converted to soluble calcium chloride that can be easily removed by washing step. We avoided to attempt the demineralization step because calcium ion is abundant in lime and calcium carbonate itself have a flocculants effect. Using carbon dioxide (CO₂) gas, calcium ion was recycled in this study which can eliminate the demineralization step. Therefore, processing length and time was significantly decreased compared to conventional methods. In the deacetylation of chitin into chitosan, strong bases (6 M NaOH) were used with high temperature. Alternative technology has been developed to replace hazardous chemical methods such as using enzymatic or microbial fermentation [16]. However, commercial enzymes are costly for the high expenses and lactic acid bacterial fermentations are relatively low chitin extraction yield [2]. Moreover, the latter method has been considered the expensive and involved potential biological contamination risk with the scale-up development because the entire process requires the sterilization condition during the fermentation. Method developed in this experiment has the advantage of being environmentally friendly, and reduce the processing time and cost.

Flocculation test

Blue crab and shrimp shell chitosan-acetate similarly showed a significant flocculants effect, which was better than a commercial product (Rapiclear[®]) made of aluminum sulfate. Commercial product seems that the required dosage is higher than blue crab and shrimp shell chitosan-acetate. Chitosan contains many amino groups (–NH₂) and hydroxyl groups (–OH) on the molecular chain [35]. These –OH and –NH₂ groups can chelate into a steady complex compounds (–N–M–O–) with metal ions [35]. Therefore, chitosan can be used for removal of metal ions such as Ca²⁺ and Cu²⁺, and protonated with H⁺ with the active amino groups (–NH₂) which has powers of static attraction and adsorption for removing COD (organic contaminant),

and SS (solid suspending substances) in water treatment [34]. The inorganic salt aluminum sulphate (alum) is one of the most widely used flocculants in conventional water and wastewater treatment. However, alum produces abundant sludge which is difficult to dehydrate. Moreover, its toxicity is still under concerning [4]. Therefore, alum based flocculants have not been considered for food processing applications. Compared with alum flocculants, chitosan as bio-flocculant has several advantages which are the less dosage requirement, a quick depositing velocity, a high efficiency of removing solids and metal ions, and no further environmental pollution. Hirohara et al. [15] suggested that a chitosan-based material is safe to animals and plants without causing environmental pollution. However, it is considered higher cost than traditional alum flocculants [34]. Therefore, cheaper composite chitosan materials such as crab and shrimp shells in this study make up the availability enlargement.

Table 1 shows that the protein and mineral content of soy solutes treated by blue crab and shrimp shell chitosan-acetate. Blue crab and shrimp shell chitosan-acetate treated soybean solutes statically exhibited less protein and mineral contents than control (soy solutes) except for calcium (Ca) and sodium (Na) content ($P < 0.05$). It might be their flocculation effect which was significantly removed the COD and SS. The higher content of Ca and Na of blue crab and shrimp shell chitosan-acetate treatments than control should be originated from shell itself which are abundant of blue crab and shrimp. Clarity testing with 0.5 g/L dosing rate were conducted and the results show in Fig. 2. The optical density (Abs 650 nm) showed 0.76 at soy solutions, 0.71 at centrifuged soy liquid, 0.16 at blue crab chitosan treatment, 0.06 at shrimp shell chitosan treatment, 0.58 at Rapiclear[®], respectively. There is no significant difference

Fig. 2 Clarity testing with 0.5 g/L dosing rate. (A) Soy solutions, (B) centrifuged soy liquid, (C) blue crab chitosan, (D) shrimp shell chitosan, (E) Rapiclear[®]

between soy solutions and centrifuged soy liquid. These results indicated that the physical gravity and centrifugal force might be ineffective in the removal of COD and SS from soybean solutes. With the same dosing rate, blue crab and shrimp shell chitosan-acetate treatment showed the almost four times higher flocculation effect than commercial Rapiclear[®] ($P < 0.05$). Shrimp shell chitosan-acetate treatment showed higher flocculants effect than blue crab. Figure 3 shows that the clarity results by different dosing rate. The optical density of blue crab and shrimp shell treated sample significantly decreased with 0.1 g/L dosing rate. The OD of shrimp shell chitosan treatment showed higher than blue crab until 0.25 g/L dosing rate, but the opposite result was observed on 0.5 g/L dosing rate. On the other hand, the effect of the commercial Rapiclear[®] was not significantly different until 1.0 g/L dosing rate. The OD of the commercial Rapiclear[®] showed 0.03 with 6.0 g/L dosing rate (data are not shown) which meant the flocculants effect of commercial product significantly low

Table 1 Protein (% wet basis) and mineral (ppm) content of soy solution treated by blue crab and shrimp shell chitosan

Contents	Treatments		
	Soy solute (control)	Blue crab chitosan	Shrimp shell chitosan
Protein (%)	1.9 ± 0.05 ^a	1.1 ± 0.05 ^b	1.2 ± 0.06 ^b
Mineral (ppm)			
P	452.71 ± 22.64 ^a	320.41 ± 16.02 ^b	442.73 ± 22.14 ^a
K	2276.02 ± 204.84 ^a	2107.16 ± 126.43 ^b	2158.21 ± 194.24 ^b
Ca	145.86 ± 8.75 ^a	254.59 ± 15.28 ^b	225.24 ± 13.51 ^c
Mg	270.09 ± 13.50 ^a	238.22 ± 11.91 ^{bc}	258.96 ± 12.95 ^c
Na	15.37 ± 0.77 ^a	136.27 ± 6.81 ^b	56.72 ± 2.84 ^c
Zn	3.31 ± 0.17 ^a	0.80 ± 0.04 ^b	2.01 ± 0.11 ^c
Fe	27.51 ± 1.38 ^a	35.12 ± 1.76 ^b	27.56 ± 1.38 ^a
Cu	0.79 ± 0.04 ^a	0.63 ± 0.03 ^b	0.52 ± 0.03 ^c
Mn	2.07 ± 0.10 ^a	1.38 ± 0.07 ^b	2.16 ± 0.11 ^{ac}

Data are expressed as mean ± SD ($n = 3$)

^{a-c}Means within a row with different letters are significantly different ($P < 0.05$)

Fig. 3 Clarity testing with different dosing rate

compared to blue crab and shrimp shell chitosan-acetate to soybean solutes. According to Mohn [23], alum based inorganic flocculants required the higher dosage rates which can result in a higher cost per unit of flocculation than organic flocculants although they are relatively cheap.

Figure 4 shows that the solid removal rate of composite blue crab and shrimp shell chitosan flocculants were about 60% with 0.5 g/L dosing rate. About 70% solid removed with 1.0 g/L dosing rate while 40% solid removal with the same dosing of commercial Rapiclear®. The solid removal rate of blue crab and shrimp shell chitosan-acetate was higher than commercial product. With physical centrifuge (4500 rpm for 15 min), about 37% solid can be removed without any treatment. Figure 5 shows that the purity test (carbohydrate/solid) of composite flocculants were not significantly different from 0.1 to 1.0 g/L dosing rate on blue crab and shrimp shell chitosan-acetate, and commercial Rapiclear®. These results show that flocculants may not significantly affect to remove the soluble carbohydrate with low dosing rate. However, purity was significantly decreased with above 5.0 g/L Rapiclear® dosing rate (data are not shown). According to the Zeng et al. [33], the carbohydrate in the water was effectively adsorbed and flocculated through adsorption, ion exchange, and subsidence by chitosan as cationic flocculants.

In conclusion, the chitosan was extracted from blue crabs and shrimp shells using calcium oxide treatment (deproteinization) followed by sodium hydroxide treatment

Fig. 4 Solid removal with different dosing rate

Fig. 5 Purity test with different dosing rate

(deacetylation) which could allow to skip demineralization step. The results of this study verified that the calcium oxide treatment for deproteinization should effectively remove protein and increase the chitin extraction yield from blue crab and shrimp shells. This new route of chitosan extraction should be a useful method for producing commercial flocculants which can apply for food processing and re-use water such as soybean solutes.

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