THE PROXIMATE ANALYSIS AND SPECTRAL PROFILE OF CHITIN EXTRACTED FROM THE SHELL OF *Portunus pelagicus* ORIGINATED FROM CIREBON, INDONESIA

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ABSTRACT

Indonesia has a great potential to be exploited for its abundant marine resources, e.g. the blue swimming crab (*Portunus pelagicus*). During 2018 in West Java, *P. pelagicus* is highly utilized (8.423,89 tons or 5.127% of the total production throughout Indonesia). However, the shell waste (approximately 40-60% of the total crab weight) is usually deserted and needs to be further exploited. In this work, we extracted chitin from *P. pelagicus* shells originated from Cirebon-West Java, Indonesia, using the natural deep eutectic solvents (NADES): a mixture of choline chloride and malic acid. The extraction was carried out twice using the same solvents. The chitin produced, by employing NADES, was proximate-analyzed and UV/IR spectrophotometric-characterized. The two times-extraction of chitin from *P. pelagicus* shells using NADES yielded chitin powder of 35.43% w/w. The proximate analysis indicates that our chitin meets the Indonesian National Standard requirements, and its infra-red spectrum confirms the β -chitin form. It could be concluded that chitin extracted from the wasted shell of *P. pelagicus* possesses a good quality, very low level of arsenic and lead, and therefore could be further developed as active pharmaceutical ingredients.

Keywords: chitin, chitosan, choline chloride, malic acid, NADES solvents, *Portunus pelagicus*

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INTRODUCTION

Indonesia, possessing an immense marine area, has a great potential to be exploited for its abundant natural resources, e.g. the blue swimming crab (*Portunus pelagicus*). The native habitat of this particular crab is the Western Indo-Pacific. A cluster analysis revealed two stock units of *P. pelagicus* in the Indonesian fisheries management area, the first stock was Southern Madura, and the other stocks were East Lampung, Lancang Island, Cirebon, and Rembang.¹ In Indonesia, especially West Java, *P. pelagicus* is highly utilized. Its utilization in 2018 reached 8.423,89 tons or 5.127% of the total production throughout Indonesia. However, the shell waste (approximately 40-60% of the total crab weight) is deserted and needs to be further exploited.²

Interestingly, this neglected crab shell contains chitin. Chitin has been widely used in agriculture as an insecticide. Moreover, pharmaceutical fields such as biomedical food wound dressings also contain chitin. Chitin and/or its deacylated product, chitosan, is used in the textile industry, to efficiently protects yarns during weaving.^{3,4,5} Factors affecting the yield and character of chitin are harvesting seasons,⁶ extraction methods,⁷ and sources used in the manufacture of chitin.^{8,9}

In this work, we extracted chitin from *P. pelagicus* shells originated from Cirebon-West Java, Indonesia, using the natural deep eutectic solvents (NADES), a solvent synthesized from natural ingredients, that is environment-friendly.¹⁰ Furthermore, the proximate content and heavy metals level in the produced-chitin were also analyzed.

EXPERIMENTAL

Instruments

Rewez multifunctional microwave oven EM820ABT, 80-1 tabletop low speed electric medical centrifuge (Jiangsu Jinyi Instrument Technology Company Limited, China), inductively coupled plasma-optical emission spectrometer (ICP-OES) (ThermoScientific iCAP PRO Series), Fourier-Transform infrared spectrophotometer (ThermoScientific, Nicolet 380), UV-visible spectrophotometry (Shimadzu UV mini-1240).

Materials and Chemicals

The blue swimming crab shell (*P. pelagicus*), Ø 13-15cm originated from Gunung Jati Cirebon, West Java, Indonesia, choline chloride CAS No. 67-48-1 (Salus Nutra Inc), DL-malic acid (Salus Nutra Inc), commercial chitin produced from crustacean (Carbosynth) used as the standard, potassium bromide analytical grade (Merck), hydrochloric acid 37% analytical grade (Merck), distilled water.

Preparation of NADES

NADES was prepared by following the method of Huang and colleagues (2018)¹¹, i.e. mixing a 1: 1 molar ratio of choline chloride and DL-malic acid. The mixture was heated to 80°C with constant stirring until a homogeneous liquid was formed.

Extraction of Chitin

The blue swimming crab shell powder (mesh-60) was mixed with NADES (1:20) to a homogenous mixture. The mixture was heated in the microwave at 700w for 9 min, during which it was taken out from the microwave every minute for 3 sec to avoid overheating. Chitin, formed as a precipitate, and NADES were separated by centrifugation.¹¹ The obtained chitin was washed with distilled water and re-extracted using the same procedure. The product was named REC (re-extracted chitin).

Proximate Analysis

Proximate content (moisture, ash, protein, fat, and carbohydrate) in the *P. pelagicus* shell powder, the commercial chitin, and REC, was strictly determined according to the protocol of the Indonesian National Standard SNI 01-2891-1992.¹²

Determination of Heavy Metals

Approximately 0.5 g of the sample was dissolved with heating in 5 mL of HNO₃, cooled to room temperature, and filtered. The solution was diluted with Milli-Q water in a 25mL volumetric flask.

The standard metal solutions and the sample were measured using inductive couple plasma-OES (ICP-OES) at the maximum wavelength of each metal, i.e. As 188.9 nm; Ca 396.9 nm; Cd 226.5 nm; Co 238.9 nm; Cr 283.6 nm; Cu 327.4 nm; Fe 238.2 nm; Hg 253.6 nm; K 766.5 nm; Mg 279.5 nm; Mn 257.6 nm; Na 588.9 nm; Pb 220.4 nm; Sn 283.9 nm.¹³

Qualitative UV-Spectrophotometric Analysis

10 mg of standard chitin and the sample in a separate flask were dissolved in 4.2 mL of HCl 37%. The solutions were immediately diluted with distilled water to get a concentration of 80 ppm. Measurement was carried out at 200-250 nm.

FTIR-Spectrophotometric Analysis

1 mg of previously dried chitin was dispersed in approximately 200 mg of potassium bromide (KBr) and was pressurized under vacuum for 10 min to form pellets. The pellets were analysed using an infrared spectrophotometer at a frequency of 4000-500cm⁻¹.¹⁴

RESULTS AND DISCUSSION

The demineralization process in the extraction of chitin is conventionally carried out using strong mineral acids (HCl, HNO₃, H₂SO₄), organic acids (CH₃COOH and HCOOH), however, dilute HCl is the most often

chosen solvent. Moreover, deproteination generally employs alkalines (sodium hydroxide, potassium hydroxide, calcium hydroxide) and salts (Na_2CO_3 , NaHCO_3 , K_2CO_3).¹⁵ However, the waste (solvent residue) could damage the environment, e.g. the severe impacts of alkaline residues on aquatic environments.¹⁶

Meanwhile, the natural deep eutectic solvent (NADES) is known as a sustainable, non-toxic, and biodegradable solvent. NADES has been developed for extracting superior quality chitin from crustacean shells.¹¹ Considering these advantages, NADES was chosen as the solvent in our work for chitin extraction (two times) from the blue swimming crab (*P. pelagicus*) shells and resulted in a fine dry powder (Fig.-1b) compared to the commercial standard chitin (Fig.-1a).

A mixture of choline chloride and DL-malic acid (NADES) has been reported to successfully extract superior quality chitin from shrimp shells.¹¹ Choline chloride can remove protein from the shells, while DL-malic acid was able to demineralize calcium carbonate compounds from the crustacean shells. This can be seen from the decomposition of calcium carbonate that dissolves in water by releasing carbon dioxide bubbles.¹⁷ The demineralization process produces salts that dissolve easily, washing with deionized water is carried out to separate salt and solids.¹⁵

Our two times-extraction of chitin from the blue swimming crab (*P. pelagicus*) shells using choline chloride and DL-malic acid yielded more chitin powder (35.43% w/w) (Fig.-1b) compared to that of chitin produced by using 1N HCl and 1N NaOH (20.24%) extracted from Malaysian *P. pelagicus*.¹⁸

Fig.-1: The Powder of (a) Commercial Standard Chitin and (b) REC (Re-extracted Chitin) from *P. pelagicus* Shells (Yield = 35.43% w/w).

Proximate Content

The proximate content (protein, moisture, ash, fat, and carbohydrate) in the *P. pelagicus* shell powder, the commercial chitin, and REC is provided in Table-1.

Table-1: Proximate Content in the *P. pelagicus* Shell Powder, the Commercial Chitin, and REC

Proximate Content	<i>P. pelagicus</i> Shell Powder (%)	Commercial Chitin (%)	REC (%)
Moisture	4.53	5.70 ± 0.0	1.93 ± 1.5
Ash	71.31	1.97 ± 0.1	30.61 ± 8.6
Protein	8.17	0.39 ± 0.2	0.30 ± 0.0
Fat	0.26	16.83 ± 20.2	12.50 ± 8.8
Carbohydrate	15.73	75.12 ± 20.3	54.67 ± 1.7

According to the Indonesian National Standard,¹² the maximum percentage of water and ash content is 12% and 5%, respectively. However, the ash contained in REC from *P. pelagicus* shells was still high (Table-1). Our result was in agreement with that of Toliba and co-workers¹⁹ who reported that the ash content in

the blue swimming crab sample was higher (71.3%) than that of the crustacean shell from Egypt (33-38%).¹⁹ High ash content indicates that the demineralization process has not been completed.^{9,20}

The protein content in REC (0.30%) resembled commercial chitin (0.39%). Moreover, although REC contains high carbohydrates (54.67%), it is less amount than that of the commercial chitin (75.12%). High protein content indicates the presence of organic and nitrogenous substances,⁹ which often trigger allergies in humans.¹⁵ Whereas high carbohydrate content is not surprising, because originally chitin is a polysaccharide composed of N-acetyl-2-amino-2-deoxyglucose units linked by β -(1 $\rightarrow$ 4) bonds.²¹

Heavy Metals Content

Chitin can absorb toxic metal ions such as Cd, Ni, Cu, Pb, and Zn,²² however, it is important to determine the heavy metal content in chitin to ensure its safety and quality. The result is presented in Table-2.

Table-2: Heavy Metals Content in Chitin

Heavy Metal	Commercial Chitin (mg/kg)	REC (mg/kg)
As	$< 0.0001 \pm 1.40$	$< 0.0001 \pm 2.67$
B	$< 0.0001 \pm 0.01$	$< 0.0001 \pm 0.01$
Ca	1736.4114 ± 0.02	1861.0928 ± 0.00
Cd	$< 0.0001 \pm 0.00$	$< 0.0001 \pm 0.03$
Co	$< 0.0001 \pm 0.06$	1.4471 ± 0.06
Cr	4.1262 ± 0.00	13.0888 ± 0.02
Cu	2.4251 ± 0.02	$< 0.0001 \pm 0.05$
Fe	89.2382 ± 0.02	466.1976 ± 0.12
K	$< 0.0001 \pm 0.00$	$< 0.0001 \pm 0.02$
Mg	1120.1151 ± 0.06	438.4930 ± 0.02
Mn	28.7790 ± 0.01	29.1966 ± 0.00
Na	300.9026 ± 0.05	369.1667 ± 0.07
Pb	7.4737 ± 0.24	$< 0.0001 \pm 0.04$
Sn	5.9413 ± 0.04	17.7944 ± 0.09
Hg	9.6613 ± 0.05	$< 0.0001 \pm 0.01$

The maximum level of As and Pb in chitin is 5 mg/kg.¹² Both REC and the commercial chitin meet this requirement. Moreover, the amounts of Cu, Pb, and Hg in REC were smaller than the commercial chitin (Table-2). These results have proven that NADES solvent was suitable for obtaining a good quality of chitin.

The ionic character of NADES, obtained from the complexation of hydrogen acceptor compounds (e.g. choline chloride) and hydrogen bond donor compounds (e.g. malic acid), is capable of extracting heavy metals in young barley with the efficiency percentage of Mn (26%), Cu (8%), Zn (24%), Mo (52%).²² The mechanism that may play a role in reducing the concentration of heavy metals in chitin is ion exchange. Choline chloride which is an alkaline compound acts as an anion exchanger, while malic acid is a cation-exchanger.²³

Qualitative UV-Spectrophotometric Analysis

The UV-spectrophotometric analysis indicates that λ max 208.5 nm is for commercial chitin and λ max 206.5 nm is for REC (depicted in Fig.-2).

An acetonitrile extract of chitin, recorded in the UV spectral region 196–248 nm, was reported showing a maximum at 212.6 nm. This band was caused by the $n\text{-}\pi^*$ electronic transition type due to the secondary amide fragment of chitin.²⁶

FTIR-Spectrophotometric Analysis

Figure-3 depicts the infrared spectrum of the chitin recorded in the spectrum field 4000-500 cm^{-1} in the form of % transmittance (y-axis) versus wavenumber (x-axis).

Fig.-3 depicts the bands of C-O aliphatic ($1,100\text{ cm}^{-1}$); C-N stretch (1380 cm^{-1}); C = O stretch (1600 cm^{-1}); the amide's carbonyl (1660 cm^{-1}); C-H alkyl ($2890\text{-}2968\text{ cm}^{-1}$); and O-H alcohol ($3400\text{-}3550\text{ cm}^{-1}$), which

are similar for both chitins. The band at 2900 cm^{-1} is usually used as a reference band in identifying chitin.²⁵ The part of spectra that confirms α -chitin is the presence of the amide's carbonyl (-C=O) band at 1660 and 1620 cm^{-1} and the amide's secondary amine (-N-H) at 1552 cm^{-1} . These two sharp peaks at 1660 and 1620 cm^{-1} are not found in β -chitin.^{26,27} Commercial chitin, extracted from Crustaceans, confirms the α -chitin form. Interestingly, the spectrum of REC from *P. pelagicus* indicates the β -chitin form.

Fig.-2: UV Spectra of Commercial Chitin (Red Spectrum; λ max 208.5 nm) and REC (Blue and Black Spectra; λ max 206.5 nm)

Fig.-3: FTIR Spectra of Commercial Chitin (Red) and REC (Blue)

CONCLUSION

Extraction of chitin from the blue swimming crab (*Portunus pelagicus*) shells, originated from West Java, Indonesia, using NADES (choline chloride: malic acid) solvent, has produced a good quality of β -chitin. This chitin reveals low moisture, protein, and fat content, however, high carbohydrate, and ash are quantified. The extracted chitin meets the requirement of heavy metals content following the Indonesian National Standard.

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