

The Preparation, Anti-inflammatory, and Antioxidant Properties of Glucosamine Hydrochloride from the Waste of *Litopenaeus vannamei* Processing Plant

ARTICLEINFO

Article Type Original Research

Authors

Alika Jafari, M.Sc.¹ Mehdi Tabarsa, Ph.D.^{2*} Hossein Naderimanesh, Ph.D.³ Hassan Ahmadi Gavlighi, Ph.D.⁴ SangGuan You, Ph.D.⁵

How to cite this article

Jafari A., Tabarsa M., Naderimanesh H., Ahmadi Gavlighi H., You S. The Preparation, Anti-inflammatory, and Anti-oxidant Properties of Glucosamine Hydrochloride from the Waste of *Litopenaeus vannamei* Processing Plant. ECOPERSIA 2024;12(2): ??-??.

DOI:

10.22034/ECOPERSIA.12.2.6

1 M.Sc., Department of Seafood Processing, Faculty of Marine Sciences, Tarbiat Modares University, Noor, Iran. 2 Associate Professor, Department of Seafood Processing, Faculty of Marine Sciences, Tarbiat Modares University, Noor, Iran. 3 Professor, Department of Biophysics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran. 4 Associate Professor, Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. 5 Professor, Department of Marine Food Science and Technology, Gangneung-Wonju National University, Gangneung, Gangwon 25457, Republic of Korea.

* Correspondence

Address: Associate Professor, Department of Seafood Processing, Faculty of Marine Sciences, Tarbiat Modares University, Noor, Iran. Tel.: +981144558151 Fax: +981144550906 P.O. Box 46414-356 E-mail: m.tabarsa@modares.ac.ir

Article History

Received:February 27, 2024 Accepted:April 25, 2024 Published: May 20, 2024

ABSTRACT

Aims: The purpose of the present study was to investigate the antioxidant and antiinflammatory properties of glucosamine hydrochloride (G-HCl), glucosamine sulfate sodium chloride (GS-Na), and glucosamine sulfate potassium chloride (GS-K) isolated from the shells of *Litopenaeus vannamei* obtained from a shrimp processing plant.

Materials & Methods: G-HCl was synthesized via hydrolysis of chitin with concentrated HCl, followed by several sequential decolorization, crystallization, and washing steps. Using G-HCl as the precursor, adding sodium and potassium sulfates at 40 °C for one hour produced GS-Na and GS-K.

Findings: The yield of chitin was found to be 19.9%, and those of glucosamine products ranged between 75.5%-82.5%. The HPAEC-PAD indicated the presence of glucosamine monomers, as compared with commercial standard, with different elution times to that of glucose. The appearance of O-H, N-H, and C-O-C characteristic signals in the FT-IR spectra further supported successful glucosamine isolation. SEM images and EDX glucosamine spectra confirmed the samples' elemental compositions and polyhedral crystalline structures. DSC and TGA thermograms indicated endothermic and exothermic peaks specific to glucosamine products. Relatively low DPPH and ABTS radical scavenging activities and ferric-reducing power were obtained for all glucosamine products. All the glucosamine derivatives indicated an anti-inflammatory effect on LPS-simulated RAW264.7 cells.

Conclusion: Glucosamine products showed no cytotoxicity and down-regulated NO release in RAW264.7 murine macrophage cells induced by LPS. Overall, the present results indicated the successful production of glucosamine with antioxidant and anti-inflammatory properties from the waste of the L. vannamei processing plant.

Keywords: Litopenaeus vannamei; Shell; Chitin; Glucosamine; Antioxidant; Anti-inflammatory.

CITATION LINKS

[1] Olsen R.L., Toppe J., Karun ... [2] López-Pedrouso M., Lorenzo ... [3] Khodabandeh S., Hassan Saje ... [4] Knorr D. Recovery and utili ... [5] Nirmal N.P., Santivarangkna ... [6] IMARC. Shrimp market: Globa ... [7] FAO. 2023. Fishery and Aqua ... [8] Suryawanshi N., Eswari J.S. ... [9] Coward-Kelly G., Agbogbo F. ... [10] Sachindra N.M., Bhaskar N. ... [11] Díaz-Rojas E.I., Argüelles- ... [12] Jayakumar R., Prabaharan M. ... [13] Soni T., Zhuang M., Kumar M ... [14] Anderson J.W., Nicolosi R.J ... [15] Houpt J.B., McMillan R., We ... [16] Luo J., Hu Y., Wu Y., Fan W ... [17] Vangsness C.T., Spiker W., ... [18] Bissett D.L. Glucosamine: a ... [19] Piperno M., Reboul P., Le G ... [20] Shikhman A.R., Kuhn K., Ala ... [21] Li Y., Chen L., Liu Y., Zha ... [22] Cheng D.W., Jiang Y., Shale ... [23] Chen Y.J., Huang Y.S., Chen ... [24] Mendis E., Kim M.M., Rajapa ... [25] Valvason C., Musacchio E., ... [26] Stadler J.O., Stefanovic-Ra ... [27] Yan Y., Wanshun L., Baoqin ... [28] Xing R., Liu S., Guo Z., Yu ... [29] Xing R., Liu S., Wang L., C ... [30] Green L.C., Wagner D.A., Gl ... [31] Brand-Williams W., Cuvelier ... [32] Re R., Pellegrini N., Prote ... [33] Oyaizu M. Studies on produc ... [34] Zhang Z., Khan N.M., Nunez ... [35] Bertuzzi D.L., Becher T.B., ... [36] Zaeni A., Safitri E., Fuada ... [37] Yu S., Zang H., Chen S., Ji ... [38] Kim H.S., Kim S.K., Jeong G ... [39] Wahab A., Khan G.M., Sharif ... [40] Al-Hamidi H., Edwards A.A., ... [41] Asare-Addo K., Šupuk E. Al- ... [42] El-Houssiny A., Ward A., Mo ... [43] Scherer R., Godoy H.T. Anti ... [44] Kamala K., Sivaperumal P., ... [45] Benavente M., Arias S., Mor ... [46] Brugnerotto J., Lizardi J., ... [47] Chen H., Zhang M., Qu Z., X... [48] Díaz-Rojas E.I., Argüelles- ... [49] Drovanti A., Bignamini A., ... [50] Gottardi, D., Hong, P. K., ... [51] Jamialahmadi K., Arasteh O. ... [52] Kraisangsri J., Nalinanon S ... [53] Li X., Li X., Zhou A. Evalu ... [54] Trung T.S., Phuong P.T. Bio ... [55] Mohan K., Muralisankar T., ... [56] Synowiecki J., Al-Khateeb N ... [57] Mojarrad J.S., Nemati M., V ... [58] Wang X., Liu B., Li X., Sun ... [59] Miao Q., Li Q., Tan W., Mi ... [60] Rao R.S., Muralikrishna G. ... [61] Meng L., Sun S., Li R., She ... [62] Liew F.Y., Xu D., Brint E.K ... [63] Azuma K., Osaki T., Kurozum ... [64] Chiu H.W., Li L.H., Hsieh C ... [65] Hwang S.Y., Shin J.H., Hwan ... [66] Wu Y.L., Lin A.H., Chen C.H ...

Introduction

Some aquaculture waste products or bycontain many biomolecules, including polysaccharides, peptides, proteins, enzymes, vitamins, and minerals with a wide variety of antioxidant, anti-aging, antiinflammatory, antimicrobial, and antitumor activity [1,2,3]. In recent years, crustacean aquaculture has become the world's largest seafood production sector. Shrimp is a crucial aquacultural product processed for meat and waste products [5]. The consumption demand for shrimp and shrimp products is increasing yearly [6]. Global shrimp production will increase to 7,28 million tons by 2025 [7]. *Litopenaeus vannamei* is cultured commercially as one of the important species in Iran. The average production of *Litopenaeus* vannamei was estimated at 57000 tons in 2021^[8]. Shrimp processing side streams, including shells, heads, and viscera, have attracted mounting attention because of their indispensable by-products, including chitin, chitosan, glucosamine, and astaxanthin, with diverse applications in food, cosmetic, and pharmaceutical industries [9]. This everincreasing interest in valorizing shrimp wastes to produce feed and food products could benefit environmental sustainability and a circular economy. Depending on the source species, chitin, protein, calcium phosphate, and calcium carbonate are the most critical components of shrimp shells [10,11,12]. Chitin, β -(1-4)-poly-N-acetyl-D-glucosamine, has received significant interest as a natural amino polysaccharide chiefly due to its non-toxic, biocompatibility and biodegradability nature [12,13]. It is one of the most abundant polysaccharides in nature, widely used as a raw material to produce chitosan and glucosamine [12]. 2-amino-2-deoxy-D-glucose Glucosamine, ubiquitously found in the matrix of shells of crustaceans and could be produced via various approaches, including chemical (e.g.,

acid hydrolysis), microbial fermentation, enzymatic and combined methodologies [14]. Different forms of glucosamines, such glucosamine sulfate, glucosamine as hydrochloride, and N-acetyl glucosamine, are commercially used as dietary supplements in treating osteoarthritis and knee and back pains [15,16,17]. Nutritional glucosamine supplements have been extensively used to treat osteoarthritis [17]. The high level of nitric oxide (NO) and phospholipase A2 as inflammatory molecules have been observed in the cartilage of osteoarthritis patients [19]. Glucosamine is believed to be suppressing pro-inflammatory mediators such as NO, IL-6, and TNF- α in chondrocytes [20,21,22]. Besides the above advantages, plenty of studies have exhibited the cellular antioxidant effect of glucosamine as a potential therapeutic for OA [23,24,25]. Glucosamine exhibited prominent protective properties on the chondrocytes and prevention of oxidative stress [26]. Oxidative stress occurs through increasing production of NO and reactive oxygen species (ROS) derived from superoxide [26,27]. Yan et al. [28] have reported glucosamine's antioxidant capacity and immunostimulatory effects in both in vitro and animal models. Few studies observed that glucosamine hydrochloride effectively inhibited reducing power and superoxide/hydroxyl radicals scavenging activity dose-dependently [29,30]. Thus, to utilize the wastes of shrimp processing plants and reduce their environmental impact, this study aimed to isolate the commercial grade of glucosamine using acid hydrolysis, evaluate the antioxidant activity then immunostimulating and properties glucosamine hydrochloride and glucosamine sulfate to produce high added-value products.

Material & Methods Materials

Pacific white shrimp (*Litopenaeus vannamei*) wastes were purchased from Shil Abzi

Company, Golestan, Iran, and transferred to the laboratory in a sealed ice box. Samples were immersed in 1% sodium hydroxide for two hours at room temperature as pretreatment to remove flesh from shrimp shells. After completing the pretreatment process, shells were washed with tap water several times at the same temperature to separate sodium hydroxide liquid and eliminate the impurities. Cleaned shells were dried at 60 °C in an oven and milled using a grinder. Afterward, the powder was sieved using a Sieve Shaker (74-µm mesh size). Finally, dried shrimp shell powder was packed in plastic bags and stored at a temperature approaching -20 °C before extraction of glucosamine hydrochloride.

Extraction of chitin

Firstly, the dry powder (1 g) was chemically demineralized. The powder was mixed with 1M HCl by stirring magnetically for two h and then subjected to centrifugation (6080 g for 5 min at four °C) to sediment demineralized shells. The supernatant was removed, and the residue was washed with distilled water for one hour under constant stirring. At the end of the treatment, the demineralization liquid was centrifuged at 6080 g for 5 min at four ^oC. Secondly, the demineralized residue was mixed with 4 % NaOH under continuous stirring at 70 °C for six hours to eliminate protein. The solution was centrifugated (6080 g for 5 min at 4 $^{\circ}$ C) and washed by agitation using distilled water for 12 h. The centrifugation was performed in the same manner as previously described. The white resulting residue was dried at 60 °C for three hours.

Preparation of glucosamine hydrochloride

Glucosamine hydrochloride was synthesized via hydrolysis of chitin with concentrated HCl by stirring under reflux conditions at 95 °C for 75 min. The liquid-to-solid ratio was 10:1. The solution was kept in a freezer at -80

 $^{\circ}$ C for 24 h. The frozen sample was thawed at room temperature for one hour. After centrifugation at 9000 rpm for 5 minutes at four $^{\circ}$ C, the wet powder was washed with 5 mL of 95% ethanol for 20 min and centrifuged as described. The white precipitate was dried at 50 $^{\circ}$ C for 3 hours, and the resulting glucosamine hydrochloride was passed through a 30 mesh sieve (535 μ m).

Preparation of glucosamine sulfate sodium chloride and glucosamine sulfate potassium chloride

G-HCl (3 g) was slowly dissolved in water (8 mL). After 5 min, 0.98 g of sodium sulfate or 1.21 g of potassium sulfate was added to the solution of G-HCl. The solution was stirred at 40 °C for 1 hour. The liquid was kept in a freezer at -80 °C for 24 hours. Samples were centrifuged for 5 min at 6080 g and four °C after thawing. Precipitates were washed with 10 mL of 95% ethanol for 20 minutes to decolorize glucosamine sulfate. The white solid crystals were centrifuged under the same conditions described and dried at 50 °C for three hours to produce GS-Na and GS-K.

HPAEC-PAD analysis

G-HCl, GS-Na, and GS-K were determined using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) analysis. The HPAEC-PAD system consisted of a GS50 gradient pump, an AS50 chromatography organizer, an ED50 electrochemical detector, and a $4 \times 250 \text{ mm}^2$ CarboPac PA1 analytical column (Dionex Corp., Sunnyvale, CA). Samples (20 mg) were dissolved in deionized water (5 mL) and filtered through a 0.22 μ m syringe filter.

Fourier transform infrared spectroscopy (FT-IR) analysis

The functional groups of G-HCl, GS-Na, and G-S-K were determined via Fourier transform infrared spectroscopy using a Tensor 27 spectrometer (Bruker Instruments, Billerica, USA). Samples were prepared in

0.5-1 mm of KBr layer. The spectra were carried out in the region from 500 to 4000 cm⁻¹ at a high spectral resolution (2 cm⁻¹) in the absorbance mode.

SEM-EDX analysis

G-HCl, GS-Na, and GS-K morphology were examined by scanning electron microscopy (SEM) (JSM-IT500HR, JEOL Co., Japan) operating at an accelerating voltage of 20 kV. Before imaging, a thin layer of gold was coated on the samples. Energy-dispersive X-ray analysis (EDX) (JSM-IT500HR, JEOL Co., Japan) was performed along with SEM to analyze the elemental contents of G-HCl and G.S.

Thermal analysis of glucosamine

Thermal properties of G-HCl, GS-Na, and GS-K were studied by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). TGA experiment was performed with 10 mg of samples. Samples were heated at 10 °C.min⁻¹ from 30 to 900 °C using TGA 500 (T.A. Instruments, New Castle, DE, USA) under a constant nitrogen flow of 25 mL.min⁻¹. The DSC analysis was conducted on a TA DSC 25 (T.A. Instruments, New Castle, USA) enriched nitrogen gas flow. 10 mg of samples were heated from 20 °C to 400 °C at ten °C.min⁻¹.

Cytotoxicity and anti-inflammatory of glucosamine

The RAW 264.7 cells were cultivated onto a T25 flask containing an RPMI-1640 medium culture plus 10% (v/v) FBS. The cells (100 μL volume of 1 × 10⁴ cells.well⁻¹) were transferred into a 96-well microplate. Various concentrations (50, 100, and 150 $\mu g.mL^{-1}$) of samples and LPS (1 $\mu g.mL^{-1}$) were chosen to mix with cell suspensions. Afterward, 100 μL of samples were added to the cells, maintained at 37 $^{\circ}C$ for 18 h. After incubation, the culture medium was collected and mixed with Griess reagent $^{[31]}$. The murine macrophage-derived nitric oxide (NO) was determined by comparing with an obtained standard curve of NaNO $_2$ (1-

200 μ mol). The absorbance of the solution was read at 450 nm. The absorbance values (A) were translated into a macrophage proliferation ratio, Eq. (1). (%) = At/Ac × 100, where At and Ac are the absorbance values of the samples and control, respectively.

DPPH radical scavenging activity

The DPPH radical scavenging activity of G-HCl, GS-NA, GS-K, and G-S was evaluated according to the slightly modified procedure of Brand Williams et al. $^{[32]}$. 0.1 mM DPPH was dissolved in 100 μ L of 95 % ethanol. 100 μ L of samples and ascorbic acid as a standard (25 to 299 μ g.mL $^{-1}$) were added to 100 μ L of the ethanolic solution of DPPH into a 96-well microplate and incubated by wrapping the microplate in the dark for 30 minutes. The optical density was recorded with a microplate reader at 517 nm. DPPH radical scavenging activity was obtained using the following equation:

DPPH scavenging effect (%) = $[(A_c-A_s)/A_c] \times 100$ Eq. (2)

Where A_s was the absorbance of samples and A_c was the absorbance of the control.

ABTS radical scavenging activity

ABTS radical scavenging activity was determined based on the protocol previously described by Re et al. [33,34]. ABTS solution was prepared by dissolving ABTS and potassium persulfate in water to 7 mM and 2.45 mM concentrations, respectively. The solution was maintained in the dark for 16 hours and diluted with ethanol to obtain an optical density of 0.7 at 734 nm. Afterward, 150 µL of ABTS was added to 50 μL of each sample and ascorbic acid at various concentrations (25 to 200 µg.mL⁻¹) in a 96-well microplate. Samples were held in the dark for 20 min, and optical density was recorded at 734 nm using a microplate reader. The inhibition of ABTS radical scavenging was obtained according to the following equation:

ABTS scavenging activity (%) = $[(A_c-A_s)/A_c]$ × 100 Eq. (3)

where A_s and A_c are the optical densities of the samples and control (150 μ L of ABTS with 50 μ L of ethanol), respectively.

Reducing power activity

The reducing power of G-HCl, GS-Na, GS-K, and G-S were determined by Oyaziu's method [35]. Initially, 500 μ L of samples at concentrations of 25 to 200 μ g.mL⁻¹ were transferred in a vial containing 500 μ L of 1% potassium ferricyanide and 500 μ L of 0.2 M sodium phosphate buffer (pH 6.6). The final mixture was incubated at 50 °C for 20 min. Then, 500 μ L TCA (10%) was added, and solutions were centrifuged at 11000 rpm for 10 min. Finally, 1 mL of supernatant was transferred to a new vial containing 1 mL distilled water and 200 μ L of ferric chloride (0.1%) and allowed to stand for 10 min. The absorbance of the sample and standard were measured at 700 nm.

Statistical analysis

The experiment's results were analyzed using SPSS (version 17.0). The one-way analysis of variance and Duncan multiple range test were carried out at a significant level of 5%.

Findings

Production of chitin and glucosamine

Chitin was isolated by a chemical procedure using hydrochloric acid and sodium hydroxide at different concentrations and temperatures to eliminate the minerals and proteins, respectively. As presented in Table 1, the weight of the dried chitin obtained in the present study was $19.90 \pm 1.02\%$. G-HCl was prepared by acidic hydrolysis of chitin using hydrochloric acid. The overall yield of G-HCl, GS-Na, and GS-K were 82.52%, 75.53%, and 82.11%, respectively.

HPAEC-PAD analyses

The HPAEC-PAD method is one of the most efficient techniques for separating monosaccharides [36]. HPAEC-PAD analysis was performed to determine G-HCl, GS-Na, GS-K, G-S, glucose, and water. The HPAEC-

PAD elution profile of components is shown in Figure 1. The CarboPac PA1 column was explicitly developed for this analysis. The results indicated that glucosamine-standard was separated in elution time of 5 minutes on a HPAEC column. G-HCl, GS-Na, and GS-K were liberated and appeared simultaneously (5 min). These results were further confirmed by the elution profile of glucose, which was observed in an elution time of 6 min. Glucosamine hydrochloride and glucosamine sulfate were identified by comparing them with G-S and glucose retention time.

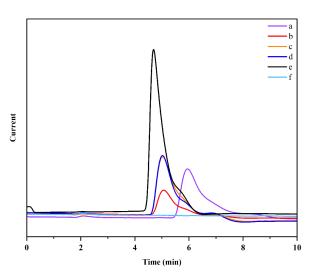


Figure 1) HPAEC-PAD of (a) glucose, (b) glucosamine hydrochloride, (c) glucosamine sulfate sodium chloride, (d) glucosamine sulfate potassium chloride, (e) glucosamine-standard and (f) water

FT-IR spectroscopy

FT-IR spectroscopy was conducted to determine the specific chemical bonds of G-HCl, GS-Na, and GS-K (Figure 2). Glucosamine exhibited peaks at around 2500-3500 cm⁻¹, which attributed to the N-H and O-H groups [37]. Zaeni et al. [38] indicated that the peaks in the spectrum at 3353 cm⁻¹ and 3293 cm⁻¹ were due to the -O.H. and N.H. amines groups, respectively. The wavenumber at 3350 cm⁻¹ corresponds to the O-H functional group in glucosamine hydrochloride. The N-H and O-H stretching

Table 1) Yield (%) of chitin and glucosamine isolated from shrimp shells.

Sample	Yield (%)
Chitin	19.90° ± 1.02
Glucosamine hydrochloride	82.52° ± 3.63
Glucosamine sulfate sodium chloride	75.53 ^b ± 2.54
Glucosamine sulfate potassium chloride	82.11° ± 3.60

Different capital letters (a, b, and v) represent statistically significant differences at (p < 0.05).

vibrations were observed at 3345 cm⁻¹ and 3275 cm⁻¹ in the structure of glucosamine [^{39]}. The NH₂ bending vibration was observed at 1540 cm⁻¹. Kim et al. [^{40]} reported a similar vibration peak of 1545 attributed to -NH₂ [^{40]}.

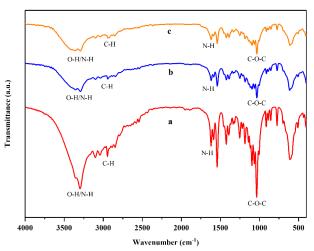


Figure 2) FT-IR spectra of (a) glucosamine hydrochloride, (b) glucosamine sulfate potassium chloride, (c) glucosamine sulfate sodium chloride.

SEM/EDX analysis

SEM images and EDX spectra of (a) G-HCl, (b) GS-Na, and (c) GS-K were obtained to understand the morphology of the surface and confirm the elemental compositions of samples, respectively. As shown in (Figure hydrochloride 3a-c), glucosamine and glucosamine sulfate exhibited polyhedral crystalline structures. It was similar to that noted by Wahab et al. [41] and Al-Hamidi et al. [42], who reported the prismatic shape (polygonal) of glucosamine hydrochloride. The quantitative analysis of glucosamine hydrochlorideindicatedthepresenceofcarbon

(4.87 %), oxygen (42.85%), and chloride (16.28%), which attributed to glucosamine hydrochloride. The analysis showed a peak in the glucosamine hydrochloride, confirming the production of glucosamine hydrochloride (Figure 3a). Moreover, the presence of sulfate (3.44%) and sodium (4.06%) in glucosamine sulfate sodium chloride (Figure 3b) and sulfate (5.52%) and potassium (13.02%) in glucosamine sulfate potassium chloride (Figure 3c) confirmed the isolation of these sulfate salts of glucosamine.

Differential scanning calorimetry (DSC)

DSC thermogram of G-HCl, GS-Na, and GS-K was measured. The peak temperatures and melting enthalpies (ΔH) of each component are displayed in Figure 4A. Glucosamine hydrochloride showed two endothermic peaks, one at 196.76 °C, which was attributed to the melting point of glucosamine hydrochloride, and the other peak appeared at 267.35 °C. The total enthalpy was 508.99 J.g-1 and 15.024 J.g-1, respectively. A single exothermic peak was exhibited at 384.67 °C with an enthalpy of 11.661 J.g-1 (Figure 4). Three endothermic peaks were appeared at 197.52 $^{\circ}$ C (Δ H = 4.517 $J.g^{-1}$), 212.86 °C ($\Delta H = 39.03 J.g^{-1}$) and 227.79 °C $(\Delta H = 98.946 \text{ J.g}^{-1})$. These melting peaks belong to glucosamine sulfate potassium chloride. The DSC diagram of glucosamine sulfate sodium chloride indicated two endothermic peaks at 223.20 ${}^{\circ}$ C (Δ H = 438.45 J.g⁻¹) and 255.88 ${}^{\circ}$ C (Δ H = 1.0326 J.g⁻¹). It is followed by an exothermic peak at 393.80 °C and 7.5273 J.g-1 enthalpy. The DSC curve of glucosamine

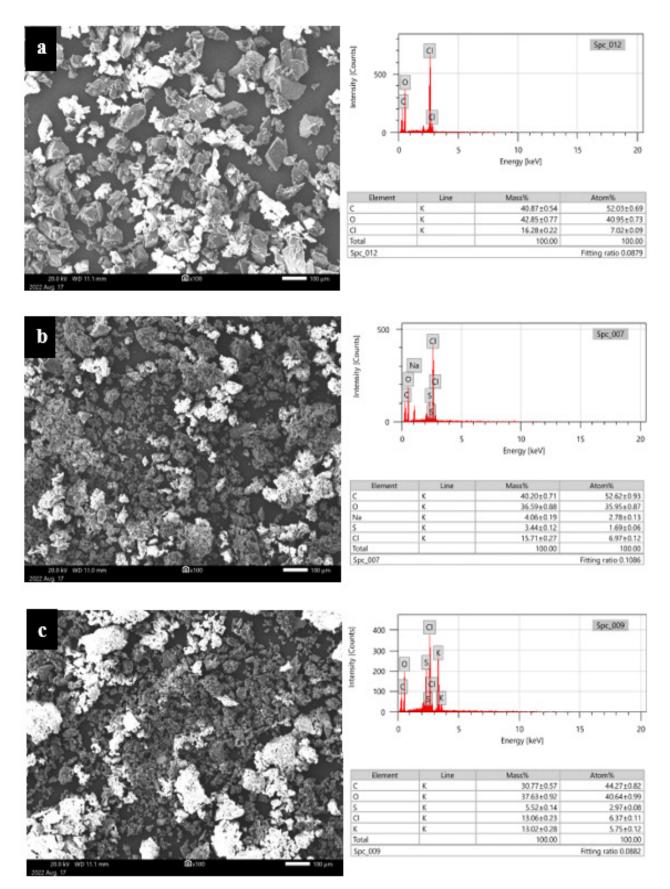


Figure 3) SEM image and EDX profile of (a) glucosamine hydrochloride, (b) glucosamine sulfate sodium chloride, and (c) glucosamine sulfate potassium chloride.

sulfate illustrated a sharp endothermic peak at 218.65 °C, attributed to the crystalline structure and melting temperature [43,44].

Thermogravimetric analysis (TGA)

TGA plots of G-HCl, GS-Na, and GS-K are depicted in Figure 4B. Based on Figure 5a, two strong exothermic peaks are found at 191.26 °C and 590 °C. The corresponding weight loss is 55.11 % and 72.86 %, respectively. In the glucosamine sulfate potassium chloride sample, the first mass loss was 35.80 % at around 207.21 °C, and the second was 82.05 % at around 843.59 °C. Glucosamine sulfate sodium chloride showed the same trend as other samples. The first weight loss was noticed at 200.24 °C (41.05 %), and the second was 78.82 % at 790.69 °C.

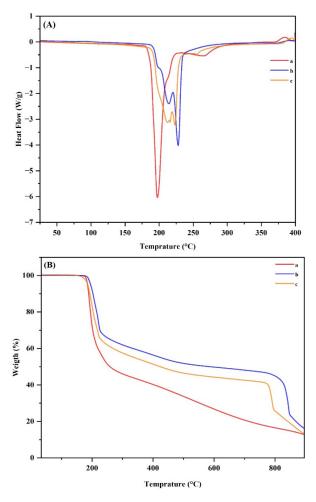


Figure 4) (A) DSC and (B) TGA thermograms of (a) glucosamine hydrochloride, (b) glucosamine sulfate potassium chloride, and (c) glucosamine sulfate sodium chloride.

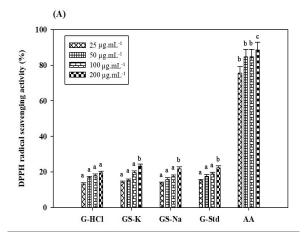
DPPH radical scavenging activity

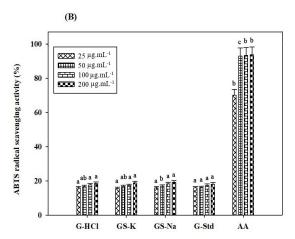
The DPPH free radical (2,2-Diphenyl-1picrylhydrazyl) is widely performed to characterize the antioxidant capacity of natural compounds [45]. Antioxidant activity of G-HCl, GS-Na, GS-K, and G-S was determined in the concentration range of 25 µg.mL⁻¹ to 200 µg.mL⁻¹ using DPPH radical scavenging (Figure 5), with ascorbic acid as the standard sample at the same concentration. Generally, DPPH radical scavenging activity increased with increasing concentration. The DPPH free radical abilities of glucosamine hydrochloride were 13.24% and 19.40% at concentrations of 25 μg.mL⁻¹ to 200 μg.mL⁻¹, respectively. Among the samples, glucosamine standard (22.38%) and glucosamine sulfate potassium chloride (23.32%) indicated higher radical scavenging activities. However, ascorbic acid showed radical scavenging capacity ranging from 75.46% to 88.43% in 25 µg.mL⁻¹ and 200 μg.mL⁻¹, respectively. This result showed that glucosamine could not reduce the free radical compared with standard ascorbic acid (75.46% to 83.43%) in concentrations ranging from 25 to 200 µg.mL⁻¹.

ABTS radical scavenging activity

ABTS radical scavenging activity G-HCl, GS-Na, GS-K, and G-S at various concentrations from 25 to 200 µg.mL⁻¹ are presented in Figure 5. Based on these results, glucosamine standard (16.06%) exhibited significant differences at a concentration of 50 μ g.mL⁻¹ (p<0.05). In all samples ranging from 25 to 200 μg.mL⁻¹, the greatest radical scavenging activity was for the glucosamine hydrochloride (19.01%) and the least for the glucosamine sulfate potassium chloride (15.51%). As shown in Fig. 6e, ascorbic acid showed 75.46 % and 88.43 % of radical scavenging capacity at the same concentrations of 25 µg.mL⁻¹and 200 μg.mL⁻¹, respectively. Our present study showed that glucosamine could not inhibit

oxidative damage at 25 to 200 $\mu g.mL^{-1}$ concentrations.





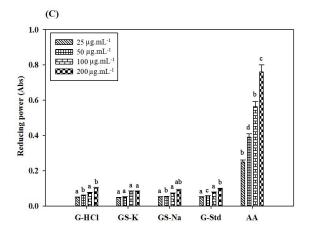
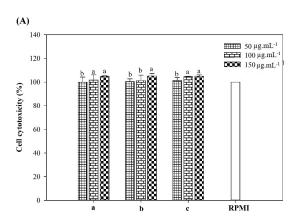


Figure 5) (A) DPPH and (B) ABTS radical scavenging activities and (C) ferric reducing power of (a) glucosamine hydrochloride (b) glucosamine sulfate potassium chloride (c) glucosamine sulfate sodium chloride, glucosamine standard (d) and ascorbic acid (e). Different small letters (a, b, and c) represent statistically significant differences at (p < 0.05).

Ferric reducing power

Ferric reducing ability of G-HCl, GS-Na, GS-K, and G-S are reported in Figure 5. On the contrary, statistically significant differences (p<0.05) were seen between samples at the concentration level of 50 µg.mL⁻¹. The absorbance of G-HCl, GS-K, G-S, GS-Na, and ascorbic acid were 0.048, 0.046, 0.050, 0.05, and 0.24, which increased to 0.1, 0.08, 0.08, 0.09 and 0.76 at the concentration level of 25 μg.mL⁻¹ to 200 μg.mL⁻¹, respectively. The result of reducing power showed the least antioxidant capacity of samples compared to the content of ascorbic acid (0.24, 0.39, 0.56, and 0.76) as standard at the concentration of 25 to 200 μg.mL⁻¹. Results indicated that the reducing power improved with the increasing concentration of glucosamine.



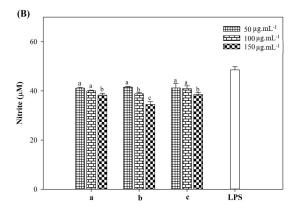


Figure 6) RAW264.7 cell (A) cytotoxicity and (B) nitric oxide production under stimulation of (a) glucosamine hydrochloride, (b) glucosamine sulfate sodium chloride, and (c) glucosamine sulfate potassium chloride. Different small letters (a, b, and c) represent statistically significant differences at (p < 0.05).

Cytotoxicity and anti-inflammatory activities of glucosamines

The murine macrophage cellline (RAW264.7) was treated with polysaccharides within the $50\text{--}150~\mu\text{g.mL}^{-1}$ concentration for 18~h to assess potential adverse effects. As illustrated in Figure 6A, no cytotoxicity effects were observed on the growth of RAW264.7 cells when cultured in a medium supplemented with G-HCl, GS-Na, and GS-K. In LPS-induced macrophages, NO production is a typical indicator of conventional inflammatory responses. As depicted in Figure 6B, the presence of $1.0~\mu\text{g.mL}^{-1}$ of LPS notably triggered NO release in macrophage cells. However, G-HCl, GS-Na, and GS-K exhibited a dose-dependent reduction in NO levels.

Discussion

The amount of chitin obtained from different sources is believed to be incredibly varied depending on the type of origin and the methodology employed for isolation. For instance, Bertuzzi et al. [37] reported 37% of chitin content isolated from shrimp shells of *Litopenaeus vannamei* using 0.5 M HCL (37%) and 1M NaOH. Kamala et al. [46] reported that the yield of chitin isolated from Parapenaeopsis styliform shells using 1000 mL of 7 % HCl (w/w) and 1000 mL of 10% NaOH (w/w) was 32%, which was much higher than that obtained in the present research. Trung and Phuong [56] reported that the content of chitin treated with 2% NaOH (w/v) and 4% HCl from shrimp wastes was 9.3% based on a dry basis. Similarly, Mohan et al. [57] reported that the overall yield of chitin isolated from *Litopenaeus vannamei* and Penaeus monodon shells was about 20%. The chitin isolated from the exoskeletons of crustaceans is also reported to range between 15 and 40% [58]. By increasing the reaction temperature to 95 ± 1 °C, chitin was hydrolyzed during the initial time (0-15 min), resulting in a dark brown color, which

caused Millard's reaction, which involved amino groups reacting with an aldehyde. The glucosamine sulfate was formed by adding sodium and potassium sulfate to glucosamine hydrochloride. Benavente et al. [47] found that the maximum yield of glucosamine hydrochloride isolated using acid hydrolysis of chitin from shrimp shells was 58%. Bertuzzi et al. [37] reported that the yield of glucosamine isolated from shrimp shells of Litopenaeus vannamei, cicada sloughs, and cockroaches were 13.70, 20.46 and 3.28%, respectively. In this study, the yield of G-HCl from shrimp shells was higher than previously reported. The efficiency of glucosamine hydrochloride was positively associated with acid concentration, solid-toliquid ratio, and reaction time [59].

Generally, it has been stated that there is a direct connection between antioxidant activities and carbohydrate concentrations [55]. Jamialahmadi et al. [53] reported that G-HCl significantly decreased the DPPH radical scavenging activity at various concentrations (5, 10, 20, and 40 mM). Wang et al. [60], Xing et al. [29], and Xing et al. [30] found that ABTS radical scavenging activities of glucosamine hydrochloride and glucosamine sulfate increased dose-dependently with increasing their concentrations. However, numerous studies on glucosamine have reported that the antioxidant activity did not increase significantly with increasing concentrations [55]. Similarly, Gottardi et al. [46] reported that glucosamine hydrochloride did not exhibit any DPPH scavenging activity at a concentration of 0.2 mM. Several factors such as molecular weight, chemical composition, extraction, and separation methods of carbohydrates closely affect their biological activities, especially antioxidant capacity [61,62,63]

The most important response of the immune system to infection and injury, which may characterized by any feeling such

as pain, heat, and redness, is inflammation [64]. In terms of the inflammatory system, glucosamine not only inhibited expressions of inducible nitric oxide synthase and IL-1\beta in lipopolysaccharide-activated microglia and the J774A macrophage cell line in vitro but also demonstrated significant improvements in inflammatory bowel disease and lung inflammation in vivo [65,66,67,68]. According to the current study, all the glucosamine derivatives, including G-HCl, GS-NA, and GS-K, exhibited some extent of anti-inflammatory effects on LPSinduced RAW2647 macrophage cells.

Conclusion

The chitin and glucosamine products were isolated from the shells of L. vannamei. Analysis using HPAEC-PAD, FT-IR, SEM, EDX, DSC, and TGA revealed the successful production of G-HCl, GS-Na, and GS-K. Despite relatively low DPPH and ABTS radical scavenging activities, as well as ferric reducing power, observed for all glucosamine products, they exhibited no cytotoxicity and down-regulated the release of NO in LPS-induced RAW264.7 murine macrophage cells. In conclusion, the results suggest that glucosamine products derived from L. vannamei possess antioxidant and anti-inflammatory properties. Nonetheless, additional investigation is required to evaluate the efficacy of obtained glucosamine animal models and enhance the bioactivity of glucosamine using chemical and enzymatic means.

Ethical Permissions: None declared by authors.

Author Contribution: Alika Jafari, leading researcher/data acquisition; Mehdi Tabarsa, conceptualization/supervision; Hossein Naderimanesh, conceptualization/supervision; Hassan Ahmadi Gavlighi, methodology; SangGuan You, methodology

Conflicts of Interest: The authors have no competing interests relevant to the content of this article.

Funding/ Supports: We are grateful for the financial support of Iran National Science Foundation (Grant No. 4005100). The authors also would like to acknowledge the support of Modares Science and Technology Park for this project.

References

- 1. Olsen R.L., Toppe J., Karunasagar I. Challenges and realistic opportunities in the use of byproducts from processing of fish and shellfish. Trend. Food Sci. 2014;36(2): 144-151.
- López-Pedrouso M., Lorenzo J., Cantalapiedra J., Zapata C., Franco J.M., Franco D. Aquaculture and by-products: Challenges and opportunities in the use of alternative protein sources and bioactive compounds. Adv. Food Nutr. Res. 2020;92: 127-185.
- Khodabandeh S., Hassan Sajedi R., Behmanesh M. Influence of Enzyme Type and Hydrolysis Time on Antioxidant Activity of Hydrolyzed Protein from Longtail Tuna (*Thunnus tonggol*) Dark Muscle. ECOPERSIA 2023;11(3): 187-195.
- 4. Knorr D. Recovery and utilization of chitin and chitosan in food processing waste management. Food Technol. 1991; 26: 114–122.
- Nirmal N.P., Santivarangkna C., Rajput M.S., Benjakul S. Trends in shrimp processing waste utilization: An industrial perspective. Trends Food Sci. 2020;103: 20-35.
- 6. IMARC. Shrimp market: Global industry trends, share, size, growth, opportunity, and forecast 2020-2025. USA: IMARC group.
- 7. FAO. 2023. Fishery and Aquaculture Statistics. Global aquaculture production 1950-2021 (FishstatJ);
- 8. Suryawanshi N., Eswari J.S. Chitin from seafood waste: particle swarm optimization and neural network study for the improved chitinase production. J. Chem. Technol. Biotechnol. 2022; 97(2): 509-519.
- 9. Coward-Kelly G., Agbogbo F.K., Holtzapple M.T. Lime treatment of shrimp head waste for the generation of highly digestible animal feed. Bioresour. Technol. 2006; 97(13):1515-1520.
- 10. Sachindra N.M., Bhaskar N. In vitro antioxidant activity of liquor from fermented shrimp biowaste. Bioresour. Technol. 2008; 99(18): 9013-9016.
- 11. Díaz-Rojas E.I., Argüelles-Monal W.M., Higuera-Ciapara I.. Hernández J, Lizardi-Mendoza J.

- Goycoolea F.M.. Determination of chitin and protein contents during the isolation of chitin from shrimp waste. Macromol. Biosci. 2006; 6(5): 340-347.
- 12. Jayakumar R., Prabaharan M., Kumar P.S., Nair S.V., Tamura H.J. Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnol. Adv. 2011; 29(3): 322-337.
- 13. Soni T., Zhuang M., Kumar M., Balan V., Ubanwa B., Vivekanand V., Pareek N. Multifaceted production strategies and applications of glucosamine: a comprehensive review. Crit. Rev. Biotechnol. 2023; 2;43(1): 100-120.
- 14. Anderson J.W., Nicolosi R.J., Borzelleca J.F. Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations, and efficacy. Food Chem. Toxicol. 2005; 43(2): 187-201.
- 15. Houpt J.B., McMillan R., Wein C., Paget-Dellio S.D. Effect of glucosamine hydrochloride in the treatment of pain of osteoarthritis of the knee. J. Rheumat. 1999; 26(11):2423-2430.
- 16. Luo J., Hu Y., Wu Y., Fan W. Effect of glucosamine hydrochloride in ameliorating knee osteoarthritis. Chinese J. Clinical Rehab. 2005; 9: 70-72.
- 17. Vangsness C.T., Spiker W., Erickson J. A review of evidence-based medicine for glucosamine and chondroitin sulfate use in knee osteoarthritis. Arthroscopy: J. Arthrosc. Relat. Surg. 2009; 25(1):86-94.
- 18. Bissett D.L. Glucosamine: an ingredient with skin and other benefits. J. Cosmetic Dermatol. 2006; 5(4): 309-315.
- 19. Piperno M., Reboul P., Le Graverand M.H., Peschard M.J., Annefeld M., Richard M., Vignon E. Glucosamine sulfate modulates dysregulated activities of human osteoarthritic chondrocytes in vitro. Osteoarthritis and Cartilage. 2000;8(3): 207-212.
- 20. Shikhman A.R., Kuhn K., Alaaeddine N., Lotz M. N-acetylglucosamine prevents IL-1 β -mediated activation of human chondrocytes. J. Immunol. 2001;166(8): 5155-5160.
- 21. Li Y., Chen L., Liu Y., Zhang Y., Liang Y., Mei Y. Antiinflammatory effects in a mouse osteoarthritis model of a mixture of glucosamine and chitooligosaccharides produced by bi-enzyme single-step hydrolysis. Sci. Rep. 2018;8(1): 5624.
- 22. Cheng D.W., Jiang Y., Shalev A., Kowluru R., Crook E.D., Singh L.P. An analysis of high glucose and glucosamine-induced gene expression and oxidative stress in renal mesangial cells. Arch. Physiol. Biochem. 2006;112(4-5): 189-218.
- Chen Y.J., Huang Y.S., Chen J.T., Chen Y.H., Tai M.C., Chen C.L., Liang C.M. Protective effects of glucosamine on oxidative-stress and ischemia/ reperfusion-induced retinal injury. Invest.

- Ophthalmol. Visual Sci. 2015;56(3): 1506-1516.
- 24. Mendis E., Kim M.M., Rajapakse N., Kim S.K. Sulfated glucosamine inhibits oxidation of biomolecules in cells via a mechanism involving intracellular free radical scavenging. Eur. J. Pharmacol. 2008;;579(1-3): 74-85.
- 25. Valvason C., Musacchio E., Pozzuoli A., Ramonda R., Aldegheri R., Punzi L.. Influence of glucosamine sulfate on oxidative stress in human osteoarthritic chondrocytes: effects on HO-1, p22 Phox, and iNOS expression. Rheumatol. 2008; 47(1): 31-35.
- 26. Stadler J.O., Stefanovic-Racic M.A., Billiar T.R., Curran R.D., McIntyre L.A., Georgescu H.I., Simmons R.L., Evans C.H. Articular chondrocytes synthesize nitric oxide in response to cytokines and lipopolysaccharide. J. Immunol. (Baltimore, Md.: 1950). 1991;147(11): 3915-3920.
- 27. Yan Y., Wanshun L., Baoqin H., Changhong W., Chenwei F., Bing L., Liehuan C. The antioxidative and immunostimulating properties of D-glucosamine. Int. Immunopharmacol. 2007; 7(1): 29-35.
- 28. Xing R., Liu S., Guo Z., Yu H., Li C., Ji X., Feng J., Li P. The antioxidant activity of glucosamine hydrochloride in vitro. Bioorganic Med. Chem. 2006;14(6): 1706-1709.
- 29. Xing R., Liu S., Wang L., Cai S., Yu H., Feng J., Li P. The preparation and antioxidant activity of glucosamine sulfate. Chin. J. Oceanol. Limnol. 2009; 27: 283-287.
- 30. Green L.C., Wagner D.A., Glogowski J., Skipper P.L., Wishnok J.S., Tannenbaum S.R. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal. Biochem. 1982;126(1): 131-138.
- 31. Brand-Williams W., Cuvelier ME., Berset C.L. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 1995;28(1): 25-30.
- 32. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med. 1999;26(9-10): 1231-1237.
- 33. Oyaizu M. Studies on products of browning reaction prepared from glucose amine products derived from bees. J. Pharm. Biomed. Anal. 1986; 41: 1220-1234.
- 34. Zhang Z., Khan N.M., Nunez K.M., Chess E.K., Szabo C.M. Complete monosaccharide analysis by high-performance anion-exchange chromatography with pulsed amperometric detection. Anal. Chem. 2012; 84(9): 4104-4110.
- 35. Bertuzzi D.L., Becher T.B., Capreti N.M., Amorim J., Jurberg I.D., Megiatto J.D., Ornelas C. General Protocol to Obtain D-Glucosamine from Biomass Residues: Shrimp Shells, Cicada Sloughs and

Cockroaches. Global Challenge. 2018; 2(11): 1800046.

- 36. Zaeni A., Safitri E., Fuadah B., Sudiana I.N. Microwave-assisted hydrolysis of chitosan from shrimp shell waste for glucosamine hydrochloride production. Journal of Physics: Conference Series. 2017; 1 (846): 012011.
- 37. Yu S., Zang H., Chen S., Jiang Y., Yan B., Cheng B. Efficient conversion of chitin biomass into 5-hydroxymethylfurfural over metal salts catalysts in dimethyl sulfoxide-water mixture under hydrothermal conditions. Polym. Degrad. Stab. 2016; 1(134): 105-114.
- 38. Kim H.S., Kim S.K., Jeong G.T. Efficient conversion of glucosamine to levulinic acid in a sulfamic acid-catalyzed hydrothermal reaction. RSC advances. 2018; 8(6): 3198-205.
- 39. Wahab A., Khan G.M., Sharifi M., Khan A., Khan A., Khan N. Preparation, solid-state characterization and evaluation of ketoprofen-glucosamine HCl solid dispersions. Arch. Pharm. Pharmaceutic. Sci. 2018; 2(1): 010-019.
- 40. Al-Hamidi H., Edwards A.A., Mohammad M.A., Nokhodchi A. To enhance dissolution rate of poorly water-soluble drugs: glucosamine hydrochloride as a potential carrier in solid dispersion formulations. Colloids Surf., B. 2010; 76(1): 170-178.
- 41. Asare-Addo K., Šupuk E. Al-Hamidi H. Owusu-Ware S. Nokhodchi A. Conway B.R. Triboelectrification and dissolution property enhancements of solid dispersions. Int. J. Pharm. 2015; ;485(1-2): 306-316.
- 42. El-Houssiny A., Ward A., Mostafa D., Abd-El-Messieh S., Abdel-Nour K., Darwish M., Khalil W. Drug-polymer interaction between glucosamine sulfate and alginate nanoparticles: FTIR, DSC, and dielectric spectroscopy studies. *Advances in* Adv. Nat. Sci.: Nanosci. 2016; 7(2): 025014.
- 43. Scherer R., Godoy H.T. Antioxidant activity index (AAI) by the 2, 2-diphenyl-1-picrylhydrazyl method. Food Chem. 2009; 112(3): 654-658.
- 44. Kamala K., Sivaperumal P., Rajaram R. Extraction and characterization of water soluble chitosan from *Parapeneopsis stylifera* shrimp shell waste and its antibacterial activity. Inter. J. Sci. Res. Public. 2013; 3(4): 1-8.
- 45. Benavente M., Arias S., Moreno L., Martínez J. Production of glucosamine hydrochloride from crustacean shell. J. Pharm. Pharmacol. 2015; 3(1): 20-26.
- Brugnerotto J., Lizardi J., Goycoolea F., Argüelles-Monal W., Desbrieres J., Rinaudo M. An infrared investigation in relation with chitin and chitosan characterization. Polymer, 2001; 42(8): 3569-3580
- 47. Chen H., Zhang M., Qu Z., Xie B. Antioxidant

- activities of different fractions of polysaccharide conjugates from green tea (*Camellia Sinensis*). Food Chem. 2008; 106(2): 559-563.
- 48. Díaz-Rojas E.I., Argüelles-Monal W.M., Higuera-Ciapara I., Hernández J., Lizardi-Mendoza J., Goycoolea F.M. Determination of chitin and protein contents during the isolation of chitin from shrimp waste. Macromol. Biosci. 2006; 6(5): 340-347.
- 49. Drovanti A., Bignamini A., Rovati A. Therapeutic activity of oral glucosamine sulfate in osteoarthrosis: a placebo-controlled double-masked investigation. Clin. Ther. 1980; 3(4): 260-272.
- Gottardi, D., Hong, P. K., Ndagijimana, M., & Betti, M. Conjugation of gluten hydrolysates with glucosamine at mild temperatures enhances antioxidant and antimicrobial properties. LWT-Food Sci. Technol. 2014; 57(1): 181-187.
- 51. Jamialahmadi K., Arasteh O., Riahi M.M., Mehri S., Riahi-Zanjani B., Karimi G. Protective effects of glucosamine hydrochloride against free radical-induced erythrocytes damage. Environ. Toxicol. Pharmacol. 2014; 38(1): 212-219.
- 52. Kraisangsri J., Nalinanon S., Riebroy S., Yarnpakdee S., Ganesan P. Physicochemical characteristics of glucosamine from blue swimming crab (*Portunus pelagicus*) shell prepared by acid hydrolysis. WJST. 2018; 15(12): 869-877.
- 53. Li X., Li X., Zhou A. Evaluation of antioxidant activity of the polysaccharides extracted from *Lycium barbarum* fruits in vitro. Euro. Polymer J. 2007; 43(2): 488-497.
- 54. Trung T.S., Phuong P.T. Bioactive compounds from by-products of shrimp processing industry in Vietnam. J. Food Drug Anal. 2012; 20(1): 64.
- 55. Mohan K., Muralisankar T., Jayakumar R., Rajeev Gandhi C.A. Study on structural comparisons of α -chitin extracted from marine crustacean shell waste. Carbohydr. Polym. Technol. Appl. 2021; 25(2): 100037.
- 56. Synowiecki J., Al-Khateeb N.A. Production, properties, and some new applications of chitin and its derivatives. 2003; 145-171.
- 57. Mojarrad J.S., Nemati M., Valizadeh H., Ansarin M., Bourbour S. Preparation of glucosamine from exoskeleton of shrimp and predicting production yield by response surface methodology. J. Agric. Food. Chem. 2007; 55(6): 2246-2250.
- 58. Wang X., Liu B., Li X., Sun R. Novel glucosamine hydrochloride–rectorite nanocomposites with antioxidant and anti-ultraviolet activity. Nanotechnol. 2012; 23(49): 495706.
- 59. Miao Q., Li Q., Tan W., Mi Y., Ma B., Zhang J., Guo Z. Preparation, Anticoagulant and Antioxidant Properties of Glucosamine-Heparin Salt. Mar. Drug. 2022; 20(10): 646.

- 60. Rao R.S., Muralikrishna G. Water soluble feruloyl arabinoxylans from rice and ragi: Changes upon malting and their consequence on antioxidant activity. Phytochem. 2006; 67(1): 91-99.
- 61. Meng L., Sun S., Li R., Shen Z., Wang P., Jiang X. Antioxidant activity of polysaccharides produced by *Hirsutella* sp. and relation with their chemical characteristics. Carbohydr. Polymer. 2015; 117: 452-457.
- 62. Liew F.Y., Xu D., Brint E.K., O'Neill L.A. Negative regulation of toll-like receptor-mediated immune responses. Nat. Rev. Immunol. 2005; 5(6): 446-458.
- 63. Azuma K., Osaki T., Kurozumi S., Kiyose M., Tsuka T., Murahata Y., Imagawa T., Itoh N., Minami S., Sato K., Okamoto Y. Anti-inflammatory effects of orally administered glucosamine oligomer in an experimental model of inflammatory bowel

- disease. Carbohydr.Polymer.2015;115:448-456.
- 64. Chiu H.W., Li L.H., Hsieh C.Y., Rao Y.K., Chen F.H., Chen A., Ka S.M., Hua K.F. Glucosamine inhibits IL-1β expression by preserving mitochondrial integrity and disrupting assembly of the NLRP3 inflammasome. Sci. Rep. 2019; 9(1): 5603.
- 65. Hwang S.Y., Shin J.H., Hwang J.S., Kim S.Y., Shin J.A., Oh E.S., Oh S., Kim J.B., Lee J.K., Han IO. Glucosamine exerts a neuroprotective effect via suppression of inflammation in rat brain ischemia/reperfusion injury. Glia. 2010; 58(15): 1881-1892.
- 66. Wu Y.L., Lin A.H., Chen C.H., Huang W.C., Wang H.Y., Liu M.H., Lee T.S., Kou Y.R. Glucosamine attenuates cigarette smoke-induced lung inflammation by inhibiting ROS-sensitive inflammatory signaling. Free Radical Biol. Med. 2014; 1(69): 208-218.