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Short Communication

Length-based variations in deposition of chitin in the exoskeleton of *Penaeus monodon* and *P. indicus*

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Abstract Shrimp waste produced at the processing factories is a burden to the environment. Nevertheless, it is a raw material for extraction of chitin which is with more commercial uses. In this study chitin was extracted from shrimp waste collected from three different length ranges (5-10cm, 10-15cm, 15-20cm) of *Penaeus monodon* and *P. indicus* from a local small-scale processing factory, to determine the most suitable length class of shrimps to obtain a higher chitin yield. Dried shrimp waste was preconditioned by soaking in acetic acid, demineralized by treating with HCl and deproteinized by treating with NaOH. Extracted chitin was odourless, and pinkish white and off-white in *P. monodon* in *P. indicus* respectively. Highest chitin yield was from the largest size range of both species, *P. monodon* (30.5%) and *P. indicus* (27.86%). Ash, moisture, fat and nitrogen of extracted chitin were 0.98%, 5.80% and 6.16 % in *P. monodon* and 0.98%, 5.72% and 6.22% in *P. indicus* of dry weight respectively, which were matched with the moisture content (5.5%), ash (0.3%) and nitrogen (6.9%) of chitin available in the market. The chitin extracted from *P. indicus* of 5-10 cm length range had a moisture content of 5.72% and a nitrogen content of 6.22%, which were the closest to the moisture and crude nitrogen in the pure commercial chitin. Ash content (0.98%) of all the extracted chitin were comparable to those of pure chitin. This indicates that chitin extracted from shrimp waste in the processing industry is comparable to commercially available chitin and therefore shrimp waste is a suitable source for extraction of chitin for commercial purposes.

Keywords: chitin, demineralization, deproteinization, proximate composition, shrimp processing waste

INTRODUCTION

Chitin is the second most abundant and most important carbohydrate after cellulose (Isa et al. 2012) and structurally aids the crustacean exoskeleton. Apart from crustaceans, chitin can be seen in fungi, yeast, marine invertebrates and arthropods where it is a principal component in the exoskeleton (Lertsutthiwong et al. 2002). It is a long-chain polymer of N-acetyl D-glucosamine and derivative of glucose and is poly-β-(1-4)-N-acetyl-D-glucosamine (Pal et al. 2014).

Chitin claims to own several biological properties such as biocompatibility, acceleration of the formation of osteoblasts which aids in bone formation, haemostatic, fungistatic, spermicidal and antitumor (Pal et al. 2014). It is a valuable ingredient for many industries such as hair gel, paper, food (Krajewska et al. 2004; Rinaudo 2006) and pharmaceuticals as wound healers, anti-cholesteric. (Rinaudo 2006). Chitin is used as an excipient and drug carrier in film, gel or powder form for applications involved with mucoadhesivity (Arabia et al. 2013).

There are two allomorphs of chitin. They are α chitin which can be found in shrimps, lobsters, crabs like crustaceans and β chitin which is in pogonophoran and vestimentiferan worms. X ray power diffraction diagrams have shown that α chitin is unaffected by hydration and also has a crystal structure with inter sheet hydrogen bonds (Rinaudo 2006). β chitin crystal structure, which is tightly bound by a number of intra sheet hydrogen bonds does not contain inter sheet hydrogen bonds. (Rinaudo 2006). It is odorless, tasteless and pinkish white, white or off white depending on the species from which it is extracted.

The annual worldwide crustacean shells production in shrimp processing factories has been estimated to be 1.2 million tonnes. Crustacean shell waste can be limited and subject to seasonal supply (Teng et al. 2001). Sri Lanka is one of the shrimp exporting countries and waste from shrimp processing industry is dumped into the environment. Shrimp waste mostly includes their exoskeletons which contain chitin, proteins and carotenoids (Lertsutthiwong et al. 2002). Current annual shrimp production in Sri Lanka is 4000 tonnes which mostly include black tiger shrimp (*Penaeus monodon*) and Indian white shrimp (*P. indicus*) and shrimp waste production in 1200 tonnes per annum. Part of it is presently exported to countries like Korea where it is turned into value added products (Yapa et al. 2006). If Sri Lanka can utilize the shrimp waste to produce exportable value-added products, the foreign exchange earnings from shrimp processing industry could be significantly increased. If the properties of chitin extracted from shrimp shell are matched with those of pure imported chitin, it could be used as a replacement for the commercial chitin. This study is a preliminary attempt to extract chitin from waste generated from *P. monodon* and *P. indicus* processing industry to determine the most suitable length class from both species that gives a higher yield of chitin with proximate compositions comparable to pure commercially available chitin.

MATERIALS AND METHODS

Sample collection

Samples of *P. monodon* and *P. indicus* were collected from different processing factories and markets in Negombo and from a local market in Colombo.

Length measurements of shrimps

Partial total length (Santhanam et al. 2011) of 40-50 individuals of *P. monodon* and *P. indicus* was measured at the processing factory. Three different length groups were selected from each species, A_1 (5-10cm), A_2 (10-15cm) and A_3 (10-15cm) and head and shell parts were collected according to the length classes.

Extraction of chitin

Flesh particles retained with the shell and the head parts from the carapace were removed. Carapace and the shell from each species and each length range were separately washed and dried under the sunlight for 2-3 days. For chitin extraction process, 100 g of dried shrimp waste were measured from each length group of each species. In order to determine the differences chitin deposition in the carapace and the shell of each species and each length group. They were weighed separately and subjected to analyses.

Method described in Sewvandi and Adikary (2011) was used to extract chitin with appropriate modifications during the process. This process involved extraction, preconditioning, demineralization and deproteinization. During the preconditioning, the dried samples of weighed shrimp waste were transferred to 2 L beakers which were labeled accordingly. Shrimp wastes were soaked in 0.05M acetic acid for 24 hours. They were removed from acetic acid and left for 24 hours before washing thoroughly with tap water and dried to remove excess water. For demineralization, dried shrimp waste was treated with 0.68M hydrochloric acid (1:10 w/v) at ambient temperature (30˚C) for 6 hours. The residue was washed with distilled water until pH reached the range of 6.5 to 7.5 and was dried under sunlight. Demineralized shrimp waste was deproteinized using 0.62M sodium hydroxide (1:10w/v) at ambient temperature (30˚C) for 16 hours. The residue was washed thoroughly with distilled water until the pH reached a range of 6.5 to 7.5 and was dried under sunlight.

Yield measurements

The weight of chitin extracted from each species were measured and the % yield (dry weight) of chitin extracted was calculated using the following equation.

$$\text{Yield (\%)} = \frac{\text{Weight of extracted chitin}}{\text{Initial weight of shrimp waste}} \text{x} 100$$

Proximate analysis

Chitin extracted from all the length groups of both species were ground separately and sieved using a 100 µm mesh for crude lipid test and using a 1 mm mesh for other tests. Moisture content, ash content, crude protein and crude lipid contents were

measured both for chitin extracted and raw material from each species and each length range using gravimetric method, dry ashing method, Kjeldhal method (AOAC 1989) and Folch method (Folch et al. 1957) respectively.

RESULTS AND DISCUSSION

Percentage mean ± SD of chitin yields from the processing waste of *P. monodon* and *P. indicus* of different length groups are given in the Table 1. There were appreciable differences in the chitin yields among the length groups of *P. monodon* and *P. indicus.*

The variation of the percentage mean ± SD of chitin yields from the carapace and shell of *P. monodon* and *P. indicus* according to the length groups are given in the Table 2. Mean ± SD values of proximate composition (% dry weight) of the extracted chitin of the whole exoskeleton of *P. monodon* and *P. indicus* are given in Table 3. Mean ± SD values of proximate composition (% dry weight) of the whole exoskeleton of *P. monodon* and *P. indicus* are given in Table 4.

Table 1 Percentage chitin yield (mean ± SD; n=3) extracted from exoskeleton (carapace and shell) of *P. monodon* and *P. indicus* in the three length groups.

Length group	*P. monodon*	*P. indicus*
A1 (5-10 cm)	11.62 ± 0.25	15.56 ± 0.06
A2 (10-15 cm)	9.76 ± 0.10	26.26 ± 0.27
A3 (15-20 cm)	30.50 ± 0.10	27.86 ± 0.27

Table 2 Percentage chitin yield (mean ± SD; n=3) from the carapace and shell by dry weight from *P. monodon* and *P. indicus* in the three length groups.

Length group	Section of exoskeleton	*P. monodon*	*P. indicus*
A1 (5-10 cm)	carapace	30.56 ± 0.90	25.98 ± 0.12
	shell	34.76 ± 0.48	17.10 ± 0.11
A2 (10-15 cm)	carapace	31.98 ± 0.15	28.14 ± 0.64
	shell	35.58 ± 0.33	19.09 ± 0.11
A3 (15-20 cm)	carapace	28.89 ± 0.33	28.90 ± 0.19
	shell	40.82 ± 0.33	20.87 ± 0.28

Table 3 Mean ± SD values (n=3) of proximate composition in % dry weight of the extracted chitin of the whole exoskeleton of *P. monodon* and *P. indicus*.

Length group	Proximate composition	*P. monodon*	*P. indicus*
A1 (5-10 cm)	moisture	5.91 ± 0.10	5.72 ± 0.06
	ash	0.98 ± 0.005	0.98 ± 0.005
	crude nitrogen	6.06 ± 0.03	6.22 ± 0.03
	crude lipid	9.95 ± 0.10	4.92 ± 0.04
A2 (10-15 cm)	moisture	9.95 ± 0.10	5.83 ± 0.07
	ash	0.98 ± 0.010	0.97 ± 0.008
	crude nitrogen	6.09 ± 0.17	6.18 ± 0.01
	crude lipid	4.93 ± 0.10	4.73 ± 0.06
A3 (15-20 cm)	moisture	5.80 ± 0.10	5.74 ± 0.12
	ash	0.98 ± 0.010	0.98 ± 0.005
	crude nitrogen	6.16 ± 0.03	6.21 ± 0.03
	crude lipid	10.01 ± 0.03	9.90 ± 0.03

Table 4 Mean ± SD values (n=3) of proximate composition in % dry weight of the whole exoskeleton of *P. monodon* and *P. indicus*.

Length group	Proximate composition	*P. monodon*	*P. indicus*
A1 (5-10 cm)	moisture	70.73 ± 0.44	66.80 ± 0.40
	ash	23.96 ± 0.19	31.00 ± 0.02
	crude nitrogen	36.69 ± 0.32	35.58 ± 0.47
	crude lipid	3.84 ± 0.02	9.47 ± 0.07
A2 (10-15 cm)	moisture	74.71 ± 0.35	68.80 ± 0.30
	ash	31.96 ± 0.04	48.45 ± 0.47
	crude nitrogen	37.88 ± 0.15	33.39 ± 0.36
	crude lipid	4.27 ± 0.24	9.79 ± 0.02
A3 (15-20 cm)	moisture	75.70 ± 0.36	67.90 ± 0.25
	ash	20.83 ± 0.25	20.56 ± 0.48
	crude nitrogen	37.96 ± 0.10	33.93 ± 0.08
	crude lipid	4.03 ± 0.04	9.60 ± 0.02

Due to the time limitations, shrimp waste could not be collected from the same populations. The highest length groups (15-20 cm) of *P. monodon* and *P. indicus* were from farm raised shrimps and the samples of lowest length groups were from wild caught shrimps. The middle length group (10-15 cm) was a combination of farm raised and wild caught shrimps. Farm raised shrimps consume formulated food perhaps with a higher nutritive value when compared to the wild caught shrimps. This could be the reason for significantly higher yield of chitin from the former group. In addition, there can be stock differences and the variations in the environmental conditions that affect the formation of the shell. According to Santhanam et al. (2011), depending on the different genetic structure of the population and different environmental conditions prevailing in the geographic areas stock differences in shrimps can occur. Furthermore, there could be differences due to the sex and age of the shrimp. The differences in deposition of chitin in the exoskeleton can change due to the variations in chitin deposition in carapace and shell and chitin deposition is turned on and off according to a frequency function that decreases with age. (Neville 1965). The higher yield of chitin from the shell compared carapace of *P. monodon* and the higher yield of chitin from the carapace than in the shell of *P. indicus* in all length groups (Table 2) could be due to the differences in the genetic structure, sex, age and environmental factors related to each of the species.

Moisture contents of chitin extracted from *P. monodon* and *P. indicus* (Table 3) were higher than in pure chitin moisture value, 5.5% reported by Qin et al. (2010). Higher moisture contents may have been resulted due to impurities in the chitin extracted from the shrimp shells. Crustaceans are known to bear α chitin which is unaffected by hydration (Rinaudo 2006) and also they are reported to contain inter sheet hydrogen bonds and crystalline structures which do not allow the entry of water molecules.

Ash content was similar (0.98%) in chitins extracted from all length groups of both species (Table 3) but it was higher than the value (0.3%) obtained from Qin et al. (2010) for pure chitin. This difference could be due to the method of demineralization. For demineralization 0.68 M HCl was used for 6 hours and if it was kept for 24 hours only a slight drop in ash has been observed. (Shahidi et al. 1999). If a higher concentration of HCl is used intrinsic properties of chitin might get affected. Further investigations are necessary to determine the most suitable concentration of HCl and duration of the treatment for reduction of ash content in chitin extracted from shrimp exoskeleton. Use of inorganic acids could bring detrimental effects on the molecular weight and degree of acetylating and negative effects on intrinsic properties of chitin (Khanafari 2008). Crude nitrogen values of chitin

extracted are lower (Table 3) than that in pure chitin, 6.9% (Benjakul and Wisitwuttkul 1994). That is may be due to impurities contributing to crude nitrogen in extracted chitin.

Highest crude lipid contents were observed in the chitin extracted from the exoskeletons of the smallest (5-10 cm) and the largest (15-20 cm) length groups of *P. monodon* and the 15-20 cm length group of *P. indicus* (Table 3). This may have been due to the differences in contaminations incorporated with fat of chitin in those length groups and also the fat binding capacity variations. According to Sharp (2013), in the extraction process of chitin through demineralization and deproteinization, only minerals and proteins were removed from the shrimp waste but further purification procedures should be done to remove the fat and the pigments.

Considering the proximate composition of the raw material used for obtaining exoskeleton for extraction of chitin, the highest moisture value has been obtained by the largest size of *P. monodon* (Table 4). This may be due to the particle size of the raw material. Larger particle sizes of raw material may contain more moisture than smaller particles (Samar et al. 2012). Highest ash (Table 4) can be seen in the 10-15 cm length class of *P. indicus*, perhaps due to access to more minerals in the habitat. Nutritious and protein diets in farm raised shrimps may have resulted in the highest crude nitrogen value in the 15-20 cm length class of *P. monodon* (Table 4). The length class of 10-15 cm of *P. indicus* had the highest crude protein value (Table 4). Higher fat contents in *P. indicus* shell have been resulted perhaps due to food availability in the area.

According to the findings, chitin can be extracted from *P. monodon* and *P. indicus* of all length groups but the most suitable length group for a higher yield is 15-20 cm length group of *P. monodon*. Moisture, ash and crude nitrogen of commercially available chitin have been reported to be 5.5%, 0.3%, and 6.9% respectively (Qin et al. 2010), which are comparable to the chitin extracted from *P. monodon and P. indicus.*

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