


# Recovery of protein hydrolysate and chitosan from black tiger shrimp (*Penaeus monodon*) heads: approaching a zero waste process

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**Abstract** Shrimp heads are considered as a potential source for the recovery of many valuable components such as chitin, protein and carotenoids. In the present study, both protein hydrolysate and chitin/chitosan were recovered using combination of physical, biological and chemical treatments. Shrimp heads were separated from liquid phase by a facile and efficient physical pretreatment. The liquid fraction was then hydrolyzed using formic acid and vacuum concentrated to obtain an astaxanthin-rich protein hydrolysate. The solid fraction was used to produce chitin by deproteination and demineralization. The hydrolysate consisted of a high astaxanthin (192 ppm) and protein (26.3 wt%) content. Chitosan prepared from chitin showed a high degree of deacetylation (85.4%) with low protein (0.25 wt%) and mineral (0.22 wt%) contents. The relative crystalline structure of the chitin and chitosan were 54.7 and 10.4%, respectively. The deacetylation of chitin was confirmed by Fourier Transform Infrared Spectroscopy. The present procedure approached to produce maximum

valuable components including astaxanthin-rich protein hydrolysate and chitin/chitosan from shrimp head waste.

**Keywords** Shrimp head · Astaxanthin · Protein hydrolysate · Chitosan · Zero waste

## Introduction

In the shrimp processing industry, the solid waste such as heads and body shells can be generated approximately 48–56 wt% of whole shrimp weight depending on the species (Sachindra et al. 2006). The shrimp waste consists of various components (dry weight basis) including protein (35–50 wt%), chitin (15–25 wt%), minerals (10–15 wt%) and carotenoids (Sachindra et al. 2006). Up to date, many studies have been made to produce chitin/chitosan, protein hydrolysate and carotenoids from shrimp wastes. However, researchers mostly focused on recovery of either chitin/chitosan (Teng et al. 2001; Cira et al. 2002; Percot et al. 2003; Sini et al. 2007; Ghorbel-Bellaaj et al. 2012; Younes et al. 2012) or protein hydrolysate and carotenoids (Sachindra et al. 2006; Guerard et al. 2007; Ogawa et al. 2007; Babu et al. 2008; Handayani et al. 2008; Sowmya et al. 2014). In the production of chitin/chitosan, protein, carotenoids and lipids are often discarded. Inversely, raw materials for chitin/chitosan production are usually considered as waste in the preparation of protein hydrolysate and/or carotenoids. Consequently, each procedure may cause the loss of some valuable components as well as environmental pollution. Therefore, it is necessary to develop efficient procedures for recovering both chitin/chitosan and valuable components (i.e. proteins, lipids and carotenoids) from shrimp waste, which are

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considered both environmentally and economically attractive.

Recently, Cahu et al. (2012) has attempted to recover both protein hydrolysate and chitin by crushing, enzyme treatment, and filtering processes. Chitosan was then prepared from chitin by chemical treatment using HCl, NaOH and  $\text{KMnO}_4$  for demineralization, deproteinization, and depigmentation, respectively. This procedure can be carried out to obtain all of the available biomolecules but large amount of chemicals and unique equipment for filtering were required that may limit it for industrial application. Moreover, it reported that the molecular weight of chitosan was considerably reduced and it was difficult to recover chitin after crushing treatment of shrimp shells (No and Meyers 1999; Gildberg and Stenberg 2001). Additionally, this process also has negative effects on the quality of protein hydrolysate due to the contamination of non-protein components such as chitin particles that limit its applications. This treatment also leads to a lower yield of chitin production and may have a negative impact on the quality of chitin and its derivative-chitosan (Aye and Stevens 2004). Moreover, it is also difficult to separate liquid fraction from solid fraction after gridding process.

Based on the above mentioned limitations, the present study presented an integrated method using a facile physical pretreatment in order to separate the raw material into liquid and solid fractions. These fractions were converted to valuable products including chitin, chitosan and astaxanthin-rich protein hydrolysate using efficient biochemical treatments. This procedure is environmentally as well as economically attractive as it is a zero-waste process.

## Materials and methods

### Materials

The black tiger shrimp (*Penaeus monodon*) heads were obtained from a local company (Nha Trang Seafoods-F17, Ltd, Nha Trang, Vietnam). The fresh heads were packaged into polyethylene bags and iced in Styrofoam boxes and stored at  $-20\text{ }^\circ\text{C}$  until use. All chemicals are analytical-grade and used as received.

### Pretreatment of fresh shrimp heads

The entire procedure for recovering protein hydrolysate and chitin/chitosan is showed in Figure S1. Pretreatment of fresh shrimp heads was conducted by a modified previous work (Minh et al. 2016). Firstly, fresh shrimp heads were pressed to separate them into two fractions (liquid 1 and solid 1) at ratio of liquid/solid 50:50. The solid 1 was deproteinized using Alcalase at different concentrations of

0.20, 0.25, 0.30, 0.35 and 0.40% (v/w) at  $55\text{ }^\circ\text{C}$  for 2, 4, 6 and 8 h. After that, the mixture was also separated into liquid 2 and solid 2 fractions. The liquid 1 and liquid 2 were combined and used for recovery of astaxanthin-rich protein hydrolysate and the solid 2 fraction was used for the production of chitin.

### Preparation of astaxanthin-rich protein hydrolysate

The astaxanthin-rich protein hydrolysate was prepared using a modified previous work (Phuong and Luyen 2013; Phuong et al. 2016). The liquid mixture (liquid 1 and liquid 2) was hydrolyzed using formic acid at different concentrations of 3, 4, 5 and 6% (v/w) at  $30\text{ }^\circ\text{C}$  for 6, 12, 18, 24, 30 and 36 h. The hydrolyzed liquid was then concentrated at a vacuum pressure of 600 mmHg at  $60\text{ }^\circ\text{C}$  for 4 h.

### Preparation of chitin and chitosan

The solid 1 and solid 2 fractions were used for chitin and chitosan production (Younes et al. 2012; Rao et al. 2000). Firstly, the solid waste was demineralized in 3% HCl solution at  $50\text{ }^\circ\text{C}$  for 10 h. Subsequently, the samples deproteinized in 2% NaOH solution at  $70\text{ }^\circ\text{C}$  for 12 h to obtain chitin. Finally, chitin was deacetylated using a 50% NaOH solution at  $90\text{ }^\circ\text{C}$  for 12 h to prepare chitosan.

### Proximate analysis of astaxanthin-rich protein hydrolysate

Moisture, protein and ash content were determined using AOAC (1990) methods. Lipid content was determined by the Bligh and Dyer (1959) method. The soluble protein content in the astaxanthin-rich protein hydrolysate was measured using the Biuret method, as described by Kelleher and Hultin (1991) and calculated by the standard curve prepared from BSA. The total volatile nitrogen (TVN) was determined using a distillation method (Smith and Hole 1991). Astaxanthin was determined according to the method of Simpson and Haard (1985). The degree of hydrolysis (DH) was determinate by spectrophotometric method of Morais et al. (2013).

### Characterization of chitin and chitosan

The viscosity of the chitosan solution was measured using a Brookfield viscometer at  $20\text{ }^\circ\text{C}$ . The average molecular weight ( $M_w$ ) of chitosan was determined according to the method described by Rinaudo (2006). The crystallinity and chemical structure of the chitin and chitosan was characterized by Fourier transform infrared spectroscopy (FTIR, Nicolet iS10, Thermo Scientific) over  $548\text{--}4000\text{ cm}^{-1}$  at a resolution of  $16\text{ cm}^{-1}$  with 32 scans using a diamond ATR

attachment and X-ray diffractometer (XRD, PANalytical, X'Pert-PRO MPD) with Cu K $\alpha$  radiation. The relative crystallinity ( $X_{Crl}$ ) of chitin and chitosan was calculated by dividing the area of the crystalline peak (19.6°) by the total area under the curve as follows (Ioelovich 2014):

$$X_{Crl} = F_{Crl} / (F_{Crl} + F_{am})$$

where  $F_{crl}$  is the area of the crystalline scattering,  $F_{am}$  is the area of the amorphous scattering.

### Statistical analysis

A statistical analysis of the data was performed using Minitab 2000 Version 13.2 statistical software (Minitab Inc. PA, USA). Means were compared by one-way ANOVA, and Tukey's Multiple Comparisons. The statistical difference was defined at  $p < 0.05$ .

## Results and discussion

### Chemical composition of shrimp heads, liquid and solid fractions after pretreatment

Table 1 shows the composition of raw shrimp head and two phases after pressing step. The shrimp heads consisted of water (81.6% wet basis), chitin (16.5% dry basis, db), astaxanthin (169.2 ppm), protein (52.3% db) and lipid (11.3% db). The results indicate that shrimp heads are a potential source for the recovery of both chitin/chitosan and astaxanthin-rich protein hydrolysate. After the pressing process, the astaxanthin and protein contents in the liquid fraction were of 335.4 ppm and 53.1% (db), respectively. This reveals that considerable amounts of protein (53.2% db) and astaxanthin (132.2 ppm) still remained in the solid fraction. Therefore, one more pretreatment is needed to

**Table 1** Chemical composition of shrimp heads and liquid and solid fractions

Parameter	Shrimp heads	Liquid fraction	Solid fraction
Water content (%)	81.6 ± 1.2	93.2 ± 1.5	71.7 ± 2.3
Protein <sup>a</sup> (%)	52.3 ± 1.4	50.2 ± 1.2	53.2 ± 1.7
TVN (mg/100 g)	–	35 ± 5.3	–
Astaxanthin <sup>a</sup> (ppm)	169.2 ± 23.0	335.4 ± 30.0	132.2 ± 15.0
Ash <sup>a</sup> (%)	22.4 ± 0.5	2.8 ± 0.1	26.5 ± 0.3
Lipid <sup>a</sup> (%)	11.3 ± 0.6	–	–
pH	–	7.3 ± 0.2	–
Chitin <sup>a</sup> (%)	16.5 ± 1.4	2.5 ± 0.2	19.5 ± 1.2
Yield (%)	–	47.3 ± 2.5	52.4 ± 1.8

(–): No analysis

<sup>a</sup> Based on dry basis

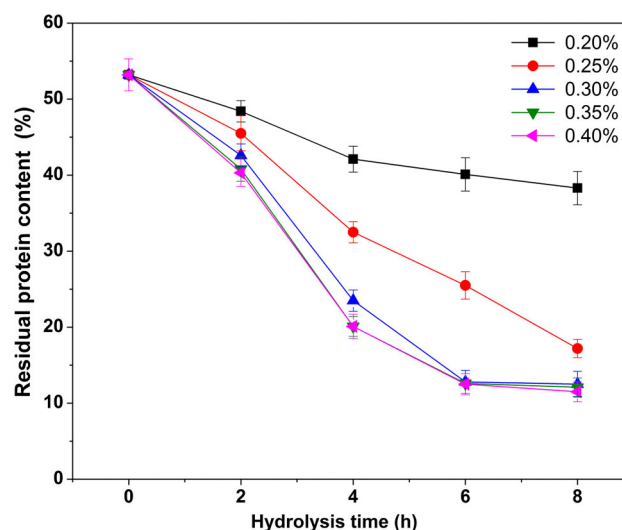
recover these valuable components before transferring to produce chitin/chitosan.

### Recovery of protein and astaxanthin in solid fraction by Alcalase

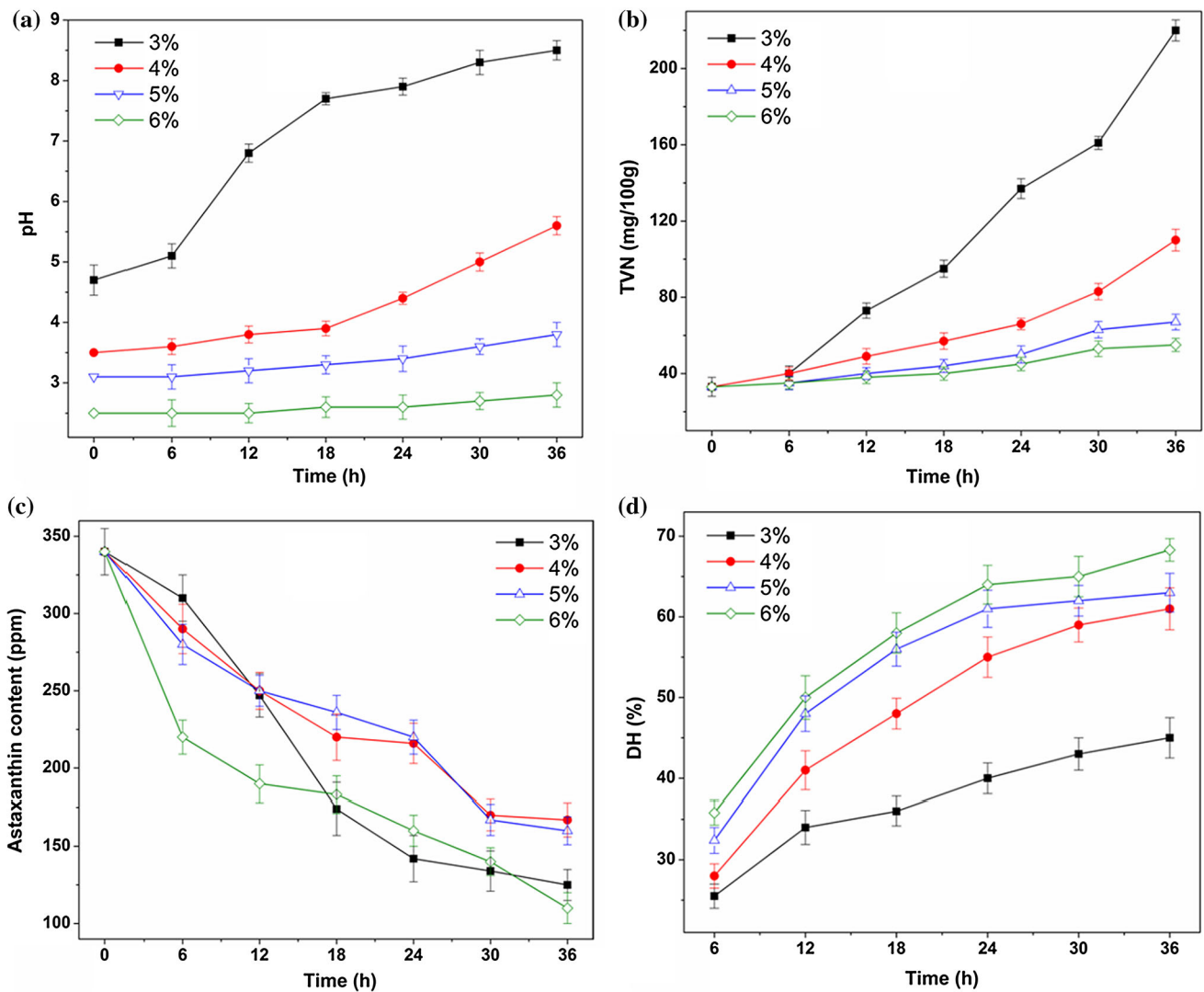
The effects of the enzyme concentration and hydrolysis time on the deproteinization efficiency were showed in Fig. 1. The deproteinization efficiency increased with increasing enzyme concentration in the range of 0.20–0.30% (v/w). However, it was unchanged from 0.30 to 0.40% (v/w). After treatment for 8 h, the residual protein contents in the solid fraction were 38.3; 17.2; 12.5, 12.1 and 11.5% for 0.20, 0.25, 0.30, 0.35 and 0.40% (v/w), respectively. Regarding the hydrolysis time, the residual protein content decreased significantly during the first 6 h of hydrolysis process but it was unchanged from 6 to 8 h. Therefore, it can be concluded that the optimal enzyme concentration and hydrolysis time were of 0.30% (v/w) and 6 h.

### Optimization conditions for preparation of astaxanthin-rich protein hydrolysate

The effects of the formic acid concentration and hydrolysis time on the pH and astaxanthin content of protein hydrolysate is presented in Fig. 2. After pressing, the initial pH value of the liquid fraction was 7.3 but it decreased significantly after adding 3, 4, 5 and 6% (w/w) formic acid (Fig. 2a). The pH values of the samples treated with 5 and 6% slightly increased throughout the hydrolysis process. However, a significant increase in pH was observed in the samples treated with 3 and 4% formic acid. The increase in



**Fig. 1** Effect of Alcalase enzyme concentration on the residual protein content in the solid fraction prepared from shrimp heads



**Fig. 2** Effect of formic acid concentration and hydrolysis time on **a** pH, **b** astaxanthin content of the hydrolysate, **c** DH, and **d** TVN content of the hydrolysate

pH may be due to the protein degradation caused by enzymatic and microbial activities. The results were in accordance with the increase in TVN content (Fig. 2b). For 5 and 6% formic acid treatments, the pH values were of 3.8 and 2.8, respectively, leading to inactive enzymes, thus inhibiting microbial growth. It is well-known that acidic pHs have an antibacterial activity (Ramasubburayan et al. 2013).

The astaxanthin content of all samples decreased significantly during the hydrolysis process by treating with different amounts of formic acid (Fig. 2c). The initial astaxanthin content of the liquid fraction was 338 ppm. However, it was 125, 167, 160, and 110 ppm after hydrolysis for 36 h with 3, 4, 5 and 6% (w/w) formic acid, respectively. It reported that acids and alkalis can cause the *cis/trans* isomerisation of double bonds in astaxanthin structure, changes in functional groups and de-

esterification (Melendez et al. 2006). Moreover, astaxanthin are exceptionally labile in alkaline medium. Therefore, in this case, it could be concluded that formic acid 5% was suitable for hydrolysis to obtain astaxanthin-rich protein hydrolysate.

The degree of hydrolysis (DH) increased remarkably with an increase in the hydrolysis time and formic acid concentration (Fig. 2d). Almost DH values increased 1.5–2 times after acid treatment for 12 h and then increased slowly up to 36 h. A significant difference in DH values were found between the samples treated with 3, 4 and 5% (w/w) formic acid. However, no significant difference in DH values was observed between the 5 and 6% (w/w) formic acid treated samples. The increase in DH is believed to be attributed to the hydrolysing activity of formic acid. Organic acids have been proved to hydrolyze proteins and peptides by cutting the peptide bonds (Connell 1995). The

TVN content of all samples increased with an increase in hydrolysis time and formic acid concentration (Fig. 2d). The TVN content of the samples treated with 5 and 6% (w/w) formic acid increased slightly during the first 30 h of hydrolysis process and increased rapidly during the subsequent hydrolysing time. But a remarkable increase in TVN content was observed in the 3 and 4% formic acid treated samples throughout the hydrolysis process. The increase in TVN could be attributed to the trimethylamine oxide (TMAO) breakdown catalyzed by endogenous enzymes (trimethylamine oxide demethylase—TMAOase) present in the samples as well as by bacterial activity (Lauzon et al. 2009). Both H<sub>2</sub>S-producing bacteria, mainly *Shewanella putrefaciens*, and *P. phosphoreum* are able to reduce TMAO to TMA but the latter is usually a considerable fraction of TVN (Cira et al. 2002). With 5 and 6% (w/w) formic acid treatments, the enzymes and bacteria were inhibited, resulting in lower TVN values as compared to those of other samples. It can be concluded, from the results, that the optimal hydrolysing time was 30 h.

### Properties of astaxanthin-rich protein hydrolysate

The astaxanthin-rich protein hydrolysate solution was concentrated using a vacuum concentrator at a pressure and temperature of 600 mmHg and 60 °C, respectively. Correspondingly the chemical composition, amino acid profile and microbial attributes of concentrated astaxanthin-rich protein hydrolysate are shown in Tables 2, S1 and S2. The astaxanthin content (dry weight basis) was 192.0 ppm and was 52.1% for the protein content. The DH of the protein was 65.0%, indicating that the majority of proteins were hydrolyzed by the formic acid. The results are in accordance with a high protein solubility value (26.3%). The concentrated astaxanthin-rich protein hydrolysate contained 8 essential amino acids (Tyr, Met, Lys, Arg, Phe, Leu, Isoleu and Cys); in which, arginine and leucine were dominant with values of 3.92 and 3.56%, respectively

**Table 2** Properties of concentrated astaxanthin-rich protein hydrolysate

Parameter	Content
Dry matter (%)	36.5 ± 1.2
Protein <sup>a</sup> (%)	52.1 ± 0.8
Ash <sup>a</sup> (%)	8.7 ± 0.3
Lipid <sup>a</sup> (%)	11.5 ± 0.4
Astaxanthin (ppm)	192.0 ± 12.0
pH	4.0 ± 0.1
DH (%)	65.0 ± 1.6
TVN (mg/100 g)	58.0 ± 5.0
Soluble protein (%protein)	26.3 ± 0.4

<sup>a</sup> Based on dry basis

(Table S1). With regard to microbial quality, the concentrated astaxanthin-rich protein hydrolysate meets the Vietnamese Standard for aquafeeds (Table S2). Therefore, the prepared product has potential for use in aquatic feed production.

### Properties of chitin and chitosan

The properties of the chitin and chitosan are described in Table 3. The chitin and chitosan had a high quality with low protein and mineral content; high degree of deacetylation (DD) and viscosity values. The protein content of chitin was about 0.43% and it decreased to 0.25% after deacetylation. The solubility of chitosan was high (99.5%) and the turbidity was low (8.2 NTU). These characteristics were better compared to those of purified chitosan prepared by enzyme (Trang and Pham 2012). Particularly, the DD value of prepared chitosan reached to 85.4% that was comparable to the chitosan prepared from this shrimp species by enzyme treatment (Trang and Pham 2012). The average molecular weight of commercial chitosan is in the range of 100–1000 kDa with a DD in the range of 60–90% and a viscosity of a solution of chitosan in acetic acid 1% in the range of 200–2000 (Li et al. 1992). Thus, the prepared chitosan from the present study can be a good candidate for application in food and agriculture industry.

The crystal structures of prepared chitin and chitosan were characterized by XRD measurements (Fig. 3a). Two clear peaks were observed in the spectrum of chitin at 2θ of 10.1° and 20.0°, which indicate a high crystallinity of prepared chitin. However, the peak at 10.0° was disappeared, suggesting an increase in amorphous property in chitosan after the deacetylation process. The  $X_{CrI}$  of the chitin and chitosan were 54.7 and 10.4%, respectively, which confirmed the reduced crystalline structure of chitosan.

The FTIR spectra of chitin and chitosan are showed in Fig. 3b, c. For the spectrum of chitin, main peaks according to the characteristic stretching vibration bands at around 3400 (O–H), 2913 (C–H), 1654 (amide I), 1553 (amide II), 1368 (C–H), and 950–1200 cm<sup>-1</sup> (C–O–C and

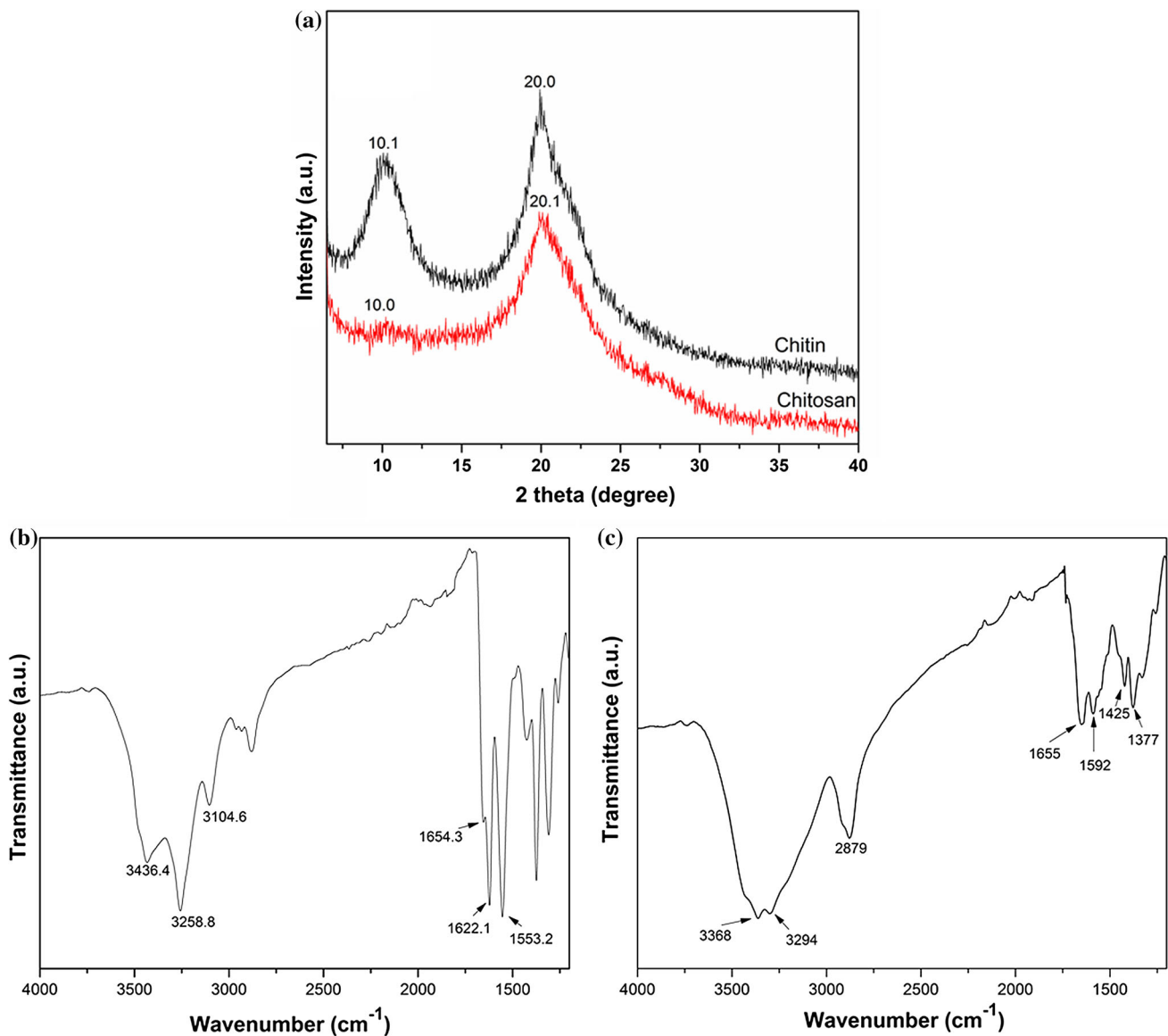
**Table 3** Properties of prepared chitin and chitosan

Properties	Chitin	Chitosan
Protein (%)	0.43 ± 0.08	0.25 ± 0.06
Minerals (%)	0.57 ± 0.07	0.22 ± 0.08
DD (%)	8.20 ± 2.40	85.40 ± 2.10
Viscosity <sup>a</sup> (cP)	–	620 ± 33
Molecular weight (kDa)	–	450 ± 25
Solubility <sup>a</sup> (%)	–	99.5 ± 0.05

(–) No analysis

<sup>a</sup> Determined in a solution of 1 wt % chitosan in 1% acetic acid





**Fig. 3** a XRD patterns of prepared chitin and chitosan and FTIR spectra of **b** chitin and **c** chitosan prepared from black tiger shrimp heads

C–O) were observed. The results were in accordance with those published by Rinaudo (2006). For the spectrum of chitosan, similar main peaks attributed to the characteristic stretching vibration bands at around 3400 (O–H), 2879 (C–H), 1655 (amide I), 1377  $\text{cm}^{-1}$  (C–H) were found. A very small peak at 1592  $\text{cm}^{-1}$  (amide II) were found. This indicates that the deacetylation process was mostly completed.

## Conclusion

Astaxanthin-rich protein hydrolysate and chitosan products could be obtained from shrimp heads using a facile pre-treatment before biochemical treatments. The optimal hydrolysing conditions for the liquid fraction were of 5%

(w/w) formic acid for 30 h. Both astaxanthin-rich protein hydrolysate and chitosan products were of high quality and met the requirements for use both in animals feeds and in the human food industry. Therefore, the present approach can be seen as a zero waste process and applied in a large scale industry for recovery of astaxanthin-rich protein hydrolysate and chitosan.

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