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To cite this article: I.F.M. Rumengan *et al* 2017 *IOP Conf. Ser.: Earth Environ. Sci.* **89** 012028

Characterization of chitin extracted from fish scales of marine fish species purchased from local markets in North Sulawesi, Indonesia

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Abstract Chitin is a biodegradable biopolymer with a variety of commercial applications, including in the food food-supplement industries as a marine-derived nutraceutical. The purpose of this study was to characterize the molecular structure of chitin extracted from fish scales of important marine fish purchased from local markets in North Sulawesi. Chitin compound material was obtained from a specific fish scale, and then sequentially carrying out a boiling treatment to separate it from a complex with collagen. From the scales of two fish species, parrotfish (*Chlorurus sordidus*) and red snapper (*Lutjanus argentimaculatus*), the rendemen of chitin obtained were 45 % and 33%, respectively. Structural characteristics of the chitin were discussed by FTIR (Fourier Transform Infrared) analysis data. FTIR analysis was done using infrared spectroscopy, which is the resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. The molecular structure of chitin, $C_{18}H_{26}N_2O_{10}$, where the hydroxyl group on the second carbon replaced by acetyl amide, was shown by the infrared spectra. In the infrared spectra, chitin from parrot fish scales indicated the amide band at 1627.13 cm^{-1}, and chitin from red snapper fish scales the amide band at 1648.09 cm^{-1} which are a typical one for marine chitin. The hydroxyl and amino bands at the ranged spectra up to 3500 cm^{-1}. The yields of chitin isolated from fish scale were relatively huge. Some treatments are necessary to confirm the molecular conformation and deacetylation behavior. All products from the extraction of fish scales could be more accessible for structural modifications to develop biocompatible materials for pharmaceutical purposes.

1. Introduction

Chitin is potential biomaterial for biotechnological industries and tissue engineering, due to some characteristics, including their polyelectrolyte and cationic nature, the presence of reactive groups, high adsorption capacities, bacteriostatic and fungistatic influences [1,2,3,4]. In Indonesia, an interest of the potential of marine-derived chitin has been mainly due to the concern on the environmental problems regarding the disposal of marine processing shellfish wastes consisting of crustacean exoskeletons.

Depending on the source, chitin can occur in the α-, β- and γ-forms. The differences among them depend on the arrangement of chains of the crystalline regions [5]. Because of these differences, each chitin polymorph has different properties specific to it. In most cases, crystallinity index provides information about the crystal state, but it is also very useful for distinguishing α-chitin from β-chitin. The crystallinity index (CI) can also be calculated by X-ray diffractograms. The molecular structure could be characterized by infrared spectrum, in which different bonds or functional groups absorb a

IOP Conf. Series: Earth and Environmental Science **89** (2017) 012028 doi:10.1088/1755-1315/89/1/012028

different range of infrared with a certain wavelength. The spectra are associated with the different vibration that occurs after deacetylation process, and therefore can be used for calculation of the degree of deacetylation (DD) as an important property that affects the biodegradability and immunological activity [6], because this parameter has effects on solubility, chemical reactivity, and biodegradability. Depending on the source and preparation procedure, DD may range from 30% to 95% [7].

Fish scales are wastes of seafood restaurants and fish markets. Fish scales could be a better source of chitin production. The extracted chitin can be used to produce chitin-derived products, such as chitosan and glucosamine. The purpose of this study was to characterize the molecular structure of chitin extracted from marine fish scales by using FTIR data. This information would be useful for further developing it as a nutraceutical compound for food industries, in particular as natural preservatives.

2. Material and Methods

The fish scales of two marine fish species, parrotfish (*Chlorurus sordidus*) and red snapper (*Lutjanus argentimaculatus*) were collected from local markets in North Sulawesi. The fish scales were washed and sun-dried for two days. The procedure of chitin extraction was adopted for collagen extraction with modification. The pre-treatment step was carried out using NaOH 0.5 M solution for 10 hours, following with hydrolysis with HCl 0.75 M solution for 24 hours. The solution was then neutralized with destilled water, and heated at $40^{o}C$ for 2 hours. The residue was separated from solution, washed with distilled water and then re-heated at $80^{o}C$ for 2 hours. The materials remaining after physical process were chitin.

Infrared spectroscopic analysis was adopted [8, 9]. Chitin samples were prepared in a potassium bromide (KBr) disk and film. Approximately 40-60 mg of chitosan powder and 120 mg of KBr were blended and triturated with agate mortar and pestle for 10 min. Approximately 40 mg of the mixture were compacted using a IR hydraulic press at a pressure of 8 tons for 60 seconds. The disk was conditioned in a desiccator placed in an oven at $80^{0}C$ for 16 hr before analysis. Sample was then inserted into ZnSe ATR cell.

3. Result and Discussion

Fish scale chitin was typically associated with a complex matrix with collagen. This collagen was not easily removed during deproteinization step. To provide a method separating a collagen, chitin compound material was separated during hydro-extraction with distilled water at $80^{0}C$ after repeatedly washed with distilled water for neutralization.

Christalinity of chitin and chitosan was generated from hydrogen bond between corresponding hydroxyl and N-acetyl groups [10]. Each crystalline peak characterizes the crystallographic the structure, which is generated from parallel and antiparallel alignments of polymeric chains or sheets. Semi-crystalline chitin and chitosan have amorphous and crystalline regions [11].

The results of FTIR spectra of fish scales are shown in Fig. 1. The IR spectra of the fish scales chitin was compared to the IR spectrum of commercial as shown in Table 1. The IR spectra of chitin of *C. sordidus* and *L. argentimaculatus* fish scales displayed similar IR spectra to that of the commercial chitin.

Table 1. Functional groups of two fish scale samples, *C. sordidus* and *L. argentimaculatus* compared to a commercial standard chitin [12].

Group	Wave length (cm^{-1}) Standard chitin	Parrot fish *C. sordidus*	Red snapper *L. argentimaculatus*
OH	3450	3437.67	3436.31
N-H stretching	3300-3250		
C-H stretching	2891,1	2925.19	2145.36
/C=O stretching	1680-1660	1627.13	1648.09
N-H bending	1560-1530	1558.41	
CH_3	1419,5	1380.63	
C-O-C	1072,3	1075.51	1082.35
N-H	750-650	602.14	606.11

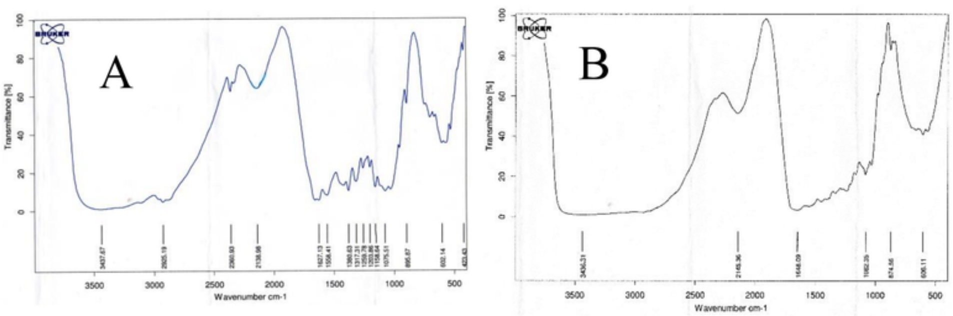

Figure.1. Infrared spectra as a wave length function displayed by fish scale chitin of parrot fish (*C. sordidus*) (A) and red snapper (*L. argentimaculatus* (B)

The FTIR spectra of both fish scale chitin exhibited a characteristic band at around 3436-3438 cm^{-1} is attributed to –NH and –OH groups stretching vibration and the band 2145-2826 cm^{-1} were an aliphatic C-H stretching bands that converges to OH stretching with N-H. The characteristic carbonyl C=O stretching of chitin at 1627 cm^{-1} are attributed to the vibrations of the amide I band. The sharp band at 1380 cm^{-1} from *C. sordidus* chitin corresponds to a symmetrical deformation of the CH_3 group. The vibrations bands at 1075-1082 cm^{-1} showed C-O-C vibration inside chitin ring and produced many peaks caused by the presence of hydroxide from chitin which contains a single bond C=O [12]. Chitin $C_{18}H_{26}N_2O_{10}$, like cellulose is a β-(1,4)-linked glycan, but is composed of 2-acetamida-2-deoxy-D-glucose (N-acetylglucosamine), while chitosan is a low acetyl form of chitin and is composed primarily of glucosamine, 2-amino-2-deoxy-D-glucose [13]. In the infrared spectra, the fish scales chitin indicated the amide II band at 1558.41 cm^{-1}, a typical one for marine chitin. A typical amide I band was found at 1627-1648 cm^{-1}.

4. Conclusion

The present findings prove that molecular structure of this marine-derived chitin as a high molecular weight polymer has a linear polyamine whose amino groups are readily available for chemical reactions [13]. For future works, it remains to explore its physical and chemical properties to develop an applicable nutraceutical biomaterial for food industries.

IOP Conf. Series: Earth and Environmental Science **89** (2017) 012028 doi:10.1088/1755-1315/89/1/012028

Acknowledgements
This work was a part of the research project sponsored by the Research Technology and Higher Education Ministry with a research scheme, MP3EI for fiscal year 2016-2017. The authors thank the Directorate Research and Community Service staff and the involved board of the ministry for financial support.

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