Physico-chemical properties of chitosan extracted from Whiteleg shrimp (*Litopenaeus vannamei*) processing shell waste in Sri Lanka

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Abstract The present study investigated the potential of whiteleg shrimp shell waste to use as a source of chitosan which has a limited focus on previous Sri Lankan studies. Comparatively higher amount of chitosan yield (33.53%) was obtained in the study with 91.78 ± 0.66 (%) of dry matter content, 8.22 ± 0.66 (%) of moisture content, 1.08 ± 0.24 (%) of ash content, 80.43% of the degree of deacetylation, 435.87 ± 1.03 cP dynamic viscosity and a good thermal stability up to 312 °C. Fourier transform infrared spectroscopy (FTIR) analysis confirmed the structure of chitin and chitosan by the presence of their characteristic IR bands and X-ray diffraction analysis further verified the crystalline structure of the extracted chitin and chitosan. Scanning electron microscopic (SEM) images of the extracted chitosan recognized the layers of flakes with porous and fibril structures. According to the energy-dispersive X-ray spectroscopy image, carbon, oxygen and nitrogen were observed as the major elements in the extracted chitosan and no calcium peaks were detected confirming effective demineralization in the extraction process. Further, DPPH (2,2-diphenyl-1-picryl hydrazy) assay revealed that the chitosan solution with the concentration of 10 mg/mL was having 66.45% of free radical scavenging activity. Thus, the present study reveals that the chitosan with high quantity and quality can be extracted from whiteleg shrimp (*Litopenaeus vannamei*) processing shell waste in Sri Lanka suggesting a solution to the waste accumulation in processing factories while proposing an alternative income generation strategy from waste.

Keywords: Chitin Extraction, Chitosan, Industrial waste, Whiteleg shrimp

INTRODUCTION

The fishing industry plays a vital role in the national economy in Sri Lanka, an island surrounded by the Indian Ocean, and the livelihood and socioeconomic condition of coastal communities. Due to increasing export demand, especially for shellfish products like crabs, lobsters and prawns, during the past decade, has generated a significant foreign exchange earning for the country. In 2017, Sri Lanka exported 24,827 Mt of fish and fishery products and earned 39,230 million LKR and of that 18% was encountered from crabs, prawns and lobsters (National Aquatic Resources Research and Development Agency 2018).

The expanding shellfish industry, consequently, poses a high amount of waste which is responsible for several environmental, social and community health issues. Further, with recent changes in waste legislation, the management of waste has become more difficult, expensive and challenging. Hence, shellfish processing factories in many developing countries like Sri Lanka used to dispose of their waste into the sea, burn, landfill or simply allow for natural degradation without proper waste management. But due to inadequate knowledge and technology to convert this shell waste into high-value-added products, regularly this shell waste is sold out to retailers for the production of low-value livestock feeds or fertilizers. In such a context,

valorization of shellfish waste which is compatible with industrial applications is an urgent requirement.

Although bio-refinery is one of such valorization techniques for shell waste to produce high-value-added bioactive compounds such as chitin, chitosan, and protein together with several minor components including lipids, astaxanthin (Yang and Yan 2018), still such technologies are not well established in many developing countries.

Among many of the bioactive compounds extracted from shell waste (Lakmini et al. 2022; Ampitiya et al. 2022), chitosan has considerable scientific and technological interest due to its natural abundance and its positive attributes for a variety of applications including excellent biodegradability, biocompatibility, bioactivity and low toxicity. Thus, chitosan is being used as an antimicrobial agent, edible film, food additive, water purifier, cosmetic, biomedical and pharmaceutical material in many countries (Manigandan et al. 2018; Shahidi and Abuzaytoun 2018). Due to this potential for various applications, the global chitosan market revenue forecast shows an increasing trend from 2013 – 2024 (Pulidindi and Pandey 2019). Although chitosan has a high demand, limited attention has been given in Sri Lanka except for a couple of studies (Allwin et al. 2011; Hewajulige et al. 2006; Sanuja et al. 2017), which probably be due to the lack of knowledge on high-end uses and technological know-how for value-addition.

Therefore, the present study investigated the potential of using whiteleg shrimp to extract chitosan which has limited considerations before in Sri Lanka. Whiteleg shrimp is a newly introduced prawn species to Sri Lanka in 2019, whereas it has already been adopted in other countries in 1990s aiming to boost shrimp exports due to its high yield. This species has been recognized as a specific pathogen-free and grown under high stocking densities (FAO 2020). It is world production was 4966.2 thousand tonnes in 2018 and it was 52% of the world's total crustacean production in that year (FAO 2020). Thus, identifying the potential of whiteleg shrimp shell waste for extracting chitosan will be an important industrial aspect for existing processing industries and potential chitosan-based products developing industries. However, extracting chitosan from *Litopenaeus vannamei* shell waste has been recorded in several other countries. Such previous attempts of chitosan extraction from *Litopenaeus vannamei* shells by chemical extraction process have been reported by Allwin et al. (2015) and Antonino et al. (2017). Further, Mittal et al. (2020) extracted chitosan from *Litopenaeus vannamei* and they conjugated the extracted chitosan with epigallocatechin gallate for better antioxidative and antimicrobial activities (Mittal et al. 2020). Meanwhile, Hongkulsup et al. (2016) have been taken a systematic attempt for using this *Litopenaeus vannamei* shells for chitin extraction by enzyme-assisted extraction method as an alternative to the conventional chemical extraction method. Therefore, it would be important to assess the possibility of using *Litopenaeus vannamei* shell waste for chitosan extraction under Sri Lankan conditions, because considerable attention had been drawn to *Litopenaeus vannamei* culture due to its high production and disease susceptibility. Therefore, the objective of this study was to evaluate the possibility of chitosan extraction from *Litopenaeus vannamei* processing shell waste and characterise its physicochemical properties to use in future applications.

MATERIALS AND METHODS

Raw material collection and preparation

The processing waste of whiteleg shrimp comprising the abdominal shells was collected from a shrimp processing plant in Dankotuwa, Sri Lanka. Shells were prepared for extraction by cleaning several times under running water, scraping free of loose tissue, oven drying at 105 ºC at 24 hours and finally by grinding the shells using a food processor (Philips HR 7761).

Proximate composition analysis

A representative waste sample was separated before the extraction for proximate composition analysis using AOAC (1990) standards. crude moisture and dry matter were quantified by oven-drying at 105 ˚C, crude protein was quantified by the micro Kjeldahl procedure and crude ash was quantified by incineration in a muffle furnace at 550 ˚C. All values were taken on a dry weight basis and analyzed in triplicates.

Extraction of chitosan

The extraction process of chitosan from shells was carried out in subsequent steps of demineralization, deproteinization, decolorization and deacetylation according to the method of Yen et al. (2009).

Initially, the preprepared shells were subjected to demineralization using 1 M HCl at the ratio of 1:30 (w/v) at room temperature for 6 hours. Then the solution was neutralized and subjected to 1 M NaOH at the ratio of 1:10 (w/v) at 100 ºC for 3 hours. Treated shells were filtered and exposed to 1% Potassium permanganate solution at 1:10 (w/v) for 1 hour followed by mixing in 1% Oxalic acid at 1:10 (w/v) for decolourization. Decolorized shells were then freeze-dried to obtain chitin. Chitin was then deacetylated into chitosan using 40% NaOH at 1:30 (w/v) for 2 hours at 100 ºC. After that, the solution was neutralized with distilled water, by washing it several times until the washed solution becomes near pH to remove all salts, and freeze-dried to obtain chitosan. Chitosan was formed as flakes and ground into small flakes and stored in a freezer at -20 ºC until taken for characterization.

Characterization of extracted chitosan

Yield, moisture and ash contents

Extracted chitosan yield was calculated by associating the weight measurements of the raw material to the chitosan obtained after the extraction. Moisture and dry matter were quantified by oven-drying at 105 ºC and crude ash was quantified by incineration in a muffle furnace at 550 ºC (AOAC 1990).

Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR was used to confirm the structure of chitin and chitosan by the presence of their characteristic IR bands. The KBr-supported samples of chitin and chitosan were used to get the FTIR spectra over the frequency range 4000- 400 cm^{-1} at a resolution of 4 cm^{-1} using IR Affinity Fourier transformed infrared spectrophotometer (SHIMADZU, Japan). The degree of deacetylation (DD) was assessed by following equations 1 and 2 using FTIR spectroscopy results (Brugnerotto et al. 2001; Kumirska et al. 2010). Absorption values relevant for the wavenumbers 1320 and 1420 are denoted as A1320 and A1420 respectively in the following equation No. 01. Degree of acetylation and Degree of deacetylation is denoted as 'DA' and 'DD' respectively in equation no. 02. A band at 1,320 cm^{-1} is characteristic of –OH, $–NH_2$, –CO groups which were chosen to measure the degree of deacetylation. The band at 1,420 cm^{-1} was chosen as the reference band.

$$A1320/A1420 = 0.3822 + 0.0313\,DA \quad (1)$$

$$DD\% = 100 - DA \quad (2)$$

X-ray diffraction (XRD) analysis

The XRD was used to detect the crystallinity of the extracted chitin and their corresponding chitosan using Cu Kα (λ=1.5405 Å) radiation using X-Ray diffraction spectrophotometer (Rigaku ultima IV).

Scanning electron microscopy (SEM) analysis and Energy-dispersive X-ray spectroscopy analysis (EDS)

The surface morphology of chitosan-coated with gold under vacuum using a sputter coater was observed using a scanning electron microscope at different magnifications (Carl Zeiss, Evo 18, Germany). The elemental composition of extracted chitosan was investigated using EDAX Element, Energy-dispersive X-ray spectroscopy.

Thermogravimetric analysis (TGA)

TGA was carried out using thermogravimetry-differential thermal analysis equipment (Thermo plus EVO 2 Rigaku TG-DTA 8122, Japan) under a continuous flow of dry nitrogen gas from 10 °C to 600 °C at a heating rate of 10 ºC min^{-1}.

Dynamic viscosity

In determine viscosity, 1% (w/v) chitosan solution was prepared with 1% (v/v) acetic acid. The prepared solution was then filtered and the viscosity was determined using an automated viscometer (PCE Instruments, B-ONE plus, United Kingdom). Measurements were made at room temperature in triplicate and the values were reported in centipoises units (cP).

Colour measurement

The colour of extracted chitosan was measured using a Chroma Meter (CR-400 KONICA MINOLTA, INC, Japan). L*, a* and b* values of extracted chitosan were taken by the chromometer after standardising with a calibration white plate. Chroma, hue and whiteness values of the sample were calculated based on the following equations (Kucukgulmez et al. 2011).

$\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$
$\text{Hue} = Arc\tan(b^*/a^*)$
$\text{Whiteness} = 100 - \{(100 - L^*)^2 + a^{*2} + b^{*2}\}^{1/2}$
where L* = Lightness; a* = Redness; and b* = Yellowness

Antioxidant activity; DPPH free radical scavenging assay

The antioxidant activity of prepared chitosan was tested using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay as described by Younes et al. (2014). Initially, chitosan solutions at different concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mg/mL) in 1% acetic acid were prepared. Of each prepared sample, 2 mL was mixed with 1,500 µL of 99.5% ethanol and 500 µL of 0.02% DPPH in 99.5% ethanol. In the control test, the chitosan solution was replaced by the same volume of distilled water. All control and test sample mixtures were then incubated for 60 min in the dark at room temperature and the reduction of DPPH free radical was measured at 517 nm. L-Ascorbic acid was used as the reference sample to compare the DPPH free radical scavenging activity of chitosan samples. The DPPH radical-scavenging activity was calculated as follows:

$$DPPH\ free\ radical - scavenging\ activity\ (\%) = \frac{(a-b)}{a} \times 100$$

where;
a= Absorbance of control
b= Absorbance of sample

RESULTS

Proximate composition of whiteleg shrimp shells

Results of the mean percentage proximate composition of the raw material comprising the shell of the abdominal segments of whiteleg shrimp are given in Table 1. All replicate resulted in values in a similar range with narrow standard deviations.

Table 1 The mean percentage proximate composition of the raw material comprising of the shell of the abdominal segments of whiteleg shrimp on dry weight basis

Composition	Mean % amount ± SD
Moisture	19.64 ± 1.03
Dry matter	80.36 ± 1.03
Crude protein	29.46 ± 2.02
Crude ash	20.39 ± 1.40
Crude fat	0.32 ± 0.09

Physicochemical characteristics of extracted chitosan

Yield, moisture and ash content

The present study resulted in 33.53% chitosan yield with a moisture content of 8.22% ± 0.66 and 1.08% ± 0.24 of ash content.

FTIR analysis

The FTIR spectrum results of whiteleg shrimp raw shell, extracted chitin and chitosan are shown in Figure 1a, 1b and 1c respectively. In isolated prawn shell chitin it was observed a prominent broadband at 3,463 cm^{-1} along with an amide-I band which has been split into two components at 1,647 cm^{-1} and 1,637 cm^{-1}. Amide-II band appears at 1554 cm^{-1} in the chitin spectrum as a single strong peak. Since a 100% deacetylation was not attained, there were notable similarities in the spectra of chitin

and chitosan. However, the band appeared at around 3463cm^{-1} in the chitin spectrum and has been further broadening in the chitosan spectrum. Amide-I and amide-II bands were indicated in the chitosan spectrum at 1656cm^{-1} and 1553cm^{-1} respectively. The degree of deacetylation was recorded as 80.43% for the extracted chitosan.

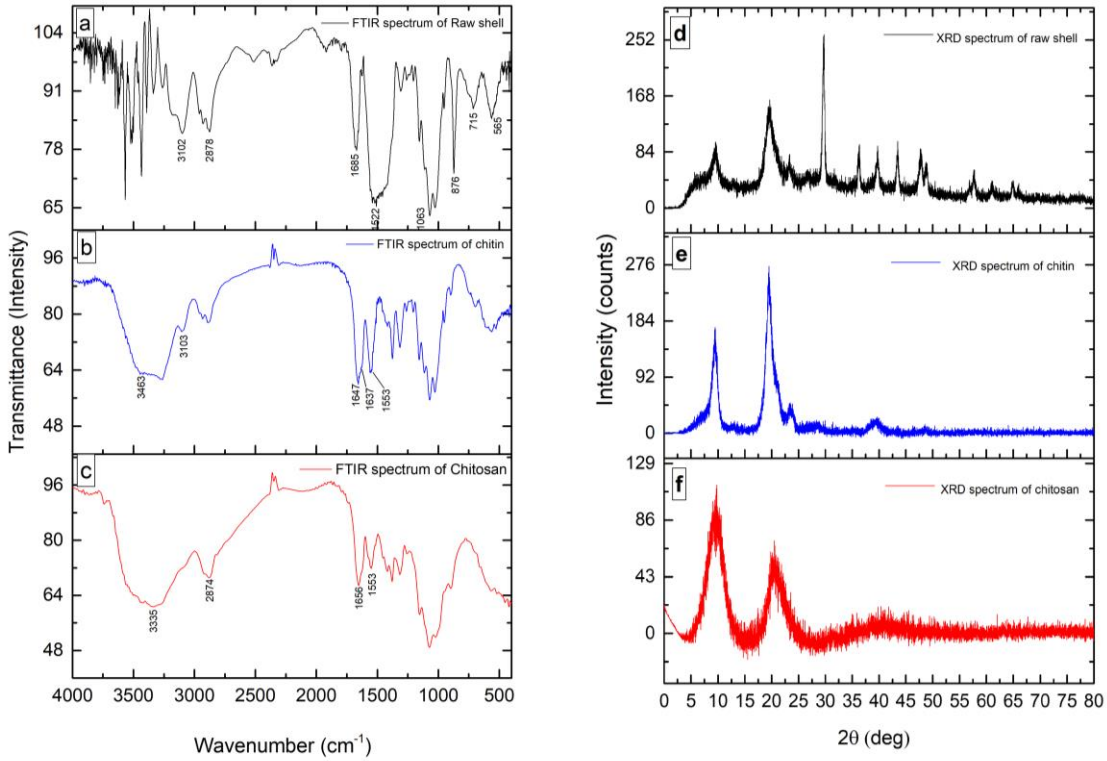


Fig 1 FTIR spectra of spectrum of raw shell (a), FTIR spectra of spectrum of extracted chitin (b), FTIR spectra of spectrum of extracted chitosan (c), XRD spectrum of spectra of raw shell (d), XRD spectrum of extracted chitin (e), XRD spectrum of extracted chitosan (f).

XRD analysis

The XRD spectrum results of whiteleg shrimp raw shell, extracted chitin and chitosan are shown in Figure 1d, 1e and 1f respectively. Numerous peaks relevant to calcite and chitin were observed in the XRD spectrum of raw shells of whiteleg shrimp. In the chitin diffractogram, two sharp crystalline reflections were indicated at 9.43 and 19.67 with two other weak peaks at 23.52 and 38.99. Only two characteristic broad diffraction peaks at (2θ) 10.01 and 20.49 were presented in the XRD pattern of synthesized chitosan from whiteleg shrimp.

SEM and EDS analysis

The morphology of extracted chitosan was studied in different magnifications as shown in Figure 2. Porous structures could be seen in the respective SEM images starting from the magnification 5.00 K X (Figure 2b and 2c). Moreover, crumbling flakes were observed with fibril structures in higher magnifications (Figure. 2d). The EDS result of the extracted chitosan is shown in figure 3. According to the EDS image, carbon, oxygen and nitrogen were presented as the major elements in extracted chitosan and a trace amount of chlorine was present as impurities. However, no calcium peaks were detected in the EDS image.

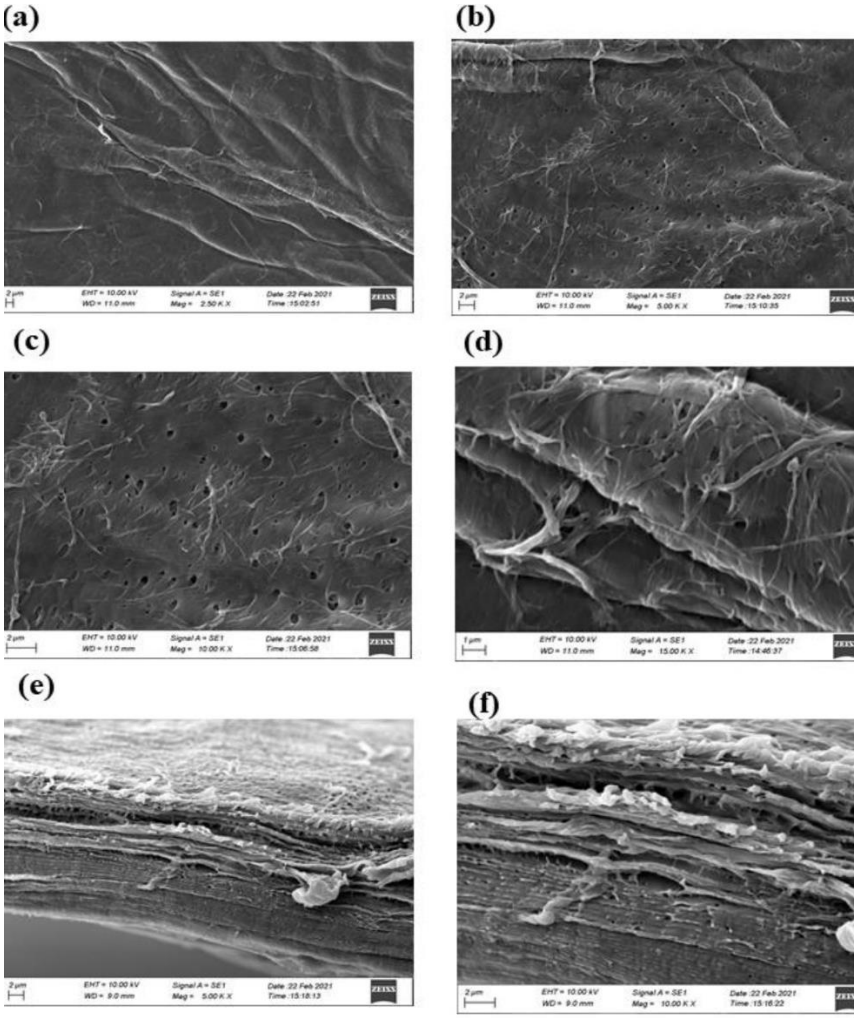
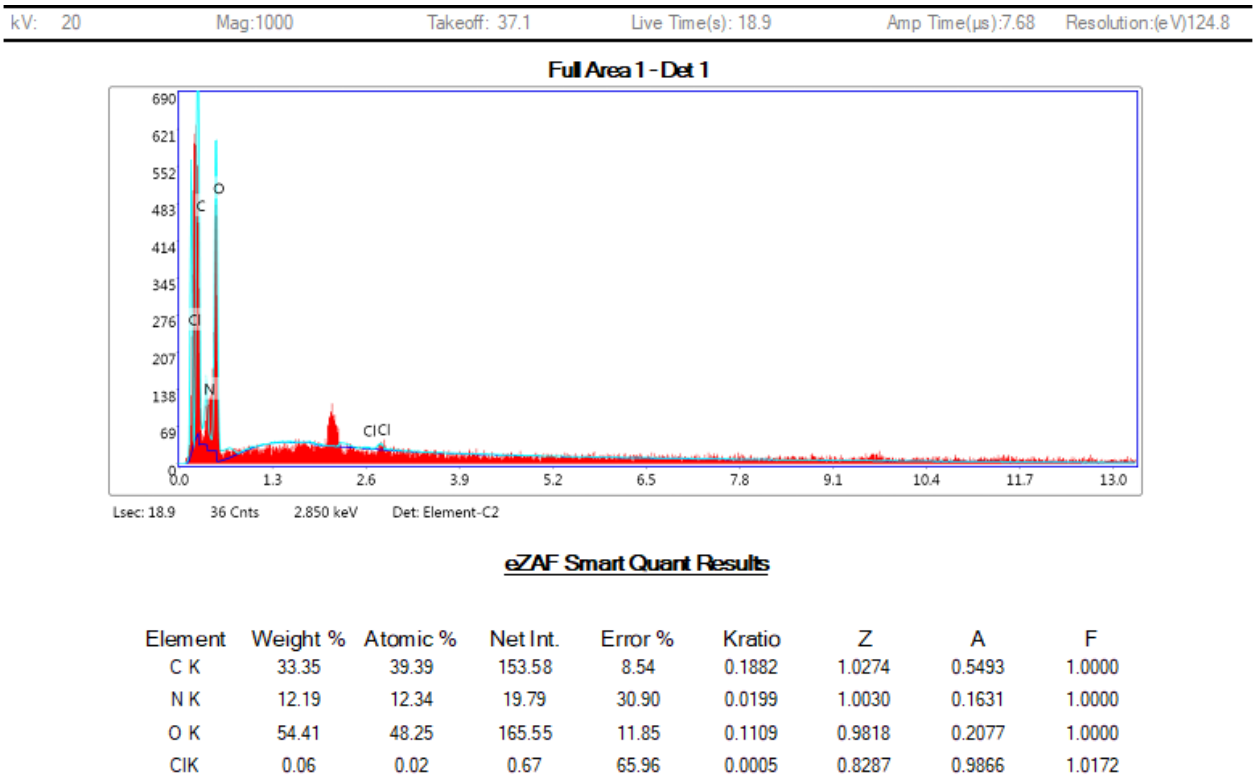


Fig 2 SEM images of extracted chitosan at magnification 2.50 K X (a), 5.00 K X (b), 10.00 K X (c), 15.00K X (d), side elevation view at 5.00 K X (e) and side elevation view at 10.00 K X (f)



eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	33.35	39.39	153.58	8.54	0.1882	1.0274	0.5493	1.0000
N K	12.19	12.34	19.79	30.90	0.0199	1.0030	0.1631	1.0000
O K	54.41	48.25	165.55	11.85	0.1109	0.9818	0.2077	1.0000
ClK	0.06	0.02	0.67	65.96	0.0005	0.8287	0.9866	1.0172

Fig 3 EDS image of extracted chitosan from whiteleg shrimp.

TGA

The TGS analysis was accomplished to inspect the thermal degradation and crystallization of the extracted chitin and chitosan samples and the results are shown in Figure 4. The thermogravimetric curves were obtained at a heating rate of 10 °C min^{-1} under a dynamic atmosphere of nitrogen in the temperature range of 32- 500 °C. The TGA curve of both chitin and chitosan reveals that decomposition takes place in two stages. Degradation of both chitin and chitosan was started in the range of 30-100 °C while the second stage of decomposition occurred in the range of 250-500 ºC. According to the DTA curve of chitin, the major exothermic peak appeared at 360.41 °C (54.48 uV) while in chitosan it appeared at 312.79 °C (28.11 uV).

Dynamic viscosity

Dynamic viscosity was recorded as 435.87 ± 1.03 cP at room temperature which was considerably a high value.

Colour measurement

The colour values of extracted and commercial chitosan are given in Table 2. Extracted chitosan showed lower whiteness values than purified chitin. Whiteness values of chitin and chitosan were reported as 87.61 ± 0.69 and 75.88 ± 0.63 respectively.

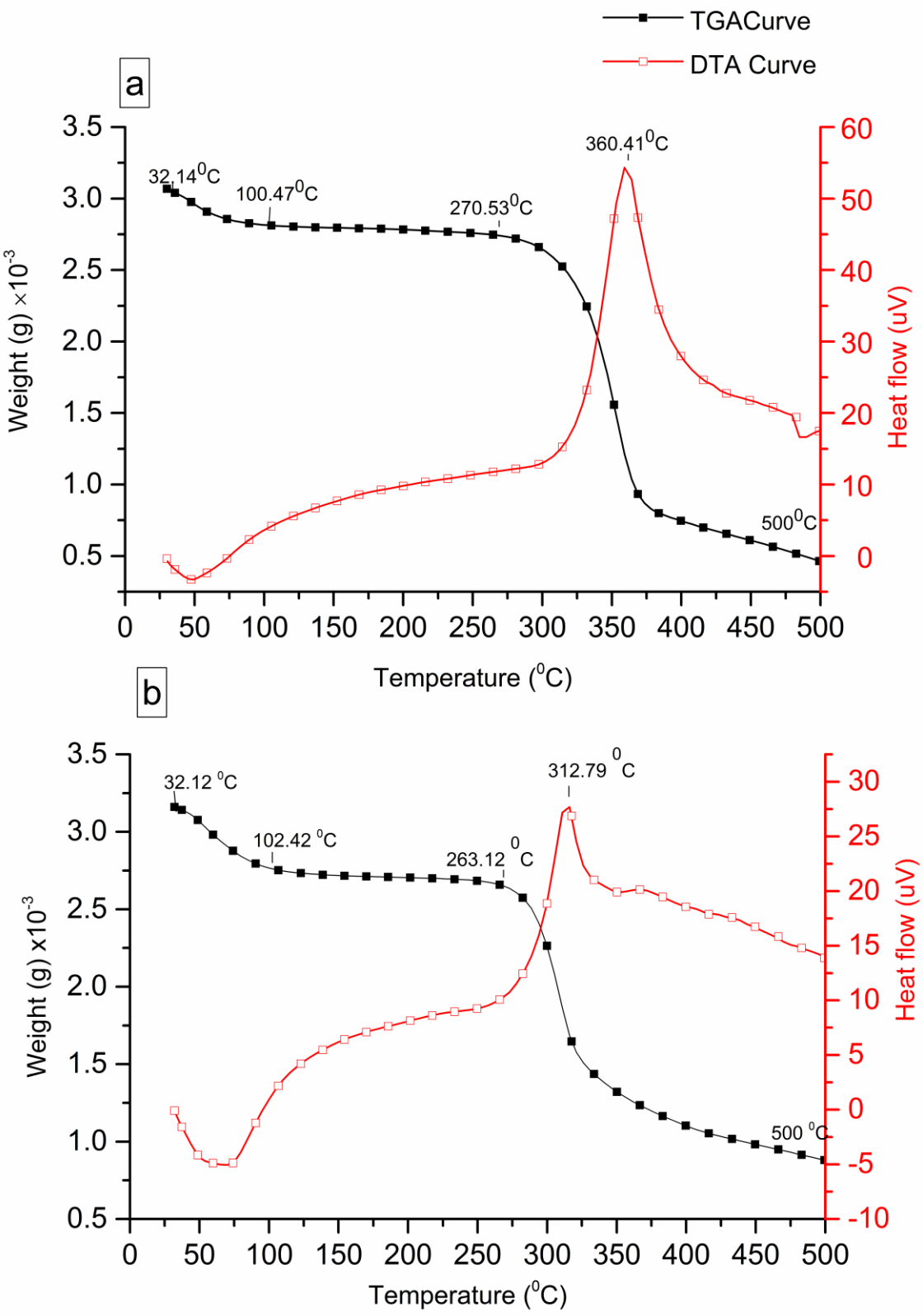


Fig 4 TGA/DTA curve of extracted chitin (a) and chitosan (b) from whiteleg shrimp.

Table 2 Color characteristics of chitin and chitosan synthesized from whiteleg shrimp (mean ± standard deviation of triplicate determinations)

	Extracted chitin	Extracted chitosan	Commercial chitosan (Kucukgulmez et al. 2011)
*L** (lightness)	89.05 ± 0.82	77.45 ± 0.56	83.14 ± 0.62
*a** (redness)	-2.21 ± 0.01	0.29 ± 0.04	0.12 ± 0.00
*b** (yellowness)	5.36 ± 0.09	8.55 ± 0.70	13.58 ± 0.02
Hue	-1.18 ± 0.00	1.54 ± 0.00	13.58 ± 0.02
Chroma	5.80 ± 0.08	8.55 ± 0.70	1.56 ± 0.00
Whiteness	87.61 ± 0.69	75.88 ± 0.63	78.35 ± 0.48

Antioxidant activity; DPPH free radical scavenging assay

According to the results of the DPPH free radical scavenging assay in Figure 5, chitosan is having the capability of scavenging free radicals and it is concentration dependent. The free radical scavenging activity of 66.45% was recorded for the chitosan solution with a concentration of 10 mg/mL. However, these DPPH free radical scavenging activities of chitosan samples were lower compared with the respective values of the reference; L- Ascorbic acid which resulted in 95.98% free radical scavenging activity at 10 mg/mL. The IC_{50} value (Half maximal Inhibitory concentration) of the L-Ascorbic acid was even below 1mg/ml whereas for chitosan it was 6.60 mg/ml.

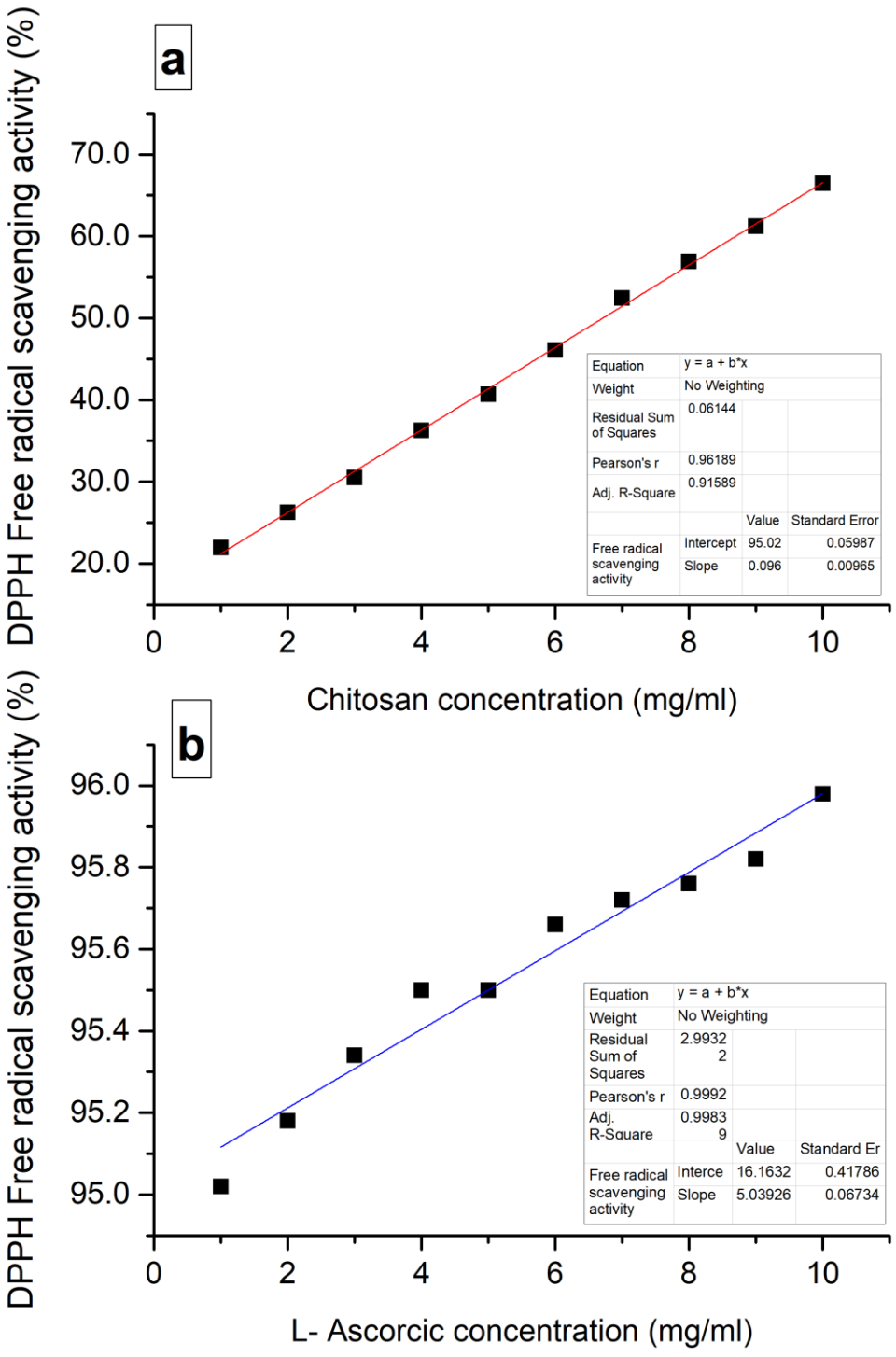


Fig 5 DPPH free radical scavenging activity of extracted chitosan from whiteleg shrimp (a) and L-Ascorbic acid (b)

DISCUSSION

Proximate composition of whiteleg shrimp shells

The proximate values of the present study were slightly different from the results of previous studies. Mittal et al. (2021) reported that whiteleg shrimp shells consist 18.11% of crude ash, 51.29% crude protein and 4.78% crude fat while Hongkulsup et al. (2016) reported 28.40% of ash and 46.76% of crude protein content in whiteleg shrimp shells. Therefore, compared with Mittal et al. (2021) and Hongkulsup et al. (2016), the present study has reported a relatively low amount of crude protein content (29.46%). However, the crude ash content was relatively high in the present study compared with Mittal et al. (2021) while it was comparatively lower compared with Hongkulsup et al. (2016). Further, Binsan et al. (2008) reported 24.24% of crude protein and 9.18% of crude ash content which were lower than the present study. These slight differences in the proximate composition of shrimp shells are possibly due to the difference in species, moulting stage and aquaculture practices, which depend upon the topography, climate, water quality and mainly feed composition as whiteleg shrimp is a culturable species (Mittal et al. 2021). The preparation method of the shell wastes at the processing plants could also affect these changes in proximate composition.

Physicochemical characteristics of extracted chitosan

Yield, moisture and ash content

The present study showed a higher chitosan yield (33.53%) over the previous studies from *Penaues monodon* shells (24.27%) by Sanuja et al. (2017) and *Metapenaeus stebbingi* shells (17.5%) by Kucukgulmez et al. (2011). However, Allwin et al. 2015 have been reported a high yield compared to the present study for *Litopenaeus vannamei* which was 47%. Although fewer studies have been conducted in other countries using this *L. vannamei* for chitosan extraction, anyhow they have not mentioned the obtained yield values (Arancibia et al. 2013, Antonio et al. 2017). Moisture (8.22 ± 0.66%) and ash (1.08 ± 0.24) contents of the extracted chitosan were in the acceptable ranges of commercial chitosan which were >10% moisture and <2.0% ash content (No and Meyers 1995). These moisture values and ash values also agreed with the study of Allwin et al. (2015) which was 7.82% and 0.98% respectively for the chitosan extraction using the same species.

FTIR analysis

The different number of bands with functional groups were detected in the FTIR spectrum of the raw shells of whiteleg shrimp due to the macromolecular character and intermolecular interactions of the polymer compound (Negrea et al. 2015). Broad peaks identified between 3,500 and 3,200 cm^{-1} are assigned to O-H stretching vibrations of water and N-H stretching vibrations of free amino groups (Gbenebor et al. 2017). The peak that appeared at 2,878 cm^{-1} represents the asymmetrically stretching vibration of C-H in the pyranoid ring structure while the peak at 1,653 cm^{-1} is caused by stretching vibrations of C=O (amide-I). Absorptions in the region of 1,200- 900 cm^{-1} are attributed to complicated vibrations mostly due to C-O and C-C stretching motions and specific band assignment is not shown in the literature for this region (Ghimire et al. 2011).

In isolated prawn shell chitin, O-H stretching vibration appeared as prominent broadband at 3,463 cm^{-1} due to the presence of extensive intermolecular hydrogen bonding in the formation of chitin (Ghimire et al. 2011). In the present study, multi-bands were observed in the range of 3,300- 3,000 cm^{-1} which is assigned to N-H stretching vibrations in chitin. Formation of either dimer with cis- or trans-conformation of secondary amides may have caused these multiple bands as suggested by Ghimire et al. (2011). In the chitin FTIR spectrum, amide-I band has been split into two components at 1,647 cm^{-1} and 1,637 cm^{-1} confirming that the extracted chitin had α form in major (Kumirska et al. 2010; Kumari et al. 2015; Suneeta et al. 2016). Two types of hydrogen bonds formed by amide groups in the antiparallel alignment present in α-chitin crystalline regions give rise to doublet in amide-I band. A single strong peak at 1554 cm^{-1} represents the NH bending vibration, known as the amide-II band (Ghimire et al. 2011; Suneeta et al. 2016).

In the formation of chitosan, the amide of chitin is replaced by the amine (NH_2) group, thereby increasing the hydrogen bonding in chitosan

compared to chitin. Therefore, a much broader hump in the frequency range of 3,335 cm^{-1} with no distinct peaks for O-H and N-H stretching was observed in the FTIR spectra of chitosan. Due to the depressing of amide content in chitosan, considerable band broadening of amide-I was observed in the frequency 1,656 cm^{-1}. However, the amide peak was not completely lost as chitin is partially deacetylated. Coupling of NH bending vibrations of NH_2 group in chitosan with amide-II band may account for the broadband in the frequency of 1,553 cm^{-1} (Ghimire et al. 2011). The degree of deacetylation was 80.43% and it was a comparatively high value with past studies which reported a degree of deacetylation of 40% by Sanuja et al. (2017), 61% by Kumari et al. (2015) and 74.82% by Mohanasrinivasan et al. (2014).

XRD analysis

Calcite peaks that appeared in the XRD of raw shells of whiteleg shrimp were absent in the chitin diffractogram and it was closely agreed with the results of Gbenebor et al. (2017). The absence of calcite peaks in chitin further verifies effective demineralization in the extraction process of chitin. Two characteristic broad diffraction peaks at (2θ) 10.01 and 20.49 in the XRD pattern of synthesized chitosan from whiteleg shrimp represent the typical fingerprints of semicrystalline chitosan which has been resulted by all most all the past studies related to chitosan extraction from shellfish waste (Mohanasrinivasan et al. 2014; He et al. 2016)

SEM and EDS analysis

Porous structures that could be observed in the SEM image of extracted chitosan have resulted from the removal of $CaCO_3$ by the acid treatment (Antonino et al. 2017). Similar morphological images were acquired for the chitosan extracted from crab chitin by Yen et al. (2009) and Kucukgulmez et al. (2011).

According to the EDS image, no calcium peaks were detected in the EDS image, thereby it can be concluded that the extraction process had undergone an effective demineralization. The nitrogen content of the molecule should be higher than 7% by weight to be confirmed as chitosan (Shahidi and Abuzaytoun 2018). By evidencing the Nitrogen content of the extracted compound as 12.19% through the EDS image, it further agreed with the literature regarding that aspect as well.

TGA

The first stage of degradation of both chitin and chitosan was recognized at around 30-100 °C and that may be resulted due to evaporation of water molecules from the samples. The second stage of decomposition occurred in the range of 250-500 ºC due to the degradation of the saccharide structure of the molecules by dehydration or the deamination process (Kumari et al. 2017; Kumar et al. 2018). Results confirmed that the chitin has good thermal stability up to 360 °C as its DTA curve shows the major exothermic peak at 360.41 °C (54.48 uV). Chitosan almost decomposes around 312 °C as its DTA curve shows the major exothermic peak at 312.79 °C (28.11 uV). Similarly, Abdou et al. (2007) also ended up with chitin and chitosan from shells of freshwater lobster (crayfish) which are having thermal stabilities of 372°C and 303°C respectively. In the study of Kumar et al. (2018) chitosan almost decomposes around 284°C (66.9 uV) which was slightly lower thermal stability than the value observed in the present study. However, they discovered that the thermal stability of chitosan can be further increased up to 481°C and 469°C by grafting pure chitosan with [Chit-g-Poly (AA-co-An)] and crosslinking with [Chit-cl-Poly (AAco-An)] respectively.

Dynamic viscosity

Dynamic viscosity of the extracted chitosan at room temperature was recorded as 435.87 cP which was considerably higher than previous studies of chitosan extraction (Jeon et al. 2002; Kucukgulmez et al. 2011). These kinds of high viscous chitosan solutions have a better preservative coating ability than low viscous chitosan solutions (Jeon et al. 2002). Chitosan with a maximum viscosity of 360 cP was attained by using 50% NaOH at 100°C for 4hrs of deacetylation in the study of Jeon et al. (2002) which was comparatively lower than the present value that may result in increased alkali concentration as explained by Cheng et al. (2020). Kucukgulmez et al. (2011) also resulted from nearly the same viscosity for the chitosan extracted from mud crab shells (383.9 cP) and commercial crab chitosan (463.25 cP). The viscosity of chitosan is

highly reliant on the degree of deacetylation and molecular weight of chitosan (Kucukgulmez et al. 2011). Generally, it is considered that chitosan with a higher degree of deacetylation and longer molecular chains have considerably high viscosity values (Cheng et al. 2020).

Colour measurement

The colour values of extracted chitosan were compared with that of commercial chitosan in Table 2. The colour of chitosan is associated with the carotenoid pigment astaxanthin. These pigments were removed in the decolorization step using oxalic acid and potassium permanganate. However, the hue and whiteness values of the synthesized chitosan were comparatively lower than commercial chitosan (Tabel 2). Extracted chitosan showed lower whiteness values than purified chitin as deacetylation made chitosan beige to brown due to its harsh chemical treatment in a hot alkali medium. Similar whiteness values (74.69) were reported in Yen et al. (2009) with the same decolourization treatment for crab shells and in Kucukgulmez et al. 2011 with hydrogen peroxide treatment for *Metapenaeus stebbingi* shrimp shells.

Antioxidant activity; DPPH free radical scavenging assay

Antioxidants are widely used in food industries to overcome the off flavours and rancidity issues related to free radicals mainly in foods with highly unsaturated food lipids. As commercially available antioxidants have potential health hazards, scientists are interested in novel natural antioxidant substances.

DPPH free radical scavenging assay is a widely used method for assessing the antioxidant activity of natural extracts. Antioxidants would provide hydrogen to DPPH free radicals converting them to more stable products and terminating their radical chain reaction. This neutralisation reaction would decolourise DPPH into light purple or pale yellow depending on the antioxidant capacity of the substance and it verifies by reducing the absorbance at 517 nm (Younes et al. 2014).

As the solution of chitosan has a concentration of 10 mg/mL resulting in 66.45% DPPH free radical scavenging activity it can be suggested that chitosan could be effectively applied as a natural antioxidant agent as an alternative to synthetic antioxidants which could create potential health hazards. However, the DPPH free radical scavenging activity of the chitosan sample was lower compared with that of the L- Ascorbic acid (reference sample) at the same concentrations. At a concentration of 10 mg/ml, DPPH free scavenging activity of L-Ascorbic acid was almost 95.98% while those of chitosan was only 66.45%.

Several previous records are also available in literature considering the free radical scavenging activity of chitosan. Trung and Bao (2015) tested the antioxidant activity of chitosan prepared from white shrimp waste by DPPH free radical scavenging activity and have been discovered that it varied from 3.7 to 16.8%, which corresponds to 1-2 mg/mL (Trung and Bao 2015). Younes et al. (2014) have resulted in 75% DPPH free radical scavenging activity at 5 mg/ml of their extracted chitosan solution which was higher than the values of the present study. Nevertheless, the study of Yen et al. (2007) obtained chitosan with 35.4–44.3% of DPPH free radical scavenging activity for fungal chitosan at 10mg/ml chitosan concentration which was comparatively lower than the values reported in the present study.

Variations in the antioxidant capacity of chitosan may depend on its degree of deacetylation and molecular weight. Kim (2018) highlights that chitosan with a high degree of deacetylation and low molecular weight shows better antioxidant capacity compared to that chitosan with low molecular weight and high molecular weight.

The mechanism behind this free radical scavenging activity has been explained by Siripatrawan and Harte (2010) and Trung and Bao (2015). Free radicals may react with residual free amino (NH_2) groups of chitosan to form a stable molecule and the NH_2 groups can form ammonium (NH_3^+) groups by absorbing a hydrogen ion from the solution (Trung and Bao 2015). However, strong intra-molecular and inter-molecular hydrogen bonds in chitosan make it difficult to dissociate its OH and NH_2 groups to bind with free radicals.

Therefore, it has also been suggested that various modifications to chitosan molecules to improve the antioxidant activity were accomplished by grafting functional groups into the molecular structure (Kim et al. 2018). The antioxidant activity of extracted chitosan can be improved by grafting chitosan with polyphenols and essential oils as

suggested by previous trials (Yuan et al. 2016). Chitosan films incorporated with green tea extract by Rahman (2017) have been tested as a product having excellent antioxidant properties. Taking these suggestions from literature, extracted chitosan in the present study also can be further developed.

CONCLUSION

The potency of using the shell waste of whiteleg shrimp (*Litopenaeus vannamei*) for chitosan extraction, which has not been considered in Sri Lanka heretofore, reveals that chitosan can effectively be extracted from whiteleg shrimp shell waste with standard quantities (yield 33.53%) and acceptable physicochemical properties (Moisture content: 8.22%, Ash content: 1.08%, DDA: 80.43%, Dynamic viscosity: 435.87 ± 1.03 cP, Whiteness value: 75.88, Thermal stability: up to 312 °C, Antioxidant activity: 66.45%) qualifying to be used in various future applications. Suggesting whiteleg shrimp waste as a source of chitosan extraction at an industrial scale would also provide a solution to the accumulative waste generated from processing plants worldwide, which often becomes a practical challenge for the industry as well as for the community health. However, a year-round investigation of the chitosan yield and its characteristics need to be further analysed together with a cost-benefit analysis for a chitosan-based industry.

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