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Production of nanochitin by combination of biochemical and physical methods: potential use for salt reduction in food products

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Abstract. Nanochitin is a product of the chitin size-reduction process. Compared to chitin, nanochitin possesses several new properties, thereby increasing its potential applications. The aim of this study was to obtain nanochitin with high solubility and the ability to interact with NaCl solutions to enhance saltiness perception. Chitin was first hydrolyzed using a chemical catalyst (acid) and a biological catalyst (enzyme), then further size-reduced through physical treatment using ultrasonic waves. In the experiment, chitin was incubated with the enzyme (at a 4 g/kg material ratio) at 50 °C for 30 minutes and then hydrolyzed with 3N HCl at an acid/chitin ratio of 25:1 (mL/g), at 80 °C for 180 minutes. The hydrolysate was subsequently subjected to ultrasound treatment at 77 % amplitude for 35 minutes with a solvent/substrate ratio of 78:1 mL/g. The final product was obtained by freeze-drying. The resulting nanochitin had an average size of 228 nm, a high solubility of 78.4 %, and a zeta potential of 29 mV. The saltiness-enhancing potential of the produced nanochitin was assessed by measuring the zeta potential of nanochitin–NaCl mixtures. Sensory analysis was conducted to compare NaCl solutions containing nanochitin with a control sample. At a concentration of 80 µg/mL, nanochitin enhanced the saltiness of a 0.65 % NaCl solution, making it comparable in perceived saltiness to a 0.69 % NaCl solution. This saltiness-enhancing effect was applied to the seasoning powder of instant noodles. Supplementing nanochitin at 1.16 % of the salt weight allowed a 5.8 % reduction in NaCl content in the seasoning packet while maintaining equivalent saltiness to the original formulation. These results suggest that nanochitin can be used as a saltiness-enhancing additive in food products, offering a promising approach for dietary salt reduction in response to current overconsumption trends.

Keywords: nanochitin, ultrasound, enzyme hydrolysis, saltiness.

Classification numbers: 1.4.2, 1.3.3.

1. INTRODUCTION

Chitin is a natural polysaccharide and a primary structural component of the exoskeletons of crustaceans and insects. It is composed of N- acetyl- D- glucosamine monomers linked by β-1,4-

glycosidic bonds. Chitin, together with small amounts of proteins and minerals, forms flat sheets known as chitin–protein planes. These planes twist and stack to form the Bouligand structure. This structural arrangement may account for chitin's insolubility in water and its limited applicability [1, 2].

Nanochitin is a nanometer-sized fragment derived from chitin, primarily produced through size-reduction techniques. Compared to chitin, nanochitin exhibits smaller particle size, higher dispersibility, and a positively charged molecular surface. This positive charge enables nanochitin to interact with negatively charged ions. In NaCl solutions, such interactions with Cl^- ions increase the mobility of Na^+ ions, thereby enhancing saltiness perception [3, 4].

Excessive salt intake has long been known to pose serious health risks. Therefore, developing saltiness-enhancing agents like nanochitin plays a key role in strategies for reducing salt consumption in the human diet.

Numerous methods for producing nanochitin have been explored, including physical approaches such as grinding [5, 6], high-pressure homogenisation [7], and ultrasonication [8, 9]; chemical methods such as mediated oxidation [10-12] Deep eutectic solvent (DES) [13] and combined physicochemical techniques [8]. Most of these studies have focused on controlling the size of nanochitin, with little attention paid to its properties or its application as a saltiness enhancer in food production. Among the published studies, methods combining chemical and physical agents have received considerable attention. However, the use of high acid concentrations, elevated temperatures (3N, 105 ^oC; 5N, 90 ^oC) [14, 15], and the relatively large size of the resulting nanochitin have greatly limited its practical applicability.

The use of acid was to cleave the β-1,4-glycosidic bonds in the polysaccharide chains. Although the number of protein bonds in chitin is relatively low, disrupting these linkages by an enzyme would facilitate more efficient cleavage of the polysaccharide chains [16, 17]. Therefore, reduce the harsh conditions of acid used for chitin hydrolysis.

This study aimed to produce nanochitin from Vietnamese white leg shrimp shells using a combination of biochemical, chemical and physical methods. The enzyme and acid method was applied during the initial stage. Following hydrolysis, the sample was further processed by a physical method involving ultrasonication to break the chitin into nano-sized fragments, resulting in nanochitin. The study comprehensively examined the processing parameters of both the hydrolysis and ultrasonication steps, as well as the critical characteristics of nanochitin, particularly its potential to enhance saltiness perception of NaCl solutions and seasoning powder. The findings would support the feasibility of using nanochitin as a saltiness enhancer in food products, thereby facilitating dietary salt reduction. The mild processing conditions of nanochitin production would help to reduce production costs and environmental impact. These advantages would establish a strong foundation for practical application, contributing to public health, promoting the value of shrimp shells by-products, and enhancing the economic potential of Vietnam’s seafood industry.

2. MATERIALS AND METHODS

2.1. Materials

Chitin used in this study was extracted from White Leg Shrimp shells (*Litopenaeus vannamei*), with protein content not exceeding 0.4 % and mineral content not exceeding 0.5 %. A commercial enzyme (SEB-Neutral PL (India)) is an enzyme with an activity of 0.5 U/g, and

table salt (provided by Hai Chau Food Processing Joint Stock Company) was used with a purity greater than 97 %.

2.2. Methods

2.2.1. Production of Nanochitin

Based on the previous studies [14, 18-20], the dissertation selected the experimental procedure for producing nanochitin as illustrated in Figure 1. In this procedure, raw chitin (size < 0.3 mm) was hydrolyzed using both a chemical catalyst (HCl) and a biological catalyst (enzyme). After hydrolysis, the suspension was washed to remove the acid solution by centrifugation at 11410.38 *x g* for 10 minutes. The hydrolyzed product was then further reduced in size using ultrasonication and collected as nanochitin by freeze-drying. At each stage, the influence of processing parameters was investigated as described below.

Hydrolysis step: Samples of 5 - 10 g chitin were mixed with the enzyme preparation at a 4 g/kg material ratio (as recommended by the provider) and incubated at 50 ℃. In this step, two incubation durations were tested: 30 minutes (U30) and 60 minutes (U60), with a non-incubated sample serving as the control (U0). After incubation, HCl solution was added to initiate acid hydrolysis. In the acid hydrolysis stage, the following parameters were investigated: temperature (50 °C - 100 °C), HCl concentration (1.0 - 4.0N), hydrolysis time (30 - 210 minutes), and HCl/chitin ratio (10:1 to 40:1 mL/g). After hydrolysis, the suspension was washed with distilled water to remove residual acid. Following enzymatic treatment and acid hydrolysis, the sample was assessed for sedimentation time and subsequently subjected to ultrasonication.

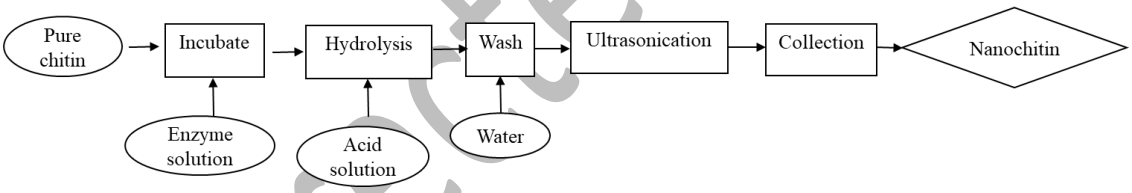

Figure 1. Diagram of the experimental process for the creation of nanochitin.

Ultrasonic step: Hydrolysed samples (1 - 6 g) were mixed with distilled water and treated using a Q700 sonicator (an ultrasonic intensity of 100 % corresponds to a power output of 700 W). Three factors were investigated during this step: ultrasonic amplitude (30 - 90 %), treatment time (15 - 45 minutes), and distilled water/substrate ratio mL/g (20:1 to 90:1). Following ultrasonication, the samples were recovered by freeze-drying with a ScanVac CoolSafe Basic & Pro system operated for 48 hours at -50 °C. The dried products were redispersed in distilled water for viscosity and solubility analysis. For each factor, the condition yielding the highest values of viscosity and solubility was selected for subsequent experiments. These single-factor results served as the foundation for experimental design and process optimisation.

2.2.2. Experimental programming and optimisation for ultrasonic step

A second-order experimental design based on the Box–Behnken model was applied for three factors: ultrasonic amplitude, sonication time, and distilled water/substrate.

The ultrasonic process was optimised using the "desirability function" algorithm proposed by Derringer and Suich [21, 22].

2.2.3. Study on Salt Reduction in Food Products

Zeta Potential Determination of Nanochitin: Nanochitin solutions were prepared at concentrations ranging from 40 to 140 µg/mL, and their zeta potentials were measured. The zeta potential values served as a basis for selecting the appropriate nanochitin concentration for further application.

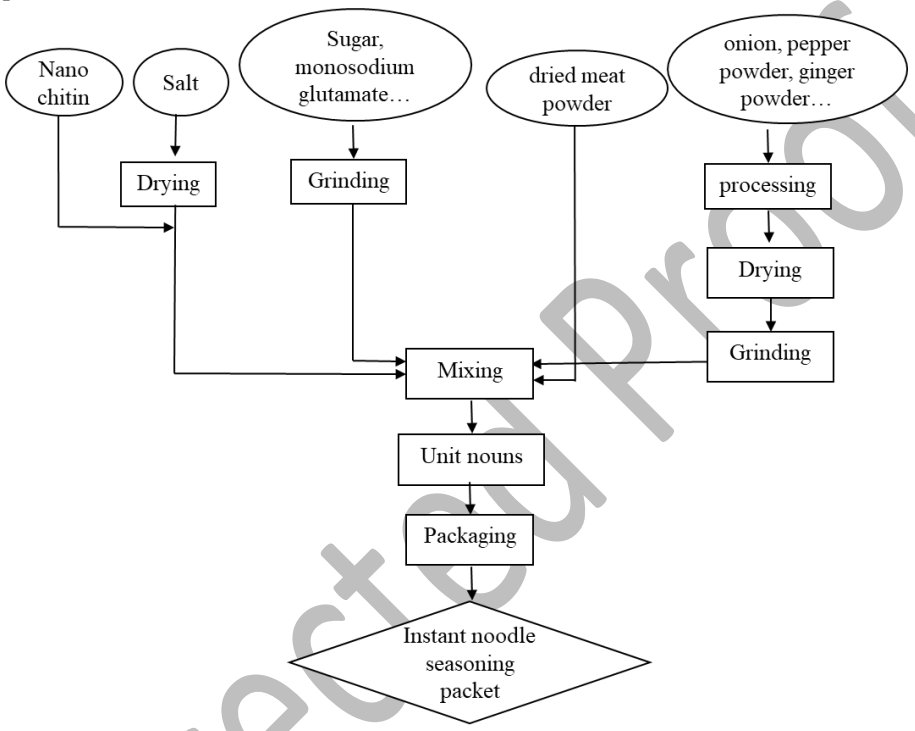

Figure 2. Flowchart of the Instant Noodle Seasoning Powder Production Process.

Zeta Potential Determination of Nanochitin–NaCl Mixtures: Nanochitin solutions with high zeta potential values were prepared and supplemented with NaCl solution. The zeta potentials of the resulting mixtures were then measured. These values were used to select nanochitin concentrations for investigating its threshold effect on saltiness enhancement.

Determination of the Effective Concentration for Saltiness Enhancement: A 0.65 % NaCl solution was prepared. This concentration reflects the typical salt level in soups and dishes consumed by individuals who do not follow a high-salt diet. Experimental trials at this concentration were conducted to reduce salt intake even for individuals accustomed to saltier foods. Experimental samples were supplemented with nanochitin at concentrations selected based on zeta potential results. The 0.65 % NaCl solution without nanochitin served as the control. Both experimental and control samples were evaluated for saltiness using a ranking sensory test. The effective nanochitin concentration that enhanced the saltiness of the 0.65 % NaCl solution was identified based on the ranking scores.

Determination of the Saltiness-Enhancing Magnitude of Nanochitin: NaCl solutions were prepared at concentrations of 0.61 %, 0.65 %, and 0.69 %. Nanochitin was added to the 0.61 % and 0.65 % NaCl solutions at concentrations previously determined as effective in enhancing saltiness. All samples, including controls without nanochitin, were evaluated using a ranking sensory test. The resulting rank orders were used to assess the magnitude of saltiness enhancement provided by nanochitin at each concentration.

Salt Reduction in Instant Noodle Seasoning Powder: The instant noodle seasoning powder was prepared following the process shown in Figure 2. The salt used was in the form of purified, delicate crystals. Sugar and monosodium glutamate (MSG) were ground into a fine powder. Black pepper was roasted, peeled, and finely ground. Shallots and ginger were cleaned, thinly sliced, dried, and ground into powder. The ingredients were formulated with the following ratios: salt 5 %, sugar 15 %, MSG 0.5 %, dried meat powder 4 %, and other spices (pepper, shallots, ginger, etc.) 0.6 % based on the weight of the noodle cake (100 g). All ingredients were mixed and dried, then weighed and packaged according to the weight of the instant noodle pack.

Experimental samples were prepared by partially substituting NaCl with nanochitin.

Seasoning powders were prepared for 100 g of noodle cakes. In the test formulation, salt content was reduced, and nanochitin was added according to the results of Experiment 3.2.4, described below:

Experimental sample E: 4.71 g NaCl + 0.058g nanochitin

Control sample 1 (E_{01}): 5 g NaCl

Control sample 2 (E_{02}): 4.71 g NaCl

All other ingredients in the three samples followed the same standard formulation. The seasoning powder was prepared according to the usage instructions and evaluated for quality through sensory analysis. Saltiness was assessed using an intensity scoring test, while color, flavor, and texture were evaluated using a hedonic scoring test. The panelists' scores were compiled and subjected to analysis of variance (ANOVA).

2.2.4. Analytical Methods

Determination of settling time: Distilled water was used to disperse the solid in a settling tube. The mixture was left undisturbed at room temperature until sedimentation was complete, and the settling time was recorded [23].

Determine relative Viscosity: The nanochitin sample was prepared at a concentration of 1.5%, and its viscosity was measured using an Ostwald viscometer. The relative viscosity (°E) was determined by calculating the ratio of the sample's flow time to that of distilled water at 20 °C [24].

Determination of solubility: Solubility was evaluated following the method described by Naiu. Approximately 0.1 - 0.2 g of the sample was dispersed in 50 mL of distilled water and centrifuged at 6000 rpm for 20 minutes. The supernatant was then collected and dried to a constant weight. The solubility (%) was determined as the ratio of the dried residue to the original sample weight [25].

Size determination: The solid fraction (hydrolyzed product), nanochitin, was dispersed in distilled water and subjected to particle size analysis using a laser scattering analyzer (LA-950V2). Particle size was determined based on the relationship between scattering intensity and angle [25].

Morphological characterization of Nanochitin: The morphology of nanochitin was analyzed using a field emission scanning electron microscope (FE-SEM, model JSM-IT80). An electron beam was directed onto the surface of the sample, and the signals generated from the interaction between the electrons and the sample surface were amplified and processed to obtain high-resolution images [26].

Zeta determined: Zeta potential was determined using dynamic light scattering (Zetasizer nano ZS90). A laser beam was directed through the nanochitin solution, where suspended particles scattered light in multiple directions. When an electric field was applied, the charged particles migrated, causing a frequency shift in the scattered light. The instrument analysed particle movement to calculate the zeta potential [27-29].

Sensory Evaluation: The panel consisted of 8 - 10 members who were expert analysts regularly involved in product evaluation and had been adequately trained and qualified to assess the sensory attributes of the samples [30, 31].

Ranking Test: A trained panel of expert analysts, routinely involved in product evaluation, was assembled to assess the sensory attributes of the samples. Panel members ranked the samples in ascending order of saltiness, 1 to 4 for four-sample tests or 1 to 3 for three-sample tests, where one indicated the least salty and the highest number indicated the saltiest sample. The ranking data were analyzed using the Friedman test to determine significant differences in perceived saltiness among the samples [31, 32].

Scoring Test: The panelists were invited to taste the samples and evaluate saltiness intensity using a descriptive scale: “no saltiness” (score 0), “very weak” (1), “slightly weak” (2), “moderately salty” (3), “slightly strong” (4), and “very strong” (5). For the hedonic evaluation of other sensory attributes (color, flavor, and texture), a 9-point scale was applied: “extremely dislike” (score 1), “strongly dislike” (2), “dislike” (3), “somewhat like” (4), “neither like nor dislike” (5), “moderately like” (6), “like” (7), “strongly like” (8), and “extremely like” (9). The data were analyzed using analysis of variance (ANOVA) to compare and interpret differences in the sensory properties of the tested samples [30, 31].

Statistical Analysis: The experiments were performed in triplicate. Data were analyzed using analysis of variance (ANOVA) and evaluated based on Fisher’s criterion and the Student's t-test at a 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Production of Nanochitin by Acid-Enzymatic Hydrolysis Combined with Ultrasonication

3.1.1. Effect of hydrolysing parameters on nanochitin’s properties

During the hydrolysis stage, the effects of temperature, HCl concentration, hydrolysis time, acid/chitin ratio, and enzymatic incubation time were investigated. When examining the effect of a single factor, the remaining parameters were held constant. For instance, in the investigation of hydrolysis temperature, the fixed conditions were a reaction time of 120 minutes, an HCl concentration of 3N, and an acid-to-chitin ratio of 20:1 (mL/g). The results indicated that a temperature of 80 °C led to the highest sedimentation time. Therefore, 80 °C was selected as the fixed temperature for subsequent experiments investigating the effect of hydrolysis time. The results are illustrated in Figure 3.

The findings indicated that all studied factors- temperature, acid concentration, hydrolysis time, acid/chitin ratio, and enzymatic incubation time-significantly affected the hydrolysis efficiency of chitin. Within the tested range, increasing these parameters led to longer settling times, reflecting improved hydrolysis. However, the settling time increased markedly to 80 °C, 3N, 180 minutes of hydrolysis, and a solvent/chitin ratio of 25:1. Further increases in these factors resulted in only marginal increases in settling time.

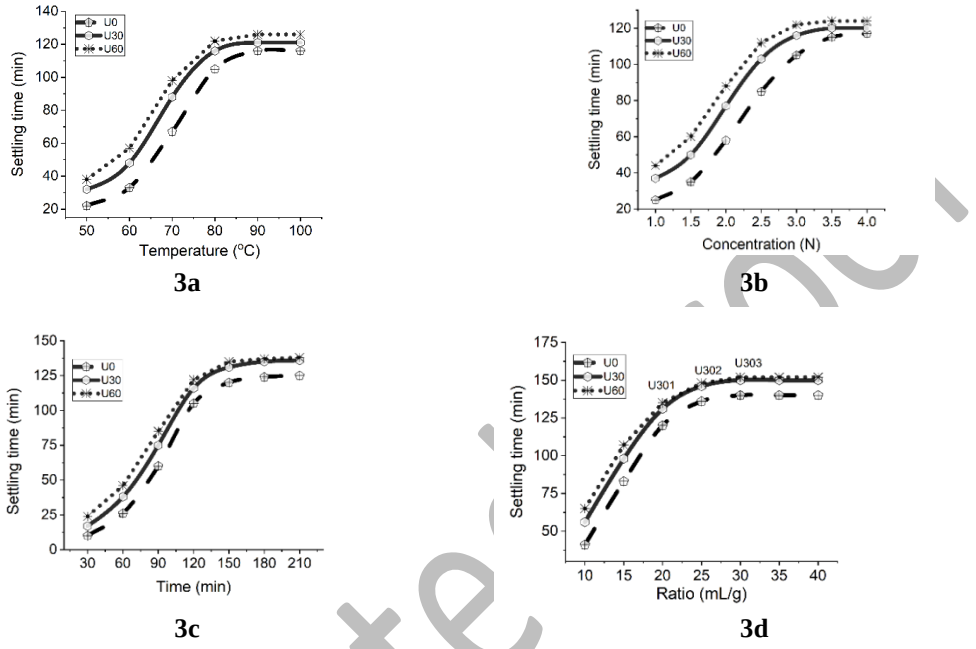

Figure 3. Effect of factors on settling time of hydrolyzed chitin.

3a: Temperature; 3b: Acid concentration; 3c: Hydrolyzing time; 3d: Acid/chitin ratio.

These results reveal a significant distinction from the previously reported acid-only hydrolysis method. Notably, the hydrolysis conditions involving enzyme–acid catalysis (80 °C, 3N HCl) were milder than those required for acid-only hydrolysis [14, 15]. Furthermore, the hydrolyzed product exhibited a longer settling time. This indicates that the enzyme effectively catalyzed the cleavage of protein-associated linkages within the chitin structure, thereby facilitating the subsequent breakdown of polysaccharide chains.

The results also indicated that enzymatic incubation for 30 minutes (U30) led to a higher settling time than samples without incubation. However, extending the incubation to 60 minutes showed minimal improvement, particularly at higher parameter levels. Based on these findings, three samples exhibiting high settling times (reflecting varying degrees of hydrolysis) from the U30 group were selected for further investigation of the ultrasonication process. These samples were designated U301, U302, and U303, with the following hydrolysis conditions and corresponding settling times: U301 (80 °C, 3N HCl, 180 min, acid/chitin ratio 20:1, settling time 131 min); U302 (80 °C, 3N HCl, 180 min, ratio 25:1, 146 min), and U303 (80 °C, 3N HCl, 180 min, ratio 30:1, 150 min).

3.1.2. Factors Influencing Ultrasonication Process

In the ultrasonication step, three influencing factors: ultrasonic amplitude, sonication time, and solvent/substrate were investigated. The effect of ultrasonic intensity was first investigated while other parameters were held constant, including an ultrasonication time of 30 minutes and a solvent-to-substrate ratio of 60:1 (mL/g). Based on the results, the ultrasonic intensity that yielded both high viscosity and solubility, while also considering processing efficiency, was selected as the fixed parameter for subsequent experiments. A similar approach was applied when evaluating the effects of other factors. The results are presented in the graph shown in Figure 4.

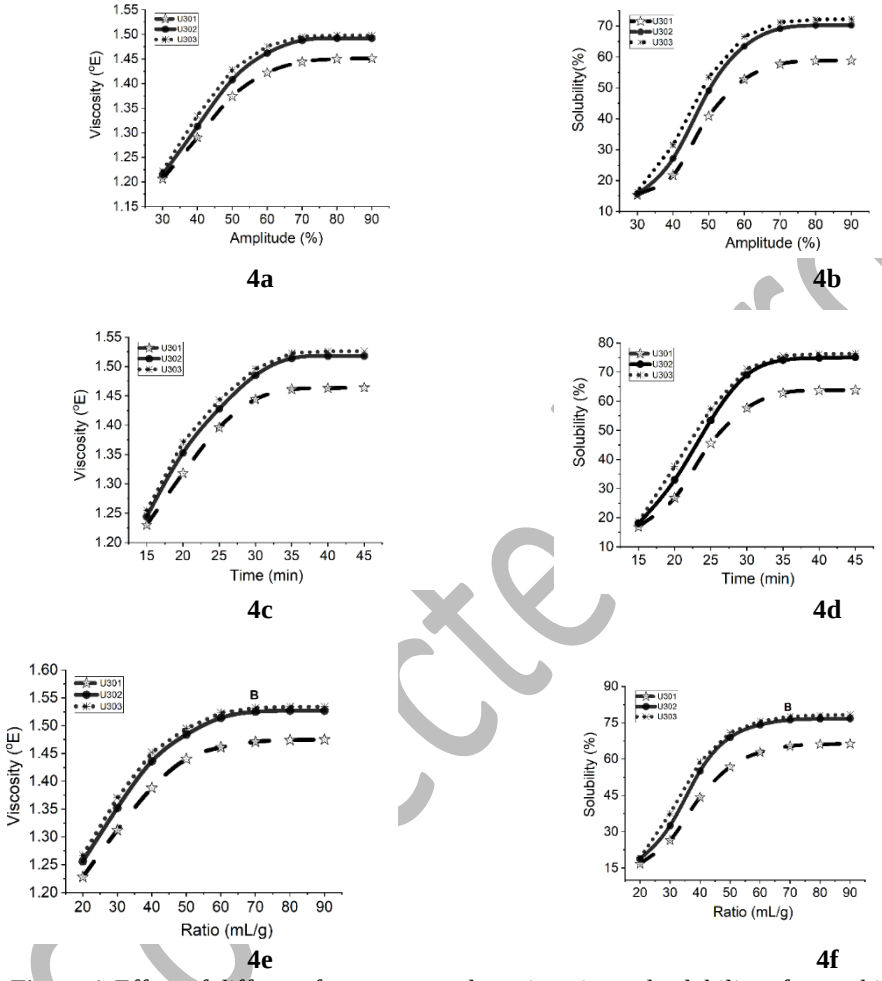

Figure 4. Effect of different factors on product viscosity and solubility of nanochitin product. 4(a-b): Ultrasonic amplitude. 4(c-d): Sonication time; 4(e-f): Distilled water/substrate.

The results indicated that, within the investigated range, increasing ultrasonic intensity, treatment time, and distilled water/substrate ratio led to an increase in the viscosity and solubility of the product. However, beyond a certain threshold, further increases in these parameters resulted in only marginal improvements. An increase in viscosity indicates that many chitin components have been broken down to the nanoscale, suggesting the potential formation of nanochitin. The improved solubility is also a significant advantage, enhancing the applicability of the nanochitin product. This trend was consistent across all three samples with different levels of hydrolysis. Among them, sample U302 exhibited higher viscosity and solubility than U301,

while the difference between U302 and U303 was not significant. Based on overall performance, U302 was selected for further study.

Considering the objective of obtaining nanochitin with small particle size and potential saltiness-enhancing properties, sample B from the U302 series, characterised by high viscosity and solubility (1.525°E; 76.16 %), was selected for further analysis. This sample was used to determine particle size, zeta potential, and other parameters that directly and clearly describe the characteristics of nanochitin.

The results showed that sample B had a small average particle size (248 nm) and high zeta potential (26.6 mV), making it suitable for application in salt reduction in food.

3.1.3. Optimization of Ultrasonication Process

The Box-Behnken design was employed with three factors, each tested at three levels determined based on the results of single-factor experiments (as shown in Table 1).

Table 1. Coding and experimental values of the ultrasonic stage experimental elements.

Variable	**Code**	**Unit**	**Level**		
			-1	**0**	**1**
Sonication time	A	min	30	35	40
Ultrasonic amplitude	B	%	60	70	80
Distilled water/substrate	C	mL/g	60	70	80

The experimental design consisted of 17 runs, including 5 replicates at the centre point, with viscosity (Y_1) and solubility (Y_2) as the response variables. Based on the experimental results, second-order regression models were developed for both responses. Regression analysis was conducted to evaluate the significance of the coefficients and the model's adequacy, confirming that the model accurately represented the experimental data.

$$Y_1 = 1.53 + 0.0156A + 0.0209B + 0.005C + 0.003AB - 0.0003AC + 0.0003BC - 0.0145A^2 - 0.0149B^2 - 0.0037C^2 \quad (1)$$

$$Y_2 = 76.21 + 2.85A + 3.95B + 1.26C + 0.1939AB - 0.0542AC + 0.0069BC - 2.31A^2 - 2.49B^2 - 0.6157C^2 \quad (2)$$

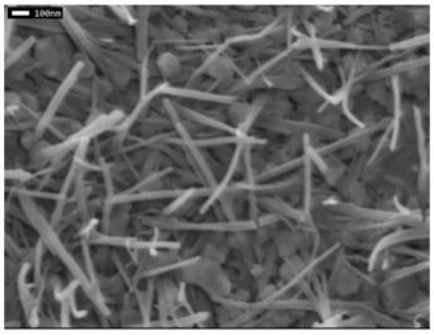

Figure 5. FE-SEM image of nanochitin. Scale bar 100 nm.

Design-Expert 11.1 software was used with the Derringer Suich desirability function to optimize the ultrasonication process. The optimal conditions, 77 % ultrasonic amplitude, 35 min sonication, and a distilled water/substrate ratio of 78 mL/g were validated experimentally. Product characteristics were then determined, including morphology (FE-SEM, Figure 5), particle size, and zeta potential. The resulting nanochitin exhibited a rod-like shape, an average particle size of 228 nm, a solubility of 78.40 %, and a zeta potential of 29 mV.

Our findings align with those reported by Naiu et al (2020), who observed nanochitin with average particle sizes ranging from 185.4 to 319.3 nm and solubility between 68.92 % and 71.72 % [25]. Beyond this agreement, our study offers several advantages.

Regarding product quality, the nanochitin obtained in our study exhibited higher solubility (78.4 %), a smaller average particle size (228 nm), and more comprehensive characterisation, including zeta potential measurement and FE-SEM imaging. Additionally, our process used milder conditions than previous acid-catalysed hydrolysis methods, with lower optimal parameters identified: acid concentration (3N HCl), hydrolysis temperature (80 °C) compared to those previously reported.

3.2. Salt reduction using nanochitin in Food Products

3.2.1. Zeta potential of the nanochitin solution

The zeta potential of nanochitin solutions was determined at various concentrations. The results are presented in Figure 6.

The results showed that the zeta potential of nanochitin solutions increased sharply with concentrations from 40 to 60 µg/mL. Between 60 and 140 µg/mL, the zeta potential increased more slowly and reached high values. Therefore, concentrations ranging from 60 to 140 µg/mL were selected for further investigation of zeta potential changes upon the addition of nanochitin to NaCl solutions.

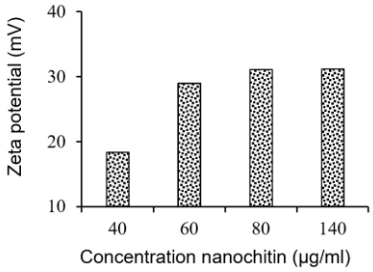

Figure 6. Zeta potential of nanochitin solution at different concentrations

3.2.2. Zeta potential of the nanochitin - NaCl solution mixture

NaCl solutions at varying concentrations were added to the pure nanochitin solution, followed by zeta potential measurement. The results are presented in Figure 7.

It was observed that the addition of NaCl to the nanochitin solution resulted in a decrease in the zeta potential of the mixture compared to the pure nanochitin solution. This indicates that Cl^- ions interacted with nanochitin molecules. The level of ionic interaction was higher at a NaCl concentration of 0.06 % compared to 0.04 %. These findings suggest that nanochitin may

enhance the mobility of Na^+ ions, thereby increasing saltiness perception. This result aligns with findings reported by other researchers, such as Jiang et al [3] and Tsai et al [27]. However, compared to previously published results, our study employed higher NaCl concentrations (while other research groups typically used 0.01 % and 0.05 % NaCl). The interaction between nanochitin and NaCl was stronger in our study, as indicated by a more significant reduction in zeta potential, yet the zeta potential of the resulting mixtures remained relatively high. This suggests that our nanochitin product has a high capacity for interaction with NaCl.

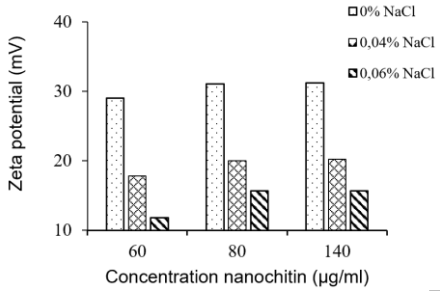

Figure 7. Zeta potential of nanochitin - NaCl mixtures at different salt concentrations.

3.2.3. Determination of the Effective Concentration for Saltiness Enhancement

The test samples, consisting of NaCl solutions supplemented with nanochitin, along with control samples, were prepared. Saltiness was evaluated using sensory analysis through the ranking test method. The converted scores were subjected to analysis of variance (ANOVA), and the results are presented in Table 2.

Table 2. Effect on saltiness perception of nanochitin.

Samples	NaCl 0.65 %	NaCl 0.65 % + nanochitin 60 µg/ml	NaCl 0.65 % + nanochitin 80 µg/ml	NaCl 0.65 % + nanochitin 100 µg/ml
Average	0.76^a	0.57^a	-0.76^b	-0.57^b

Note: The letters assigned to the exponents of the mean values in Table 2 indicate differences between those values.

The results indicated that nanochitin at a concentration of 80 µg/mL enhanced the saltiness of the 0.65% NaCl solution. Increasing the nanochitin concentration to 100 µg/mL produced a similar saltiness-enhancing effect as observed at 80 µg/mL.

3.2.4. Determination of the Saltiness-Enhancing Magnitude of Nanochitin

Table 3. Saltiness levels of experimental samples with 0.65% NaCl concentration

Sample	M_{01} (NaCl 0.65 %)	M_{02} (NaCl 0.69 %)	M_1 (NaCl 0.65 % + Nanochitin 80 µg/ml)	M_2 (NaCl 0.65 % + Nanochitin 110µg/ml)
Column sum	8^b	23^a	24^a	25^a

Note: The letters assigned to the exponents of the mean values in Table 3 indicate differences between those values.

From the results in section 3.2.3, considering the potential for increasing saltiness with efficiency, we selected a sample (NaCl 0.65 % + nanochitin 80 μg/ml) named N to determine the level of saltiness increase.

We also arranged sample M_2 to consider whether there is a better saltiness increase ability when using more than 100 μg/ml of nanochitin. Sample M_3 was designed according to the ratio of sample N, with the NaCl concentration reduced to 0.61 %. The saltiness perception of NaCl solutions at concentrations of 0.61 % and 0.65 % with nanochitin added at different concentrations was compared with each other and with the control sample (pure NaCl solution). The scores were analyzed according to the Friedman standard for the results, as presented in Tables 3, 4.

The results presented in Table 3 indicate that nanochitin at a concentration of 80 μg/mL enhanced the saltiness of a 0.65 % NaCl solution to a level comparable to that of a 0.69 % NaCl solution. Increasing the nanochitin concentration to 110 μg/mL did not further enhance saltiness perception.

Table 4. Saltiness levels of experimental samples with 0.61% NaCl concentration

Sample	M_{03} (NaCl 0.61 %)	M_{01} (NaCl 0.65 %)	M_3 (NaCl 0.61 % + Nanochitin 75 μg/ml)
Column sum	9^d	18^e	21^e

Note: The letters assigned to the exponents of the mean values in Table 4 indicate differences between those values.

The results presented in Table 4 indicate that nanochitin at a concentration of 75 μg/mL, nanochitin elevated the saltiness of a 0.61 % NaCl solution to a level equivalent to that of the 0.65 % NaCl solution. These findings serve as a basis for calculating the NaCl reduction rate in the salt-reduction application experiments for food products.

3.2.5. Salt Reduction by nanochitin supplement in Instant Noodle Seasoning Powder

The seasoning powder was prepared according to the usage instructions and evaluated for quality through sensory analysis. Saltiness was assessed using an intensity scoring test, while color, flavor, and texture were evaluated using a hedonic scoring test. The panelists' scores were compiled and subjected to analysis of variance (ANOVA), with the results presented in Table 5.

Table 5. Saltiness Scores of the Seasoning Powder

Samples	**E_{01}**	**E_{02}**	**E**
Saltiness	3.0^a	2.0^b	2.9^a
Color	5.6^c	5.5^c	5.8^c
Flavor	7.0^d	6.8^d	7.1^d
Texture	6.4^g	6.3^g	6.5^g

Note: The letters assigned to the exponents of the mean values in Table 5 indicate differences between those values.

The results indicate that the addition of 0.058 g nanochitin (equivalent to 1.16 % of the NaCl amount) to the formulation of an instant noodle seasoning sachet (sample E) enabled a reduction of 0.29 g NaCl (5.8 % of the total NaCl content) without compromising the saltiness perception compared to the control (sample E_{01}). Other sensory attributes, including color, overall flavor, and texture, remained unchanged relative to the control.

These findings are consistent with previous studies by Jiang *et al*.[3], Hsueh *et al.* [33] and Tsai *et al.* [27], reporting the saltiness-enhancing effect of nano chitin. For example, at a concentration of 80 µg/mL, nanochitin was found to enhance the saltiness of a 0.3 % NaCl solution. In our study, nanochitin improved the saltiness of NaCl solutions at concentrations of 0.61 % and 0.65 %, which are typical salt levels in food. Furthermore, the seasoning powder trial confirmed the feasibility of applying nanochitin as a salt-reducing agent in food products

4. CONCLUSION

This study demonstrated that purified chitin from white leg shrimp shells could be efficiently converted into nanochitin using combined biochemical (enzyme-acid hydrolysis) and physical (ultrasonication) methods under milder processing conditions. Chitin was mixed with the enzyme SEB-Neutral PL at a concentration of 4g/kg material ratio and incubated at 50 °C for 30 minutes, followed by acid hydrolysis using 3N HCl at 80 °C with an acid/chitin ratio of 25:1 for 180 minutes. The hydrolysate was then washed and subjected to ultrasonication at 77% amplitude for 35 minutes, using a solvent-to-substrate ratio of 78 mL/g, and nanochitin with an average particle size of 228 nm, the solubility of 78.4 %, and a zeta potential of 29 mV was obtained. Nanochitin showed saltiness-enhancing potential at a concentration of 80 µg/mL in 0.65 % NaCl solution. When incorporated into instant noodle seasoning powder, the addition of 0.058 g nanochitin (equivalent to 1.16 % of the NaCl content) enabled a 0.29 g (5.8%) reduction in NaCl without altering other sensory characteristics of food products. These findings highlight the promising applicability of nanochitin as a potential solution for reducing dietary salt intake in modern human diets.

Declaration of competing interest: The authors declare that they have no conflict of interest.

***CrediT authorship contribution statement*:** Nguyen Thi Cha: Methodology, Investigation, manuscript writing. Ho Phu Ha: methodology, supervision, manuscript editing. Tien-Thanh Nguyen: methodology, manuscript editing, supervision

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