JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Sonication-Assisted Extraction of Chitin from Shells of Fresh Water Prawns (*Macrobrachium rosenbergii*)

GUNNAR T. KJARTANSSON,[†] SVETLANA ZIVANOVIC,*[,‡] KRISTBERG KRISTBERGSSON,[§] AND JOCHEN WEISS[†]

Department of Food Science, 234 Chenoweth Laboratory, University of Massachusetts, 100 Holdsworth Way, Amherst, Massachusetts, Department of Food Science and Technology, 101 McLeod Hall, University of Tennessee, Knoxville, Tennessee, and Department of Food Science, University of Iceland, Reykjavik, Iceland

The effect of sonication during chitin extraction from freshwater prawn shells on yield, purity, and crystallinity of chitin was investigated. Dry prawn shells were suspended for 4 h in 0.25 M HCl at 40 °C while they were sonicated for 0, 1, and 4 h. Demineralized shells were lyophilized, resuspended in 0.25 M NaOH, and sonicated again for 0, 1, and 4 h for protein removal. The yield of chitin decreased from 8.28 to 5.02% for nonsonicated and sonicated samples, respectively, which was attributed to losses of depolymerized materials in the wash water. The application of ultrasound enhanced the removal of proteins. In nontreated shells, the amount of protein was 44.01% and was reduced to 12.55, 10.59, and 7.45% after 0, 1, and 4 h of sonication treatments. The glucosamine content slightly decreased with sonication probably because of losses due to depolymerization. The crystallinity indices of chitins decreased as the time of sonication increased. The degree of acetylation of chitins was unaffected by sonication, but the degree of acetylation of chitosans produced from sonicated chitin decreased from 70.0 to 68.7 and 61.4% for 1 and 4 h sonicated samples, respectively.

KEYWORDS: Chitin; chitosan; sonication; high intensity ultrasound; freshwater prawns; *Macrobrachium rosenbergii*

INTRODUCTION

Worldwide shrimp production from cultured and captured prawns increased to over 4.2 million tons in 2001 (*1*). Byproducts from marine food production, mainly shrimp shells, comprise almost 40% of total prawn mass and have become a major environmental concern due to their slow degradation (*2*). On the other hand, crustacean byproducts are a major source of raw materials for chitin production. Chitin, the second most abundant biopolymer in nature, differs from cellulose due to the presence of the acetamido instead of the hydroxy group on the C2 atom of the glucose subunits. However, naturally occurring chitin has some (<30%) of the functional groups on C2 deacetylated, making it a natural linear copolymer of β-(1→4)-2-amino-2-deoxy-D-glucan and β-(1→4)-2-acetomido-2-deoxy-D-glucan (*3*).

The extraction of chitin from crustacean shells is a time-consuming process that involves extensive demineralization and deproteinization treatments. Many processes have been implemented with various treatment times, temperatures, concentrations of acid and alkali solvents, and solid-to-solvent ratios. Regardless of the variations, all of these methods have in common that they require high concentrations of strong acids and strong alkali applied at high temperatures (*4*, *5*). Demineralization is most frequently carried out with hydrochloric acid and deproteinization with sodium hydroxide. The order in which these two steps are carried out may vary, although demineralization is typically performed first. The use of enzymes, such as trypsin and pepsin, for protein removal has been examined, but the process requires substantially more time and appears to be less efficient than the equivalent NaOH treatment (*6*, *7*). The choice of processing conditions may be governed to some extent by the purpose for which the chitin is required, since partial deacetylation during deproteinization is not a disadvantage if the chitin is subsequently to be converted to chitosan, while some hydrolysis of the polymer chain during the demineralization process can be tolerated if chitin is to be used in the form of particles or converted to microcrystalline chitin (*7*). Further treatment of extracted chitin with hot concentrated NaOH causes deacetylation and results in conversion of chitin to chitosan. Chitosan is, thus, a heterogeneous polymer consisting of a minimum of 70% of D-glucosamine and, consequently, a maximum 30% of *N*-acetyl-D-glucosamine units.

Chitin and chitosan have shown potential for numerous applications in the pharmaceutical and food industry (*5*), but the effectiveness of the polymers greatly depends on molecular

* To whom correspondence should be addressed. Tel: 865-974-0844. Fax: 865-974-7332. E-mail: lanaz@utk.edu.
[†] University of Massachusetts.
[‡] University of Tennessee.
[§] University of Iceland.

10.1021/jf052184c CCC: $33.50
Published on Web 04/04/2006

weight and degree of acetylation (DA) of the polymers. The standard deacetylation procedure involves treatment with hot concentrated NaOH, while commonly applied depolymerization treatments include acid (*3*), enzymatic hydrolysis (*7*), and, recently, sonication (*8*). High intensity ultrasound has been shown to cause depolymerization of macromolecules due to mechanical effects associated with cavitation by temporarily dispersing aggregates and breaking covalent bonds in polymeric chains (*9*−*13*). The application of ultrasound to enhance pectin yield from apple pomace has been suggested by Panchev et al. (*14*). The authors showed that 1−1.2 W/cm^2 sonication treatments were sufficient to cause an increase in pectin yield by 28% as compared to the standard extraction. The application of ultrasound on chitin has recently indicated that presonicated chitin is more deacetylated than untreated chitin due to a higher accessibility given by the ultrasound treatment (*8*).

The application of ultrasound may lower the amount and concentration of solvents used in the extraction process, which would, in turn, reduce the depolymerization of chitin molecules. The objective of this research was to investigate the effects of high intensity ultrasound on yield and physicochemical characteristics of chitin extracted from freshwater prawns (FWPs).

MATERIALS AND METHODS

Raw Material. FWPs (*Macrobrachium rosenbergii*; 82 prawns, 12.1 ± 1.3 cm long) were kindly provided by the University of Tennessee Highland Rim Experiment Station (Springfield, TN). The FWP shells were heated in boiling saltwater (4% NaCl) for 4 min and cooled in tap water. All visible meat was peeled from the shells, and the shells were extensively washed in running hot water. The clean shells were freeze-dried, weighed, grinded, and sieved to obtain a fine powder (60 μm; Wiley Mill, Thomas Scientific, Swedesboro, NJ).

Extraction of chitin from FWP shells followed the method of Percot et al. (*15*) with slight modifications (**Figure 1**). FWP shell powder (4 g) was suspended in 0.25 M HCl (1:40 solid-to-solvent, w/v) at 40 °C for 4 h. The control treatment that simulated conventional extraction consisted of prawn shell suspensions that were stirred but not sonicated during demineralization. Sonication treatments were conducted for 1 and 4 h at 41 W/cm^2 using an ultrasonic processor (model 501, Cole Parmer, Vernon Hills, IL) with a 1.27 cm (1/2 inch) diameter stainless steel probe. The ultrasonic intensity (I) was calculated from the slope of the initial rise in temperature (dT/dt_a), the slope of heat loss after the sonicator was turned off (dT/dt_b), the sample mass (m), the heat capacity of the solvent (c_p), and the radius (r) of the ultrasonic probe.

$$I = \frac{mc_p}{\pi r^2}\left[\left(\frac{dT}{dt}\right)_a - \left(\frac{dT}{dt}\right)_b\right] \quad (1)$$

Samples that were sonicated for 1 h were left in the acid suspension for an additional 3 h at 40 °C with constant stirring. The demineralized powders were extensively washed with deionized water to neutral pH, lyophilized, weighed, and milled again. To determine the effects of ultrasound on the removal of proteins (deproteinization), the fraction of obtained powder was soaked in 0.25 M NaOH (1:15 solid-to-solvent, w/v) at 40 °C. The samples were sonicated for 0, 1, and 4 h, and after 4 h, they were extensively washed, lyophilized, weighed, and milled again. The concentration of NaOH applied for deproteinization was lower as compared to the industrially used solvents. On the basis of our preliminary experiments, this appeared to be necessary because of a rapid deterioration of the sonicator probe tip that occurred when used in more concentrated alkali solutions. All samples were additionally soaked in 1 M NaOH for 2 h at 90 °C, extensively washed with water, freeze-dried, and kept in a desiccator at room temperature until analyses.

Chitin was converted to chitosan without sonication. The samples were mixed with 12.5 M NaOH in a 1:20 solid-to-solvent ratio and stirred for 4 h at 100 °C. After deacetylation, the samples were extensively washed with deionized water, freeze-dried, and kept as previously described.

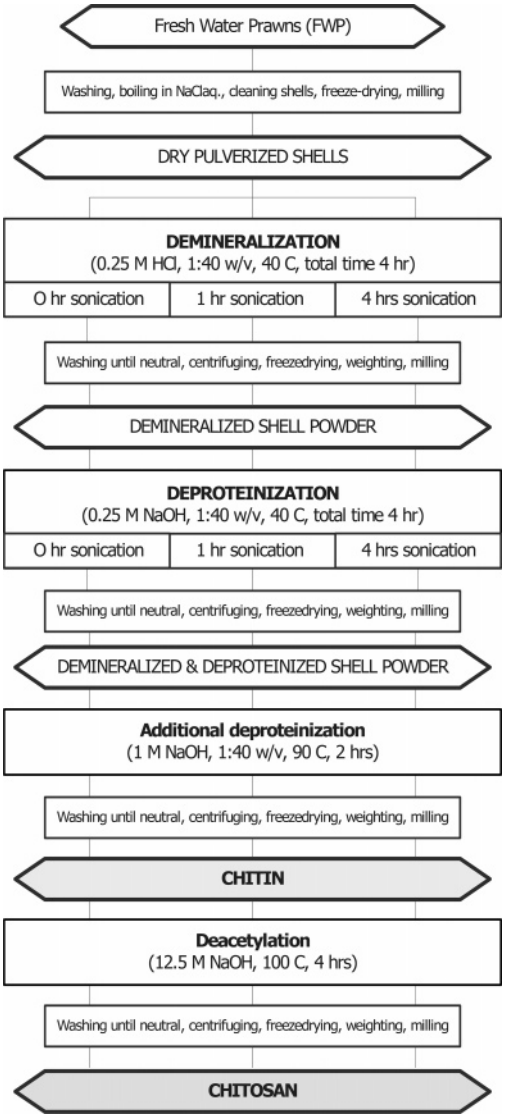

Figure 1. Schematic overview of the chitin extraction process from FWP shells.

Analyses. All freeze-dried powered samples obtained during extractions were kept in desiccators at room temperature. The yield of each treatment was determined gravimetrically, immediately following lyophilization of the treated samples. The mineral content (ash) was assessed gravimetrically after ashing at 600 °C for 6 h. Residual proteins in freeze-dried samples were solubilized in 1 M NaOH at 40 °C and determined by the Lowry method (*16*) with bovine serum albumin as a standard. The chitin content in the material was determined as total glucosamine (TGA) following the method of Tsuji et al. (*17*) after acid hydrolysis with 6 N HCl at 110 °C (*18*) and neutralization with sodium acetate (*19*). Liberated glucosamine was determined spectrophotometrically with 3-methyl-2-benzothiazolone hydrazone hydrochloride and $FeCl_2$ at 650 nm (HP 8453 spectrophotometer, Hewlett-Packard, Ramsey, MS.).

DA. A Nexus 670 Fourier Transform Infrared (FTIR) spectrometer (Thermo Electron Co., Mountain View, CA) with an attenuated total reflection accessory and ZeSe crystal was used to record infrared spectra of samples between 4000 and 700 cm^{-1} with 64 scans at a resolution of 4 cm^{-1}. The DA (%) was calculated using the OMINC 6.1 software (Thermo Electron Co.) from absorbance areas of the bands at 1655 and 3450 cm^{-1} (*20*):

$$\%\ N\text{-acetylation} = (A_{1655}/A_{3450}) \times 100/1.33 \quad (2)$$

Samples were scanned three times, and the average DA values were reported.

Crystallinity. A series of measurements were performed on the freeze-dried powder obtained during extractions to determine changes in crystallinity of produced powders. The X-ray diffraction patterns were obtained using a wide-angle X-ray diffractometer (Philips PW1729 Diffractometer, Philips Electronics, Almelo, Netherlands) using Kα Cu radiation. The 2θ angle was scanned from 5.025 to 54.975°, and the counting time was 2 s at each angle step (0.05°). The operating voltage and current of the tube were 45 kV and 40 mA, respectively. The crystallinity index (Cr I_{peak}) was determined by a method proposed for cellulose (*21*) and applied to chitosan (*22*) using the following formula:

$$\text{Cr } I_{peak} = (I_{110} - I_{am})/I_{110} \quad (3)$$

where I_{110} is the maximum intensity (arbitrary units) of the (110) lattice diffraction pattern at $2\theta = 20°$ and I_{am} is the intensity of amorphous diffraction in the same units at $2\theta = 16°$.

Sizes of Crystallites. The apparent crystal size, D_{app} at $2\theta = 20°$ of the samples, in the direction perpendicular to the (110) crystal plane, was calculated using Scherrer's equation (*23*):

$$\Delta(2\theta) = \frac{k\lambda}{B \times \cos(\theta B)} \quad (4)$$

where 2θ is the average crystallite size (nm) in the direction perpendicular to the $2\theta = 20°$ plane, λ is the wavelength of the Kα, Cu (1.5405 Å), B (in radians) is the full width of half the maximum of the reflection corrected for instrumental broadening (fwhm measured after a correction by subtracting the baseline for the amorphous region), k is a constant, indicative of crystalline perfection, here assumed to be 0.9 (*23*), and $2\theta_B$ is the peak angle (radian). The fwhm due to the equipment (source diffraction) was measured on boron lanthanum and was systematically subtracted from the experimental values (*23*).

Scanning electron micrographs of powdered samples were recorded with a LEO 1525 Field Emission with Link Oxford EDS system at 20 kV and 15 Pa (scanning electron microscope, LEO 1525 VP, LEO Electron Microscopy, NY). The samples were coated with gold to ensure sufficient electron refraction. A series of images were taken of samples at each processing step using magnification between 100× and 5000×.

Statistical Analysis. To determine interactions between extractions steps (i.e., treatment of material and levels of sonication 0, 1, and 4 h), results were subjected to statistical analyses using SAS (version 8, SAS Institute Inc., Cary, NC). Analysis of variance for yield, mineral, and TGA contents was performed using a mixed procedure (proc mixed) and, for protein contents, in samples using both a mixed procedure and general linear model (proc glm) procedure. Mean separation was accomplished by orthogonal polymonial contrast.

RESULTS AND DISCUSSION

True yields of all extraction steps are shown in **Table 1**. The "true yield" is expressed as a percent of the extracted material from the material that was used for extraction while the "relative yield" shows the amount of extracted material (g) on initial 100 g shell dry weight basis. In the demineralization and deproteinization treatments, the differences among sonication treatment levels were significant ($p < 0.0001$). For demineralization, the relationship between sonication and yield was quadratic. Demineralization with 0.25 M HCl solubilized calciumcarbonate and other salts from the shells and resulted in reduction of shell weight by 28.07, 35.77, and 39.27% for 0, 1, and 4 h sonication-assisted demineralized material, respectively. The treatment with 0.25 M NaOH (deproteinization) caused even more solubilization, yielding only 8.37−15.07% of the material from the previous step. Additional NaOH treatment further extracted alkali soluble compounds and resulted in 75.88−83.65% of previously deproteinized material ($p = 0.0154$). For both steps of deproteinization, the relationship between sonication and yield was linear. Calculated on the initial dry FWP shell basis, the final chitin yield was 8.28, 7.55, and 5.03% for nonsonicated, 1, and 4 h sonicated samples, respectively (**Table 1**). The chitin yield in conventional extractions generally ranges from 5 to 8% (*7*), and our results agree with the expected values. However, the yield of all three extraction steps decreased with an increased sonication time. Although the lowest chitin yield obtained in our experiment (5.03%, 4 h sonication) was still similar to the yield obtained in industrial settings, the yield reduction from the control (8.28%) was probably due to depolymerization of chitin molecules during the acid treatment; that is, small solvent soluble chitin fragments (chitin-oligosaccharides) may have been produced (*24*). Thus, ultrasonication, while aiding in the elimination of minerals and proteins, increased chitin losses.

Table 1. Yield of FWP Shells Extraction

	true yield		
sonication time (h)	demineralization (% of initial FWP shell powder)	deproteinization (% of demineralized FWP powder)	additional NaOH treatment (% of deproteinized FWP powder)
0	71.93 ± 0.45	14.80 ± 0.61	75.88 ± 1.75
1	64.23 ± 0.26	15.07 ± 0.30	77.94 ± 0.99
4	60.73 ± 3.66	8.37 ± 2.17	83.65 ± 4.55
significance[a] ($P \leq F$)	<0.0001	<0.0001	0.0154
linear	<0.0001	<0.0001	<0.0001
quadratic	0.0007	0.4330	0.7359

	relative yield		
	g of extracted material from initial 100 g of FWP shell powder		
sonication time (h)	demineralization	deproteinization	additional NaOH treatment
0	71.93	10.65	8.28
1	64.23	9.68	7.55
4	60.73	5.08	5.03

[a] Linear = significance of a linear relationship; quadratic = significance of adding the quadratic arguments to the linear relationship.

To remove minerals from the initial material, we treated FWP shell powder with 0.25 N HCl for 4 h at 40 °C. Sometimes, different acids are used for this purpose, including acetic, formic, hydrochloric, and nitric acids, but hydrochloric acid is most commonly used. The usual concentrations of HCl are in the 0.25−2 M range, and the treatments last from 1 to 48 h at temperatures ranging from 0 to 100 °C (*15*, *25*−*27*). It has been recognized that harsh acid treatments can cause depolymerization of chitin as a side effect of demineralization. Percot et al. (*15*) found that the intrinsic viscosity of chitosan obtained after 3 h of demineralization in 0.25 M HCl at room temperature was lower as compared to the viscosity of chitosan treated for 1 h. The authors further showed that only 15 min of the same conditions was sufficient for complete removal of minerals when the solid-to-solvent ratio was 1:20. This is the mildest successful demineralization treatment published so far, and it offers possibilities for shorter treatments, especially in combination with the application of sonication.

The largest weight losses in our experiment occurred during the deproteinization step (approximately 55−60 g of initial 100 g dry shell powder). Commonly applied deproteinization conditions include treatment with 0.125−2.5 M NaOH solution (1 M being the most common) at 65−100 °C for 1−72 h. Thus, our control treatment with 0.25 M NaOH and no sonication was fairly mild and had to be supplemented with additional NaOH treatment to eliminate residual proteins. However, 4 h of sonication-assisted treatment enhanced deproteinization prob-

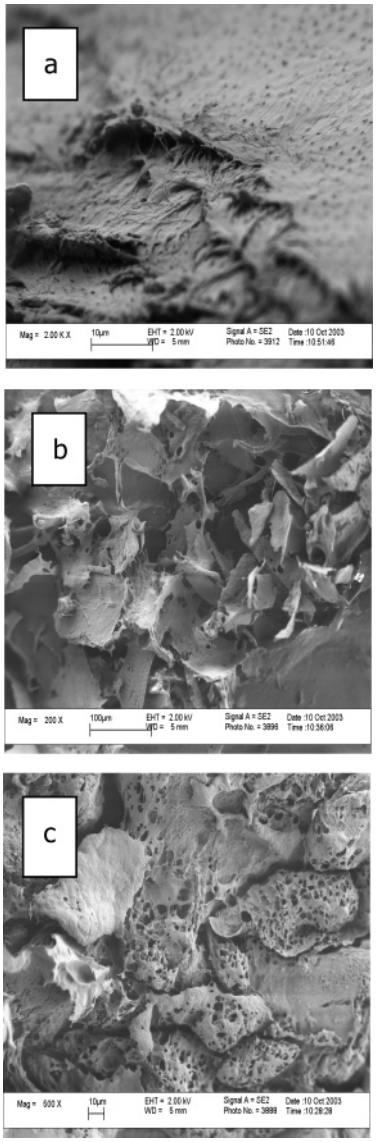

Figure 2. Scanning electron micrographs of FWP shell powder after (**a**) traditional extraction, (**b**) 1 h of sonication extraction, and (**c**) 4 h of sonication extraction.

ably due to breaking of hydrogen bonds and other intra- and intermolecular interactions. Additionally, the rigid structure of α-chitin, which exists in crustacean shells, may have been disrupted by high intensity ultrasound and promoted solubilization of compounds that comprise the shell structure (see also **Figure 2**).

Scanning electron micrographs (**Figure 2**) clearly show the effects of sonication on the morphology of FWP shell powder. The removal of minerals and proteins by traditional extraction left chitin fibers in the form of stacked layers. However, the application of 1 h of sonication during demineralization and deproteinization fractured chitin sheets, while 4 h of sonication resulted in extensive perforation of the shell fragments. These "spongelike" structures had significantly larger surface areas than nonsonicated fragments, which allowed for enhanced extraction but also resulted in lower final chitin yields.

The mineral content in FWP shells was initially 22.21% and treatment with 0.25 M HCl and no sonication reduced it to 1.82% (**Table 2**). Differences between sonication levels were highly significant after demineralization ($p < 0.0001$) and deproteinization ($p < 0.0001$) but not after additional deproteinization ($p = 0.0868$). For both demineralization and deproteinization, the relationship between sonication and mineral content was quadratic; however, an additional protein extraction step resulted in a linear relationship (**Table 2**). As mentioned earlier, Percot et al. (*15*) determined that the same concentration of acid successfully eliminated minerals from shrimp shells after only 15 min at room temperature. Further alkali treatments in our experiment resulted in a higher content of minerals in the solids (expressed on a % basis). This apparent increase may be the result of a significant reduction of proteins and not due to the accumulation of minerals. However, all sonicated samples had significantly higher levels of ash after each extraction step as compared to the control ($p < 0.0001$). The possible reason for this increase may be in a considerable deterioration of the sonication probe's stainless steel tip during prolonged sonication, as was evidenced by the formation of microholes in the tip. Thus, one of the solutions to effectively reduce the mineral fraction in the final product is to use short, 15 min or less, demineralization treatments with brief sonication.

Table 2. Mineral Content in FWP Material after Each Extraction Step

sonication time (h)	% ash in material		
	FWP powder		FWP powder after additional NaOH treatment
	demineralized	deproteinized	
0	1.82 ± 0.08	2.12 ± 0.44	2.38 ± 0.47
1	3.89 ± 0.20	3.20 ± 0.24	3.52 ± 1.01
4	4.18 ± 0.24	7.82 ± 0.36	5.54 ± 0.45
significance[a] ($P \leq F$)	<0.0001	<0.0001	0.0868
linear	<0.0001	<0.0001	0.0004
quadratic	<0.0001	0.0129	0.9134

sonication time (h)	ash (g) remaining from 100 g of initial FWP shell powder		
	FWP powder		FWP powder after additional NaOH treatment
	demineralized	deproteinized	
0	1.31	0.23	0.20
1	2.50	0.31	0.27
4	2.54	0.40	0.28

[a] Linear = significance of a linear relationship; quadratic = significance of adding the quadratic arguments to the linear relationship.

The initial protein content in FWP shells was 44.01%. Overall, the differences among sonication levels and among treatment levels were significant, with p values of 0.0016 and <0.0001, respectively. Conventional demineralization reduced the protein fraction by about 3% while 4 h of a sonication-assisted process resulted in about 10% reduction (**Table 3**). Deproteinization further decreased the protein content to 12.55, 10.59, and 7.45% for 0, 1, and 4 h sonication treatments, respectively, indicating that sonication significantly improved this extraction step. For both demineralization and deproteinization, the relationship between sonication and mineral content was linear; however, an additional protein extraction step resulted in a quadratic relationship (**Table 3**). A NaOH concentration (0.25 M) was apparently insufficient to remove all proteins from the material, and an additional treatment with 1 N NaOH was applied to complete the deproteinization. Even though sonication was not applied during this additional NaOH step, previous sonication treatments (during demineralization and deproteinization) had already weakened the shell structure, thereby enhancing the protein solubilization. Thus, chitin from the 4 h sonication-assisted extraction had less than 50% of the proteins found in chitin produced by the control extraction.

TGA Content. Contrary to minerals and proteins, the TGA content increased in the material after each step in the extraction

Table 3. Protein Content in FWP Material after Each Extraction Step

	% protein in material		
	FWP powder		
sonication time (h)	demineralized	deproteinized	FWP powder after additional NaOH treatment
0	41.07 ± 4.92	12.55 ± 0.97	5.72 ± 0.37
1	36.23 ± 1.28	10.59 ± 0.17	2.90 ± 2.18
4	34.34 ± 2.17	7.45 ± 2.37	2.67 ± 0.57
significance[a] ($P \leq F$)	0.0011	0.0437	0.9535
linear	0.0634	0.0022	>0.0001
quadratic	0.1811	0.1498	0.0219

	proteins (g) remaining from 100 g of initial FWP shell powder		
sonication time (h)	after demineralization	after deproteinization	after additional NaOH treatment
0	29.54	1.34	0.47
1	23.27	1.03	0.22
4	20.85	0.38	0.13

[a] Linear = significance of a linear relationship; quadratic = significance of adding the quadratic arguments to the linear relationship.

Table 4. TGA Content in FWP Material after Each Extraction Step

	% glucosamine in material		
	FWP powder		
sonication time (h)	demineralized	deproteinized	FWP powder after additional NaOH treatment
0	19.33 ± 0.95	54.26 ± 2.59	63.94 ± 1.82
1	24.38 ± 2.88	54.60 ± 2.12	63.37 ± 2.44
4	27.85 ± 1.97	63.74 ± 0.80	57.02 ± 2.09
significance[a] ($P \leq F$)	<0.0001	<0.0001	0.0922
linear	<0.0001	0.7750	0.0048
quadratic	<0.0001	0.0011	0.4880

	glucosamine (g) remaining from 100 g of initial FWP shell powder		
sonication time (h)	after demineralization	after deproteinization	after additional NaOH treatment
0	13.90	5.78	5.29
1	15.66	5.29	4.78
4	16.91	3.24	2.87

[a] Linear = significance of a linear relationship; quadratic = significance of adding the quadratic arguments to the linear relationship.

process (**Table 4**). For conventional extraction, the TGA content was 19.33, 54.26, and 63.94% in demineralized material, deproteinized material, and solids after additional NaOH treatment, respectively. The application of ultrasound during demineralization and proteinization resulted in a higher TGA content due to enhanced extraction of impurities. Differences among sonication levels were significant after demineralization ($p < 0.0001$) and deproteinization ($p < 0.0001$) but not after additional deproteinization ($p = 0.0922$). When the results are expressed on the absolute values basis (g TGA resulting from the initial 100 g FWP shell powder), it appears that 4 h of sonication-assisted extraction resulted in a lower TGA yield (2.87 as compared to 5.29% for control). This is probably the consequence of the long acid treatment during demineralization that may have reduced the molecular weight of the polymer and resulted in "leaching" of chitin oligomers. Shorter demineralization and/or sonication times would probably lessen depolymerization and still ensure sufficient removal of minerals.

Table 5. Crystallinity Index of FWP Solids

	crystallinity index		
	FWP powder		
sonication time (h)	demineralized	deproteinized	FWP powder after additional NaOH treatment
0	66.0	80.6	91.6
1	68.9	84.1	83.7
4	56.8	71.5	80.6

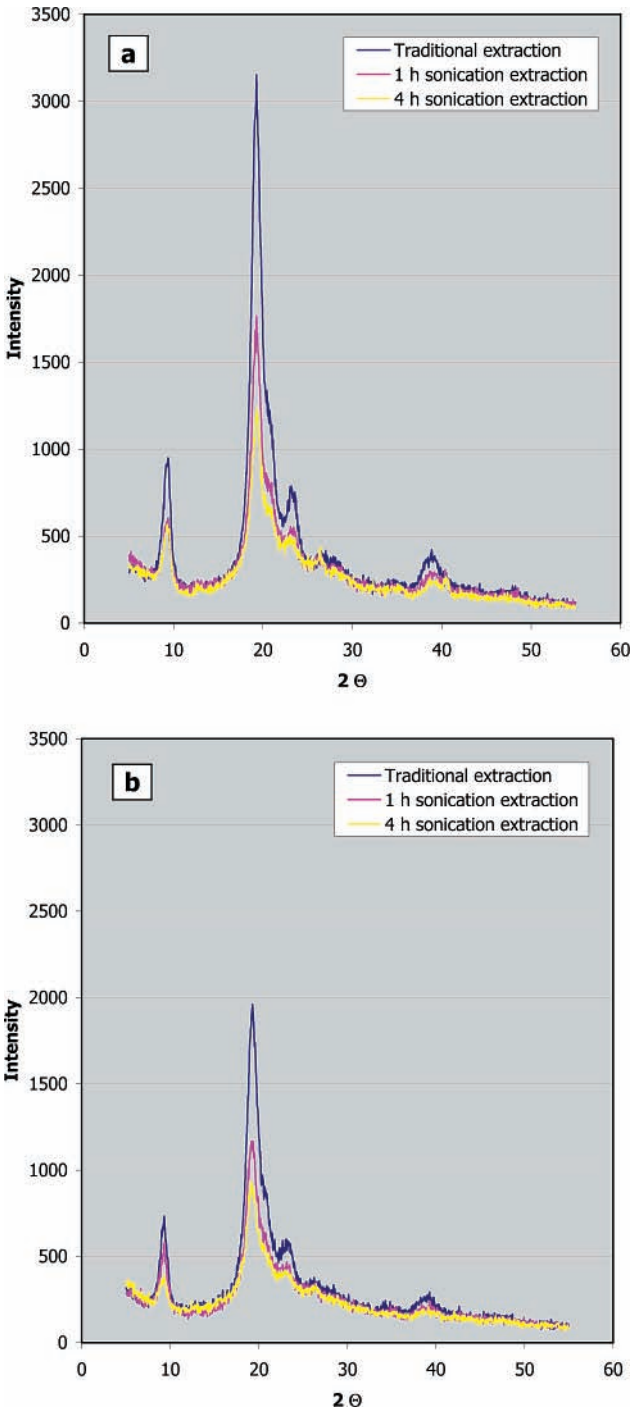

Figure 3. X-ray diffractographs of (**a**) FWP chitin and (**b**) chitosan after traditional extraction (blue line), 1 h of sonication extraction (pink line), and 4 h of sonication extraction (yellow line).

Crystallinity Index. The crystallinity of the material increased with the elimination of impurities from the FWP powder (**Table 5** and **Figure 3**). In crustacean shells, chitin fibers are

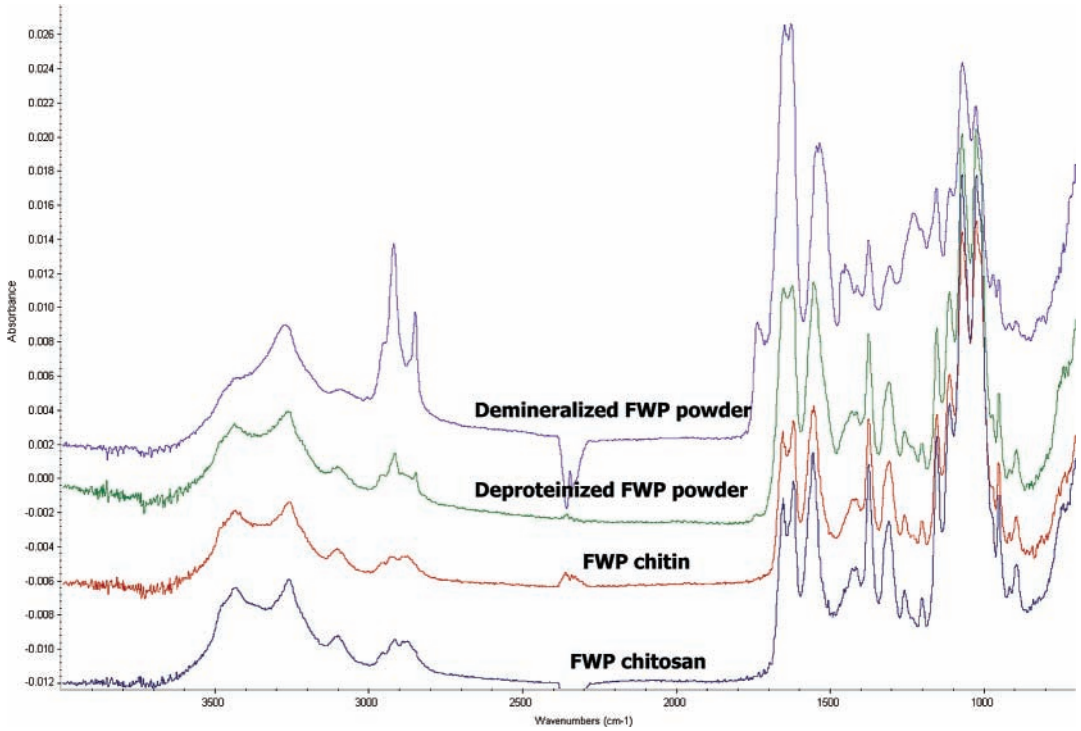

Figure 4. Infrared spectra of FWP shell material obtained during extraction.

embedded in a protein matrix and this high molecular weight complex is reinforced with large amounts of calcium salts. By removing the minerals and, more significantly, by removing the protein amorphous matrix, chitin fibers became exposed and the crystallinity index increased. Further deproteinization with an additional 1 N NaOH treatment resulted in a higher crystallinity as compared to the crystallinity of material after the first alkali treatment (91.6 vs 80.6). Interestingly, the crystallinity index detected in sonicated samples was lower than in the control. Crystallinity in polysaccharide molecules, such as in chitin, has been attributed to extensive hydrogen bonding between hydroxyl, carbonyl, amino, and amido groups of neighboring chains. Sonication may have disrupted these interactions thereby reducing the crystallinity index. Deacetylation with 12.5 M NaOH further reduced crystallinity, progressively more so as the sonication time increased during chitin extraction (**Figure 3b**). Thus, chitin samples with a crystallinity index of 91.6, 83.7, and 80.6 and 0, 1, and 4 h sonication-assisted extraction produced chitosans with crystallinity indices of 86.4, 83.7, and 73.1, respectively. This is consistent with results from Li et al. (*28*) who observed that increasing the contact time of α-chitin with hot NaOH solution resulted in a lower crystallinity index of the material. A low crystallinity index is very important in subsequent chemical modification steps such as deacetylation and hydrolysis. Efficiency of these processes typically increases with decreased crystallinity of raw materials (Kurita, 1993). On the basis of our results, sonicated materials appear to be more accessible and prone to modifications, indicating that they have an excellent potential to be used in the production of chitin derivatives.

Size of Crystallites. The sizes of the crystals corresponding to the angle $2\theta \sim 20°$ were found to be between 0.42 and 3.07 (**Table 6**). Resolutions of other peaks were less resolved, and determination of fwhm of other peak angles was not consistent. Nevertheless, it can be seen that with the extraction process the sizes of the crystallites increase as the contact time of samples with sodium hydroxide solution increases. The order of magnitude of the crystallites' size in our samples is consistent with data from Jaworska et al. (*29*) who found that chitosans extracted from shrimp shells had a fwhm between 2.02 and 3.38 nm.

Table 6. Size of Crystallites in FWP Solids

sonication time (h)	size of crystallities (nm)		
	FWP powder		FWP powder after additional NaOH treatment
	demineralized	deproteinized	
0	1.08	2.16	2.44
1	0.42	2.24	2.28
4	1.09	2.13	3.07

FTIR Scans. Characteristic bands for chitin and chitosan in the FTIR spectra include a ~3265 cm^{-1} band assigned to N−H stretching vibrations, a band in the neighborhood of 1655 cm^{-1} attributed to C═O stretching absorption (amide I band), and an amide II band appearing between 1555 and 1520 cm^{-1}. Consequently, a decrease in peak intensity at 1655 cm^{-1} and an increase of peak intensity at 1555 cm^{-1} are expected as the deacetylation of chitin progresses. However, the differences in the scans between chitins and their corresponding chitosans were not as large as expected. Thus, the DAs of 75.9 ± 1.9, 72.4 ± 5.3, and 75.7 ± 1.5% were determined for 0, 1, and 4 h of sonication-assisted extracted chitins, respectively, while corresponding chitosans had DAs of 70.0 ± 6.4, 68.7 ± 2.2, and 61.4 ± 3.2%.

Interestingly, peaks in the area between 3000 and 2800 cm^{-1} were dominant in demineralized FWP shell samples but considerably smaller after deproteinization. Because this region is characteristic for the $-CH_2$ stretching absorptions (~2930 and 2870−2840 cm^{-1}) and $-CH_3$ vibrations (~2930 and 2885−2865 cm^{-1}), the peaks were probably the result of a long hydrocarbon chain of lipids and carotenoids present in dried shell powder. Astaxanthin is a dominant pigment of crustacean exoskeleton with an average concentration of 25 μg/g on a dry weight basis (*26*). As the exoskeleton carotenoids are embedded in the protein matrix, alkali treatments apparently removed not only proteins but lipid fractions as well.

In conclusion, our results showed that although chitin yield of sonicated-assisted extractions was lower as compared to traditional procedures, high intensity ultrasound provides a simple technique for shorter extraction treatments and production of less crystalline polymers susceptible to various modifications.

ACKNOWLEDGMENT

We thank Primex, Iceland, and the United States Freshwater Prawn and Shrimp Growers Association for their advice and assistance.

LITERATURE CITED

(1) FAOSTAT. *Total Amount of Shrimp and Prawn Harvested 2002*; FAO Statistical Databases, Food and Agricultural Organization of United Nations: Rome, Italy, 2002; http://faostat.fao.org/.

(2) Zakaria, Z.; Hall, G. M.; Shama, G. Lactic acid fermentation of scampi waste in a rotating horizontal bioreactor for chitin recovery. *Process Biochem.* **1998**, *33*, 1−6.

(3) Muzzarelli, R. A. A.; Jeauniaux, C.; Gooday, G. *Chitin in Nature and Technology*; Plenum Press: New York, 1986.

(4) Muzzarelli, R. A. A. Human enzymatic activities related to the therapeutic administration of chitin derivatives. *Cell. Mol. Life Sci.* **1997**, *53*, 131−140.

(5) Wiley, J. *Chitin and Chitosan: An Expanding Range of Markets Await Exploitation*; John Wiley & Sons: New York, 1998.

(6) Broussignac, P. Chitosan, a natural polymer not well-known by the industry. *Chim. Ind. Genie Chim.* **1968**, *99*, 1241−1247.

(7) Roberts, G. A. F. *Chitin Chemistry*; The Macmillan Press Ltd.: London, United Kingdom, 1992.

(8) Cardoso, M. B.; Signini, R.; Campana, S. P. On the sonication of chitin: Effects on its structure and morphology and influence on its deacetylation. *Polym. Bull.* **2001**, *47*, 183−190.

(9) Crum, L. A. Comments on the evolving field of sonochemistry by cavitation physicist. *Ultrason. Sonochem.* **1995**, *2*, s142−s147.

(10) Mason, T. J.; Cordmas, E. D. Ultrasonic intensification of chemical processing and related operations—A review. *Trans. Inst. Chem. Eng.* **1996**, *74*, 511−516.

(11) Mason, T. J. Ultrasound in synthetic organic chemistry. *Chem. Soc. Rev.* **1997**, *26*, 443−451.

(12) Stephanis, C. G.; Hatiris, J. G.; Mourmouras, D. E. The process (mechanism) of erosion of soluble brittle materials caused by cavitation. *Ultrason. Sonochem.* **1997**, *4*, 269−271.

(13) Masselin, I.; Durand-Bourlier, L.; Laine, J. M.; Sizaret, P.; Chasseray, X.; Lemordant, D. Effect of sonication on polymeric membranes. *J. Membr. Sci.* **2001**, *181*, 213−220.

(14) Panchev, I. N.; Kirtchev, N. A.; Kratchanov, C. G.; Proichev, T. Improving pectin technology. II. Extraction using ultrasonic treatment. *Int. J. Food Sci. Technol.* **1988**, *23*, 337−341.

(15) Percot, A.; Viton, C.; Domard, A. Optimization of chitin extraction from shrimp shells. *Biomacromolecules* **2003b**, *4*, 12−18.

(16) Lowry, O. H.; Rosbrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

(17) Tsuji, A.; Kinoshita, T.; Hoshino, M. Analytical chemical studies on amino sugars II: Determination of hexoamines using 3-methyl-2-benzothiazolone hydrazone hydrochloride. *Chem. Pharm. Bull.* **1969**, *17*, 1505−1510.

(18) Chen, G. C.; Johnson, B. R. Improved colorimetric determination of cell wall chitin in wood decay fungi. *Appl. Environ. Microbiol.* **1983**, *46*, 13−16.

(19) Plassard, C. S.; Mousain, D. G.; Salsac, L. E. Estimation of mycelial growth of Basidiomycetes by means of chitin determination. *Phytochemistry* **1982**, *21*, 345−348.

(20) Domszy, J. G.; Roberts, G. A. F. Evaluation of infrared spectroscopic techniques for analyzing chitosan. *Die Makromol. Chem.* **1985**, *186*, 1671−1677.

(21) Segal, L.; Creely, J. J.; Martin, A. E.; Conrad, C. H. An empirical method for estimating the degree of crystallinity of native cellulose using X-ray diffractometer. *Text. Res. J.* **1959**, *29*, 786−794.

(22) Struszczyk, H. Microcrystalline chitosan. I: Preparation and properties of microcrystalline chitosan. *J. Appl. Polymer Sci.* **1987**, *33*, 177−189.

(23) Klug, H. P.; Alexander, L. *X-ray Diffraction Procedures*; John Wiley & Sons: New York, 1974.

(24) Baxter, S.; Zivanovic, S.; Weiss, J. Molecular weight and degree of acetylation of high-intensity ultrasonicated chitosan. *Food Hydrocolloids* **2005**, *19*, 821−830.

(25) Percot, A.; Viton, C.; Domard, A. Characterization of shrimp shell deproteinization. *Biomacromolecules* **2003a**, *4*, 1380−1385.

(26) Synowiecki, J.; Al-Khateeb, N. Production, properties, and some new applications of chitin and its derivatives. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 145−171.

(27) Tolaimate, A.; Desbrieres, J.; Rhazi, M.; Alagui, A. Contribution to the preparation of chitins and chitosans with controlled physicochemical properties. *Polymer* **2003**, *44*, 7939−7952.

(28) Li, J.; Revol, J.-F.; Marchessault, R. H. Effect of N-sulfonaton on the colloidal and liquid crystal behavior of chitin crystallites. *J. Colloid Interface Sci.* **1997**, *192*, 447−457.

(29) Jaworska, M.; Sakurai, K.; Gaudon, P.; Guibal, E. Influence of chitosan characteristics on polymer properties. I: Crystallographic properties. *Polym. Int.* **2003**, *52*, 198−205.

Received for review September 6, 2005. Revised manuscript received February 20, 2006. Accepted March 8, 2006. This research was supported by a U.S. Department of Agriculture National Research Initiative Grant (U.S. Department of Agriculture 2005-35503-15428), Hatch funds from the Tennessee and Massachusetts Experiment Stations (TEN 264 and MAS 0911), the University of Iceland, and The Icelandic National Research Fund (RANNIS 030930003).

JF052184C