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Deproteinization Process of Chitin from Dried Shrimp Shells (*Litopenaeus vannamei*) Using Papain and Nanochitin Characterizations

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Abstract

Background and Objective: Chemical treatments in chitin extraction from shrimp shell wastes have affected the environment. Shrimp shell primarily bonds chitin with inorganic salts, lipids, proteins and pigments. Extraction of chitin from shrimp shells involves protein separation processes. Deproteinization process of chitin from dried shrimp (*Litopenaeus vannamei*) shells with papain enzyme was optimized and nanochitin as a derivative product of chitin was characterized.

Material and Methods: Effect of hydrolysis time, temperature and enzyme concentration were optimized using RSM Box-Behnken method to maximize chitin yields. Nanochitin was prepared using dialysis and ultrasonic methods and characterized for physical characteristics using scanning electron microscope, particle size analysis and Fourier transforms infrared spectroscopy.

Results and Conclusion: Optimum conditions using enzymatic hydrolysis at 6 h, 50 ^{o}C and 1.25% papain decreased the protein content from 33.66 to 2.31% and produced a high chitin yield (46.03%). Deproteinization using enzymatic hydrolysis method was more efficient than that using fermentation. Data of scanning electron microscope, particle size analysis and Fourier transforms infrared spectroscopy showed that the characteristics of chitin and nanochitin products were similar to those of chemical treatments for chitin products.

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1. Introduction

Litopenaeus vannamei is one of the shrimp species that includes high commercial values and produces abundant shrimp shell wastes. Production of shell wastes from crustaceans was predicted to be 3.14 million metric tons per year worldwide [1]. Hundreds of shellfish wastes are generated from seafood manufacturing and daily Asian consumption [2]. Shell wastes from crustaceans contain a high quantity of chitin, a polysaccharide material that is important in biological functions and is biodegradable and compatible. Chitin and its derivatives are used in various fields such as pharmaceutical, food, textile and waste water-treatment industries [3]. Chitin in the shrimp shells is bonded with majorly inorganic salts, calcium carbonate, proteins, lipids and pigments. Therefore, isolation of chitin from shrimp shells involves protein separation processes and mineral separation [4]. Structure of chitin is arranged with N-acetylated glucosamine and glucosamine units, linked by β(1,4) covalent bonds. Corresponding to this structure, chitin is stable to chemical and biological actions and the linkage of chitin is similar to the linkage of

cellulose [5]. Generally, chitin is extracted through demineralization using acid treatment and deproteinization using alkali treatment. These treatments affect the environment and finding an alternative process that is more friendly to the environment is still necessary. Deproteinization process for chitin extraction from shrimp shells can be carried out via chemical, enzymatic and microbial processes [6, 2]. The chemical treatment involves mineral acid at high temperatures, resulting in high volumes of polluted waste containing mineral acids in the washing process. These treatments are harmful to the environment due to high concentrations of mineral acids [7]. Deproteinization with enzymes is a zero waste system resulting in high yields of chitin products. Protease hydrolyzes proteins in the matrix efficiently [8]. Commercially purified enzymes such as alcalase, papain, pepsin and trypsin have been used in chitin extraction studies to remove protein from crustacean shells [9].

Chitin is a biopolymer containing microfibrillar and semicrystalline structures. Based on data of the infrared (IR) spectra and X-ray crystallography (XRD), chitin is naturally in the forms of α-chitin, β-chitin and ɣ-chitin. Characteristics of chitin such as solubility, porosity and surface area restrict its uses. To solve this problem, various derivatives such as chitosan, chitin nanofibers and chitin nanowhiskers are produced [10]. Chitin nanofibers have been prepared via several methods such as ultrasonication, mechanical treatment, gelation and electrospinning [11]. Chitin nanofibers from species such as crabs, prawns and mushrooms have been prepared using mechanical and chemical treatments. The acidic medium was verified in the decrease of chitin nanofibers extracted from crab shells [12]. Under certain extraction conditions, chitin microfibrils are isolated in the form of nanocrystals and nanofibers. Their unique characteristics have been studied and used in food, cosmetics and medical industries [13]. Characteristics of chitin depend on the organisms and chitins may lay in α and β allomorphs shapes. These forms were assessed by the orientation of microfibrils that could be characterized using infrared, nuclear magnetic resonance (NMR) spectroscopy and XRD analysis [14]. Probiotic microorganisms have been studied for the demineralization treatment of crustacean shells. Chitin extraction using microorganisms was carried out simultaneously. Shrimp shells (*Penaeus monodon*) were fermented with lactic acid bacteria (LAB) and chitin was separated by adding carbohydrates [10]. Based on XRD and NMR data, chitin extraction via enzymatic process is an alternative method to preserve its native structure [1]. Shelma et al. reported the chitin nanofiber preparation via acid hydrolysis of the chitin powder followed by dialysis and ultrasonication [15]. Chitin from *P. vannamae* byproducts was prepared by associating enzymatic acid-alkaline strategies to achieve further sustainable processes [16]. Moreover, chitosan was produced through papain extract to help deproteinization process. Papain is achieved from the papaya plant with the endopeptidase, dipeptidase and exopeptidase activities. The optimum condition of this process was at 7 h of enzymatic hydrolysis and 25% of papain [8]. The current study was aimed to optimize deproteinization process of chitin from dried shrimp (*L. vannamei*) shells using low concentration papain (0.75–1.25%) and to achieve nanochitin, which was prepared via dialysis and ultrasonic methods. Furthermore, nanochitin products were characterized through physicochemical characteristics to verify their quality.

2. Materials and Methods

2.1. Materials

Dried white-shrimp (*L. vannamei)* shells were provided as byproducts of a shrimp processing industry at Muara Gading City Bekasi, West Java, Indonesia. Commercial papain (CAS no. 2323.627-2) (Xian Arisun ChemParm, Shaanxi, China) was purchased in powder form. All chemicals used included laboratory grades.

2.2. Chitin extraction from the shrimp shells

Chitin from the sample was extracted using method of Hongkulsup et al. [1] with some modification. The extraction was carried out at two steps, including demineralization and deproteinization. In demineralization process, shrimp shells were ground to achieve a size of 100 mesh. Shrimp shell powder was extracted using 1.5 M HCl (ratio 1:10, w/v) at 25 ^{o}C for 6 h and at 150 rpm. Mixture was filtered using vacuum filter and the residue was mixed with distilled water (DW) to achieve neutral pH. Then, residue was dried at 50 ^{o}C for 6 h. Dried residue was mixed with 0.75–1.25% w/v papain in a phosphate buffer pH 7 and heated at 40–50 ^{o}C for 3–6 h. Hydrolysis was stopped at 90 ^{o}C and set for 20 min. Mixture was filtered and the residue was mixed with DW until neutral pH was achieved. Then, residue was dried at 50 ^{o}C for 6 h. Total residue was assessed gravimetrically and the soluble proein content in the residue was analyzed using modified Lowry method. Briefly, 1 g of residue was diluted with DW up to 1 ml and filtered using Whatman filter papers. Then, 0.5 ml filtrate was mixed with 5.5 ml of alkaline $CuSO_4$ reagent and incubated at room temperature (RT) for 10 min. Solution was mixed with 0.5 ml of folin phenol reagent. Then, sample solution was mixed with 3.5 ml of DW and the absorbance was measured at 650 nm. The protein soluble content was assessed by plotting bovine serum albumin (BSA) standard curve [17].

2.3. Optimization of deproteinization of shrimp shells using Box-Behnken method

Optimum condition of the deproteinization process was predicted using response surface methodology (RSM)-

Box-Behnken method. Optimization of deproteinization was carried out using three factors of effects of hydrolysis time, temperature and enzyme concentration (Table 1). Proportions of total residue, chitin and protein concentration were used as the responses data. Fifteen trials were carried out indiscriminately. The center value was chosen based on the references, which were 1% papain, 45 ^{o}C and 6 h [18, 19]. Design Expert 13.0 software was used in this study.

2.4. Assessment of chitin

Chitin content was assessed using adaptation of the Morrow method [20] with some modification. One gram of the sample was mixed with 40 ml of 1 M HCI and mixed at RT for 2 h. Chitin residue was separated using vacuum filter with a porous sintered glass disc and washed several times with water to reach a neutral pH. The residue was washed off and transferred into a beaker containing 40 ml of 5% NaOH and stirred at 100 °C for 2 h. Chitin product was separated using filter paper (Whatman no. 41, USA) and then rinsed with water until a neutral pH was achieved. Content of the chitin (%) was assessed gravimetrically.

2.5. Nanochitin preparation

The selected chitin sample, which was prepared at optimized conditions, was soaked in 3 M HCl for 90 min at 90 ^{o}C. Suspension was precipitated by centrifugation at 6000 rpm for 10 min. Nanochitin from the precipitated fraction was prepared for dialysis and ultrasonic treatments using Mincea method [11] with modifications. Suspension of chitin was transferred to a dialysis bag (cellulose membrane with cut-off proteins mol. wt $\geq$ 12,000) and dialyzed in DW by changing the water every 2 h for three times. Dialysis was carried out until pH 6 was reached. Ultrasonic treatment of the chitin sample was carried out at pulse of 1/1 and amplitude of 60% (750 W, 20 kHz) for 6 h to 0.1% (w/v) of the suspension. Based on the modification of Wu and Meredith method [21], these samples were freeze-dried at -60 ^{o}C for 10 h.

2.6. Microstructure identification

Microstructure of the freeze-dried samples was assessed using scanning electron microscope (SEM) (JSM-IT30, Jeol., Akhishima, Tokyo, Japan). These samples were put in a sample holder and layered with a thin layer of gold (±10 nm). Observation was carried out by accelerating voltage at 20 kV based on a previous method.

2.7. Particle size distribution

Particle size distribution of the samples was analyzed using particle size analyzer (Zetasizer Nano ZS Malvern,UK) based on Shelma et al. method [15] with modifications. Sample was dispersed in Tween 80 (0,4%; w/v) with a ratio of 1:4.

2.8. Fourier transforms infrared spectroscopy (FTIR)

Spectra of the samples were analyzed using Fourier transforms infrared spectroscopy (FTIR 1000, Perkin-Elmer, USA) at mild conditions and method of KBr pellet scanning. Based on previous studies, KBr (100 mg) and the sample (1 mg) were mixed entirely until KBr pellet was formed. Then, samples were scanned at spectral ranges of 400, 4200 and 4200 cm-^{1}.

Table 1. The hydrolysis conditions and their responses

Run	Hydrolysis condition			Response		
	Time (h)	Temperature (°C)	Papain (%)	Total residue (%)	Chitin (%)	Soluble protein (%)
1	6	40	0.75	77.29	46.52	6.82
2	9	40	1.00	73.19	49.06	5.75
3	3	45	0.75	74.87	45.65	6.75
4	3	40	1.00	76.43	46.40	5.72
5	6	50	1.25	76.71	46.03	2.31
6	9	45	0.75	80.76	41.52	5.1
7	9	50	1.00	75.90	46.55	6.15
8	6	50	0.75	74.16	44.97	5.39
9	9	45	1.25	72.59	45.95	6.29
10	6	45	1.00	75.29	43.02	4.55
11	6	40	1.25	73.21	44.17	5.51
12	3	50	1.00	75.29	44.63	5.35
13	3	45	1.25	74.14	36.81	3.53
14	6	45	1.00	76.43	41.93	4.74
15	6	45	1.00	76.86	42.60	3.23

3. Results and Discussion

3.1. Optimization of deproteinization of the shrimp shells

The optimum conditions of the enzymatic hydrolysis in the deproteinization process of white shrimp shell powder were predicted using RSM. Fifteen trials were carried out based on the RSM-Box Behnken design. The Box–Behnken design (BBD) is a widely used RSM design that is useful for ascertaining cause-and-effect correlations between factors and responses in experiments. The BBD needs three levels and can be used for factors of 3–21 [22]. Hydrolysis factors and their responses are provided in Table 1. Data showed that the total residue of the products ranged 74.14–80.76%, chitin content ranged 41.52–49.06% and protein content ranged 2.31–6.82%. Analysis of variances (ANOVA) was calculated and *p*-values of the total residue, soluble protein and chitin content are present in Table 2. Papain concentration (C) and its interaction with temperature (AC) and hydrolysis time (BC) significantly ($p < 0.05$) affected the total residue of shrimp shell powder. The hydrolysis time (A) and its interaction with the papain concentration (AC) significantly ($p < 0.05$) affected the chitin content. However, *p*-values of the soluble protein contents showed that treatments were not significant ($p > 0.05$). The equation for estimating the optimal condition for all responses (Y1, Y2 and Y3) from the shrimp shells is present in Table 3. Total residue included the yield of the dried product after the deproteinization process with the papain enzyme. Chitin extraction via enzymatic hydrolysis needs removing proteins from the crustacean shells, minimizing the deacetylation and depolymerization processes. This process may be carried out before or after the demineralization step of solid materials for accessibility of the reactants. Efficiency of the enzymatic treatments is inferior to chemical methods ranging 5–10% of the residual protein attached to chitin [9]. Commercial enzymes such as alcalase, econase, pancreatin and other proteases were used in the chitin extraction of shrimp and crustacean shells. The objective of these treatments was to eliminate the protein contained in the waste of shells. Proportion of the chitin ranged 16.5–22% [7]. Combination of the chemical agents and enzymes has been studied to increase yields of the chitin products. Use of sodium sulfite and alcalase was the best treatment for protein recovery. Characteristics of the chitin sample were similar to those of the commercial food-grade products [6].

Three-dimensional (3D) response surfaces of the response; of which, one of the factors is fixed at the central point and the other is varied, are present in Figure 1. The highest predicted chitin content is indicated by the surface confined in the smallest ellipse in two-dimensional (2D) contour plots. This indication was correlated with the interaction between hydrolysis time and papain concentration significantly. This was similar to the results of ANOVA analysis (Table 2). The 2D contour plots showed effects of hydrolysis time (A) in the chitin content prediction (Fig. 1c). However, stagnation was observed in the chitin content with increasing temperature (Fig. 1b). To achieve the optimum condition of the deproteinization process, an optimization process was analyzed using Design Expert 13.0 RSM optimizer software. The three factors (time, temperature and papain concentration) were adjusted in the importance level 3 (+++) and responses (total residue, soluble protein and chitin yield) were adjusted in the importance level 5 (+++++). The optimum condition with desirability of 0.619 was observed for chitin extraction at 6 h, 50 ^{o}C and 1.25% papain. Further, all the responses of the products were validated through laboratory experiments. The experimental and predicted values are present in Table 4. Data showed that the experimental and predicted values were in the range (95% prediction interval); thus, reliability of the optimized condition was verified. The RSM-Box Behnken design was successfully used to assess effects of hydrolysis time, temperature and papain concentration on deproteinization process to produce higher chitin contents. Chitin from the molted shrimp shells was extracted using a chemical method. The optimum condition of deproteinization was achieved in 3% NaOH at 50 ^{o}C for 6 h with a residual protein content ≤ 1% [23]. Yulirohyami et al. (2024) reported that chitosan was prepared through processes, including depigmentation, demineralization, deproteinization and deacetylation. The optimum condition of deproteinization process was reached at 25% of papain for 7 h of hydrolysis. This study showed that the hydrolysis time of chitin deproteinization affected deacetylation degrees of chitosan [8].

Proteases have been used for chitin extraction from shrimp byproducts. Residual proteins in shrimp wastes included 1.3 and 2.8% after treatment with chymotrypsin and papain enzymes. Combination of papain with other proteases that was used for deproteinization of shrimp wastes showed that the protein removal rates were low [24]. As an alternative to chemicals and decreasing shrimp wastes, 0.2% alcalase was shown to include activities in decreasing protein contents in shrimp wastes from 49.43 to 4.12% [25]. The enzymatic deproteinization of shrimp processing wastes has limited chitin yields nealy 4.4 to 7.9% of the total weight. This might be due to the residual of short peptides appropriately bonded to the compound of chitin. Use of combination agents with protease significantly decreased the protein fraction. Through this combination, protein fraction significantly decreased, assuming that protease degraded disulfide bonds of the

shrimp head waste proteins that facilitated entry of sulfite ions [6]. Use of exoenzymes and proteolytic bacteria in deproteinization of demineralized shells produced liquid protein and solid chitin fractions [2]. Papain is a commercial enzyme, which includes endopeptidase, dipeptidase and exopeptidase activities. Binding affinity and catalytic efficiency of papain are affected by the substrate, temperature and incubation time [8].

Table 2. The *p*-values of the total residue, soluble protein and chitin contents

Source	The total residue (%)	Soluble protein (%)	Chitin (%)
Model	0.0521	0.2029	0.0046*
A (h)	0.6844	0.5140	0.0158*
B (^{o}C)	0.6430	0.1569	0.1981
C (%)	0.0325*	0.0678	0.0865
AB	0.2131	0.7098	0.7119
AC	0.0311*	0.0737	0.0009*
BC	0.0485*	0.4066	0.1315
A2	-	0.1092	0.2763
B2	-	0.3060	0.0008*
C2	-	0.6379	0.2532
Lack of Fit	0.2240	0.6379	0.1955
R2	0.7254	0.7966	0.9624

*significant at 5% level ($p<0.05$)

Table 3. Equations for estimating the optimal conditions

Response	Equation
The total residue (Y1)	$0.2125A + 0.2427B – 1.30C + 0.9643AB – 1.86AC + 1.66BC$
Soluble protein (Y2)	$0.2425A – 0.5750B – 0.8025C + 0.1925AB + 1.10AC - 0.4425BC + 0.9896A^2 + 0.5796B^2 + 0.2546C^2$
Chitin (Y3)	$1.20A – 0.2963B – 0.7125C - 0.1850AB + 3.32AC + 0.8525BC + 0.6017A^2 + 3.54B^2 – 0.6358C^2$

Table 4. Optimized deproteinization conditions with the corresponding responses

Deproteinization conditions			Responses		
Temperature (°C)	Time (h)	Papain (%)	Total residue (%)	Chitin (%)	Soluble protein (%)
50	6	1.25	76.21	44.60	3.03
			76.71	46.03	2.31
			95% PI: 72.09-80.34	95% PI: 41.38-47.83	95% PI: 0.29-6.36

Data in rows 1 and 2 represent predicted and actual values, respectively

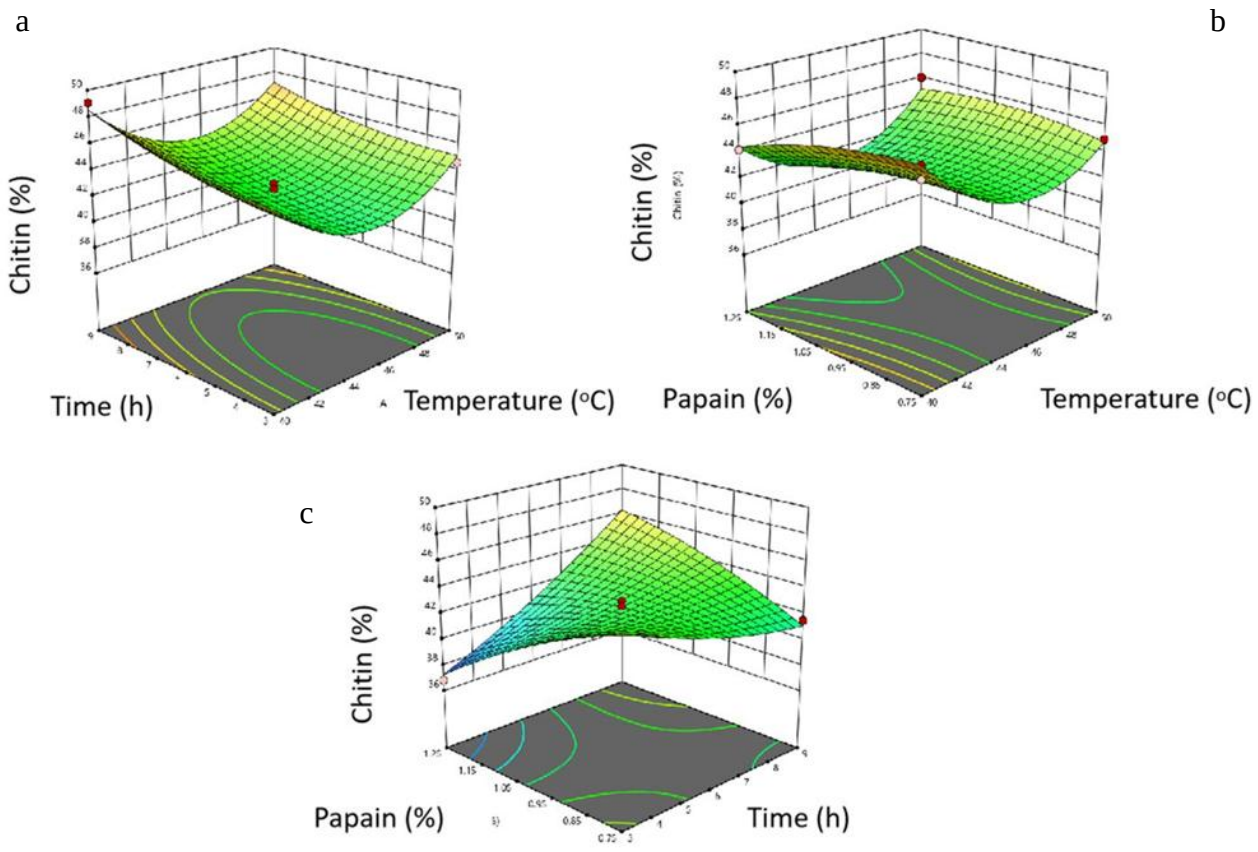

Figure 1. Three-dimensional surface plots of interactions between the factors (a, b, and c) and chitin contents of the shrimp shells

3.2 Physicochemical characterization of Chitin

Characteristics of chitin, including degree of deacetylation, morphology and molecular mass, vary depending on the extraction method and origin of chitin [14]. For example, chitin achieved by the chemical extraction showed a tightly packed morphology, while a slightly microfibrillar structure was shown by chitins extracted via enzyme treatment [1]. Use of chitin increased significantly due to the prominent characteristics of its derivatives and nanostructure configuration, which are met for industrial processing. Techniques have been developed to produce chitin derivatives. For example, dialysis and ultrasonic methods to produce nanochitin from shrimp shells; similar to those of the present study. Surface morphologies of the prepared chitin and nanochitin are present in Figure 2. Accordingly, porous-like honeycomb structure with no nanofibers on the surfaces was observed in the chitins (Figure 2a) and nanochitin achieved via dialysis process (Figure 2b). The only difference between these two products was in the pore size as the pore width of chitin (3 µm ±5) was smaller than that of nanochitin (5–15 µm). This result indicated that during the acid hydrolysis process of nanochitin preparation, the amorphous part of chitin was removed, leaving the crystalline side and leading to increases in pore size. The nanochitin generated from the ultrasonic technique (Figure 2c) showed a nanofibrillar structure with a diameter of nearly 160 nm. This reveals that hydrolysis with a strong acid followed by the ultrasonication treatment further facilitated dissolution process of the amorphous chitin [16]. Ultrasonication is a method to change the natural cithin into chitin nanofibers. Fibrillating chitin at 900–1000 W and 20 kHz in water (pH ±7) created nanofiber widths of 25–120 nm. High frequency of ultrasonication induced startling waves on the chitin surface that promoted their factorization with the axial way [26].

Chitin is naturally detected in crystalline microfibrils as a structural component, serving as a functional material that is needed by many organisms [14]. The pH of a solution in chitin treatment affects the surface morphology of chitin nanostructures, as a previous study demonstrated that the nanofiber structures of chitin were destroyed to small irregular shapes under high alkaline environments [23]. Furthermore, chitin nanofibrous structure formed due to chitin nanofibers are not soluble and result in versatile porous structures of the products by adjusting the freezing

temperature. Freeze-drying technique includes the potential for the assembly of the nanofibrous structure of water-dispersible materials [21].

Particle size distribution is an important characteristic that affects functionality of the chitin products. The chitin sample included two peaks in the spectra, which were dissolved in 0.4% of Tween 80 solution (Fig. 3a). The Z-average of chitin from the shrimp shells was 511.7 nm and the highest intensity was 21.8%. Moreover, nanochitin samples showed three peaks with Z-averages of 101.7 (Fig. 3b) and 345.4 nm (Fig. 3c), respectively. Nanochitin produced via dialysis method showed a Z-average of the particles smaller than that produced by the ultrasonic method. However, the intensity of nanochitin products was still lower than that of untreated chitin samples. Particle size distribution of the chitin nanofibers demonstrated a bimodal curve with majority sizes of 20–300 nm [15]. In this study, additional peaks in nanochitin products were assumed as degraded chitin products. Temperature of the experiments affected number of the peaks in spectra. For higher temperatures, large particles were observed, which might be caused by degradation of the chitin particles. The lower temperature of ionic liquids was further favorable, resulting in a narrow particle range of particle size distribution spectra [27]. Ionic liquids could change the chitin structure, able to modify the particle size [28].

The FTIR spectrum of chitin is present in Figure 4. Chitin sample showed similar spectra with nanochitin, which was prepared via dialysis and ultrasonic methods. Spectra at 3258 and 2924 cm^{-1} were recognized as N-H and C-H stretching vibrations. The amide I band was distributed into two peaks of 1652 and 1621 cm^{-1}. Absorption at 1557cm^{-1} was assigned to N-H bend and C-N to 1310 cm^{-1}. Peaks at 1069 and 1010 cm^{-1} were recognized as C-O stretching. Chitin from the shrimp shells has been extracted via two-step extraction using citric acids and deep eutectic solvents (DESs). The study showed that spectra of DESs-extracted chitin included N-H stretching, which was limited by the bonds of intermolecular hydrogen and the bonding of NH groups. Band of amide I was generated by bonding between the intra-chain hydrogen with the NH groups and the bonding between inter-chain hydrogen with the primary OH [29]. Moreover, two absorption bands at 1018 and 1172 cm^{-1} were identified for C-O stretching vibration of chitin from snail shells [30]. Two-step fermentation method was used for demineralization and deproteinization of chitin extraction from shrimp shell powder. The FTIR spectra of the samples showed characteristic peaks corresponding to the amide I (1652 and 1620 cm^{-1}) and amide II (1554 cm^{-1}) regions. Peaks at 1375 and 950–1200 cm^{-1} were C-H, C-O-C and C-O bonding [31]. Nanochitin was produced using microwave method to observe the difference of α and β structures in amide I. Two bands at 1654 and 1621 cm superscript were assigned as single H-bonded and double H-bonded α structures. A unique single band at 1631 cm^{-1} was assigned as β structure of nanochitin [13]. Structure of α chitin is known stable than chitin due to strong hydrogen bonding in inter and intra-sheets [32].

Based on the characteristic data of nanocithin from white shrimp shells (*L. vannamei*), the product is potential for creating nanochitin-based materials. Morphological and chemical characteristics, including helical and its structure, encourage material developments. Nanochitin is a promising product as the support material at various dimensional aspects [26]. Chitin nanofiber includes potential uses such as in biomedical and biodegradable materials and waste treatments. Limitations of nanochitin production include high-energy demands, high catalyst costs and unstable yields [33]. Thus, further studies should focus on improving yields of chitin and assessing nanochitin uses in biomedical functions.

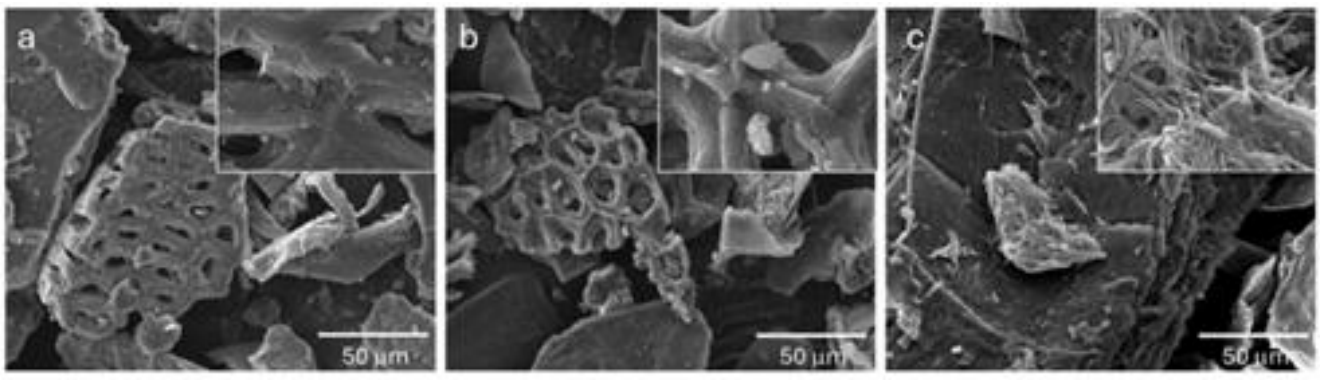

Figure 2. Scanning electron microscope images of the chitin (a), nanochitin prepared by dialysis (b) and ultrasonic (c) methods from the shrimp shells. Inset presents the magnifications of each figure.

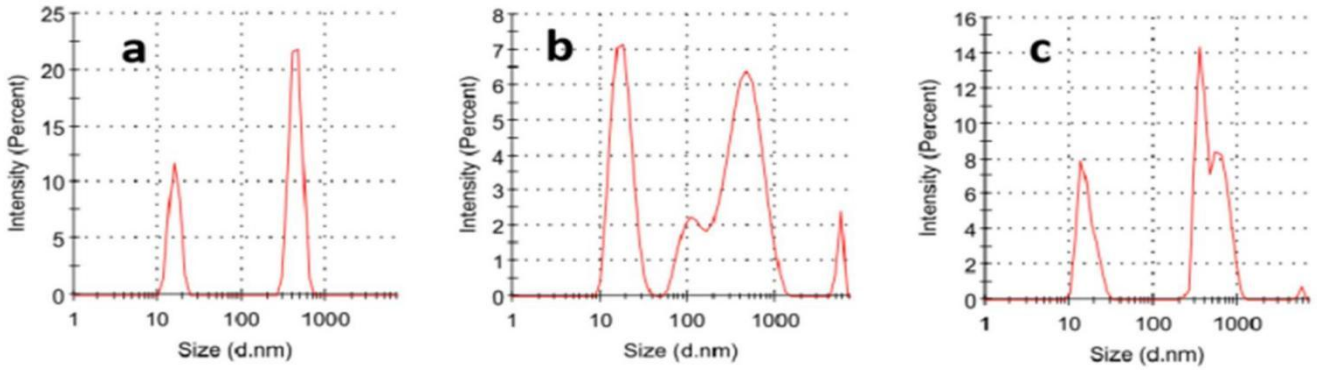

Figure 3. Particle size distributions of the chitin (a), nanochitin prepared by dialysis (b) and ultrasonic (c) methods from the shrimp shells

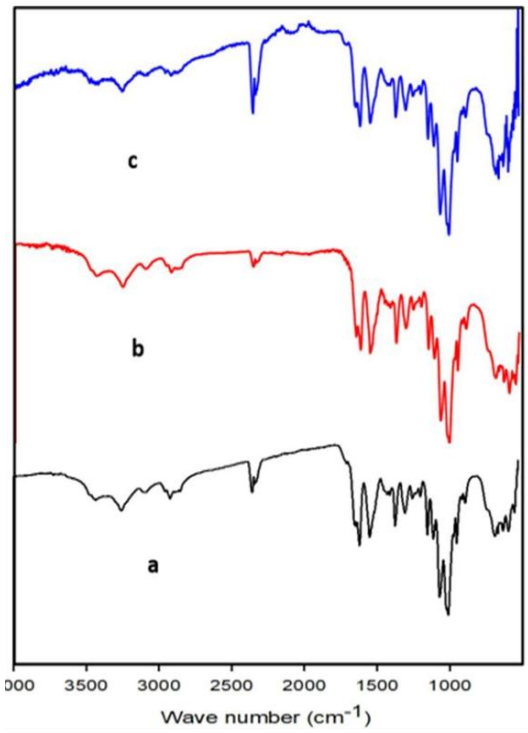

Figure 4. Fourier transforms infrared spectroscopy spectra of the chitin (a), nanochitin prepared by dialysis (b) and ultrasonic (c) methods from the shrimp shell

4. Conclusion

This study assessed effects of the deproteinization process of chitin from dried shrimp shells using enzymatic hydrolysis as an alternative method of fermentation and chemical processes. In this study, deproteinization process was optimized using RSM-Box Behnken design to maximize the chitin yield. Results of this study represented the optimum condition of chitin deproteinization process from dried shrimp shells using papain enzyme, with a chitin content ranging 41.52–49.06%. Interactions between the hydrolysis time and papain concentration included the most significant effect on the chitin content. Removing protein content in chitin extraction through protease enzymes was verified as a further efficient process, compared to the fermentation process. Use of chitin in industries needs specific characteristics to meet the industries' needs. The SEM analysis showed that acid hydrolysis affected surface morphology of the chitin nanostructure and ultrasonication treatment demonstrated nanofibrillar structure of the chitin. Nanochitin products included similar spectra with chitin samples, which included typical groups of the chitin structure. The present study indicates that alternative method of chitin production may decrease effects of chemical residues on the environment.

5. Acknowledgements

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6. Conflict of Interest

The authors report no conflicts of interest.

7. Authors Contributions

Conceptualization and Methodology, SP and DZA; Investigation: DAW, WK and DR; writing—original draft preparation: SP, IP; review and editing: CR, IP.

8. Using Artificial Intelligent chatbot

The authors declare no artificial intelligent chatbot use.

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فرآیند پروتئین زدایی کیتین حاصل از پوسته میگوی خشک شده (*Litopenaeus vannamei*) با استفاده از خصوصیات پاپین و نانوکیتین

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چکیده

سابقه و هدف: تیمارهای شیمیایی برای استخراج کیتین از ضایعات پوسته میگو بر محیط زیست تأثیر می‌گذارد. در پوسته میگو اساسا کیتین با نمک های معدنی، لیپیدها، پروتئین‌ها و رنگدانه ها پیوند دارد. استخراج کیتین از پوسته میگو شامل فرآیندهای جداسازی پروتئین است. فرآیند پروتئین‌زدایی کیتین حاصل از پوسته میگوی خشک شده (***Litopenaeus vannamei***) با آنزیم پاپائین بهینه شد و ویژگی‌های نانوکیتین به عنوان یک محصول به‌دست آمده از کیتین تعیین شد.

مواد و روش ها: اثرات زمان آبکافت[1]، دما و غلظت آنزیم با استفاده از روش RSM Box-Behnken برای به‌حداکثر رساندن راندمان کیتین بهینه شد. نانوکیتین با استفاده از روش‌های دیالیز و فراصوت تهیه و ویژگی‌های فیزیکی با استفاده از میکروسکوپ الکترونی روبشی[2]، آنالیز اندازه ذرات و طیف‌سنجی مادون قرمز تبدیل فوریه[3] تعیین شد.

یافته‌ها و نتیجه‌گیری: شرایط بهینه با استفاده از آبکافت آنزیمی در ۶ ساعت، دمای ۵۰ درجه سلسیوس و ۱/۲۵درصد پاپائین، محتوای پروتئین را از۳۳/۶۶ به ۲/۳۱ درصد کاهش داد و راندمان بالای کیتین (۴۶/۰۳ درصد) را موجب شد. پروتئین‌زدایی با روش آبکافت آنزیمی کارآمدتر از تخمیر بود. داده‌های میکروسکوپ الکترونی روبشی، آنالیز اندازه ذرات و طیف‌سنجی مادون قرمز تبدیل فوریه نشان داد که ویژگی‌های کیتین و محصولات نانوکیتین مشابه تیمارهای شیمیایی محصولات کیتین است.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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واژگان کلیدی
- کیتین
- پروتئین‌زدایی
- آنزیم
- *L. vannamei*
- RSM

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[1] Hydrolysis
[2] Scanning electron microscope; SEM
[3] Fourier transforms infrared spectroscopy:FTIR