

Structural Characteristics of Chitin and Chitosan Isolated from the Biomass of Cultivated Rotifer, *Brachionus rotundiformis*

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Abstract: We characterized the molecular structure of chitin and chitosan isolated from the biomass of cultivated rotifer (*B. rotundiformis*). Chitin and chitosan are potential biomaterials for biotechnological industries due to their structural and functional characteristics. Zooplankton seems to be a better source of chitin, associated with the very low degree of calcification and sclerotization of the cuticles. Cultivation of local strain rotifer from North Sulawesi waters has been successfully conducted in a very simple medium with raw fish input without aeration in fiber tanks. This mass production technology has enabled to harvest 45-48 million individuals with estimated 5 to 48 g rotifer biomass in each production cycle. From this rotifer biomass, 4.6% chitin was extracted and 52.7% chitosan could be derived by deacetylation of chitin. Structural characteristics of the rotifer chitin and chitosan were discussed on the basis of the X-ray and infrared analysis data. X-ray analysis was done using X-Ray Diffraction (XRD) with CuK radiation at a voltage of 40 kV and 30 mA. X-ray diffraction diagram of chitin indicated the molecular form at three strongest peaks, 8.1, 9.2 and 19 2θ , differed from that of chitosan at peaks, 9.6, 19.5 and 21.1. Crystallinity of chitosan (47.06%), was higher than that of chitin (33.94%). 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, the rotifer chitin indicated the amide II band at 1558.48 cm^{-1} , a typical one for marine chitin. A marked difference was observed for the amide I bands, at 1651.07 cm^{-1} . Chitosan showed no amide band, but hydroxyl and amino bands at the ranged spectra up to 3500 cm^{-1} . The yields of chitin isolated from rotifer biomass and its deacetylated products (chitosan) were relatively small and as a polymer of N-acetyl D-glucosamine, both molecules showed difference in specific functional groups. Chitin is composed of 2-acetamido-2-deoxy-D-glucose, while chitosan composed primarily of glucosamine, 2-amino-2-deoxy-D-glucose. Some treatments are necessary to confirm the molecular conformation and deacetylation behavior. Chitosan could be more accessible for structural modifications to develop biocompatible materials for pharmaceutical purposes.

Keywords: Chitin, chitosan, rotifer, structural characteristics

INTRODUCTION

Chitin and chitosan are potential biomaterials for biotechnological industries and tissue engineering, due to a number of characteristics, including their polyelectrolyte and cationic nature, the presence of reactive groups, high adsorption capacities, bacteriostatic and fungistatic influences (Islam *et al.*, 2011; Hasri, 2010; Khoushab and Yamabhai, 2010; Aranaz *et al.*, 2009). In Indonesia, an interest of the potential of marine 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 in the crystalline regions (Jang *et al.*, 2004). 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 on the basis of X-ray diffractograms. The molecular structure could be characterized by infrared spectrum, in which different bonds or functional groups absorb different range of infrared with certain wavelength (Hang, 2009). The spectra are associated with the different vibration that occurs after deacetylation process and therefore can be used for calculation of Degree of Deacetylation (DD) as an important property that affects the biodegradability and immunological activity (Fernandez-Kim, 2004), 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% (Synowiecki and Al-Khateeb, 2003).

Zooplankton seems to be a better source of chitin, associated with the very low degree of calcification and

sclerotization of the cuticles, with mean chitin production values of 1 g chitin/m²/year (Jeuniaux *et al.*, 1988). As a cosmopolitan zooplankton, rotifer has molecular potential for biotechnological industries (Rumengan, 1997, 2007). Culture of local strain of rotifer (*Brachionus rotundiformis*) from North Sulawesi waters has been established since 1995 in Laboratory of Marine Biotechnology, Faculty of Fisheries and Marine Science, Sam Ratulangi University (Rumengan *et al.*, 1998, 2007). Recent cultivation of this rotifer in mass culture scale has been developed by using rawfish input, without microalgae and aeration (Dewanto *et al.*, 2012; Limbong *et al.*, 2012) has enabled to harvest 45-48 million individuals with estimated 5 to 48 g rotifer biomass in one production cycle. Harvested rotifer has been currently developed as the source of biomass for chitin production. From this rotifer biomass, 4.6% chitin was extracted and 52.7% chitosan could be derived by deacetylation of chitin (Modaso *et al.*, 2013). The purpose of this study was to characterize the molecular structure of chitin dan chitosan isolated from the biomass cultivated rotifer (*B. rotundiformis*) by using X-ray diffraction for determining the crystallinity

of chitins and chitosans and infrared spectra analyses for molecular structure.

MATERIALS AND METHODS

The rotifer biomass from the culture system (Dewanto *et al.*, 2012) was harvested as described by Rumengan *et al.* (2012). The method of sample preparation for chitin extraction was previously reported (Modaso *et al.*, 2013). Sample for X-ray diffraction analysis adopted from Khan *et al.* (2002) was nondestructive (except for grinding). Chitin and chitosan samples were prepared in the form of potassium bromide (KBr) disk and film. The patterns were recorded using X-ray diffractometer with CuK radiation (40 kV, 30 mA) until scanning with XRD spectrum (Carpenter, 2011). Data were collected with the scan angle from 2° to 70°. On the basis of X-ray diffractograms, the Crystallinity Index (CI) was calculated as adopted from Xu *et al.* (2005) as follows:

$$\text{Crystallinity Index (\%)} = \left(\frac{\text{K} \alpha \text{ Intensity profile}}{\text{Intensity Smoothing Profile}} \right) \times 100$$

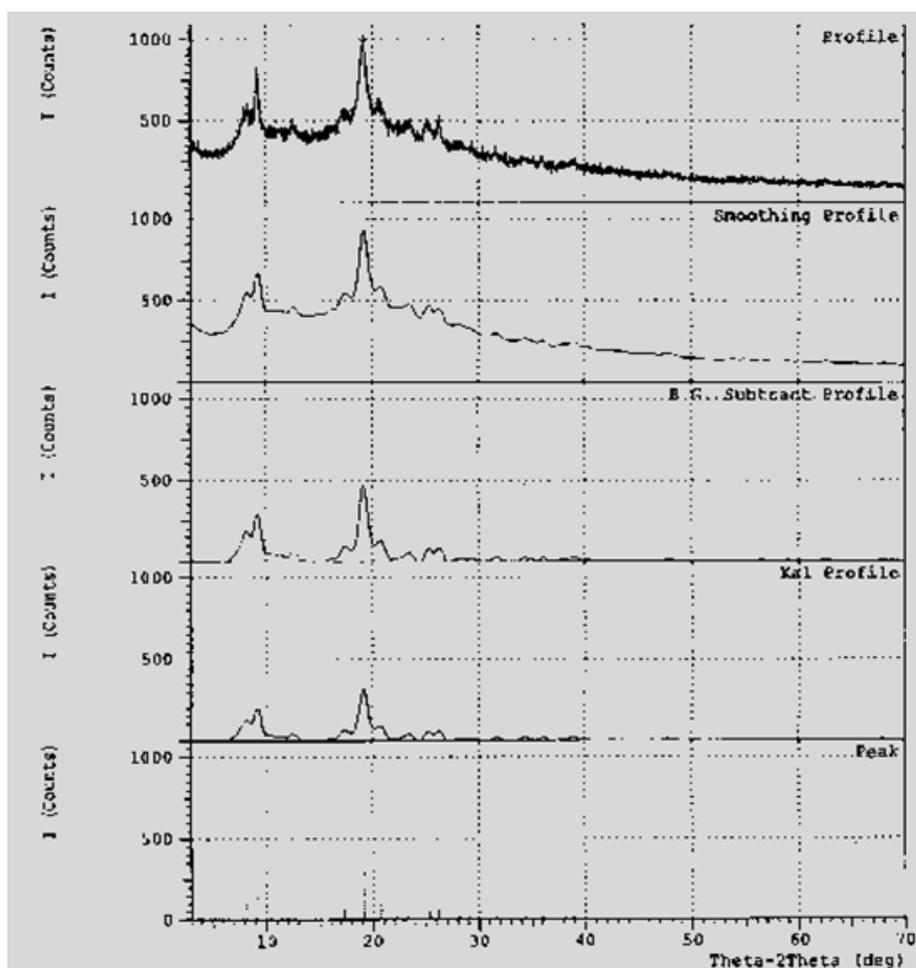


Fig. 1: XRD diffractogram of rotifer chitin

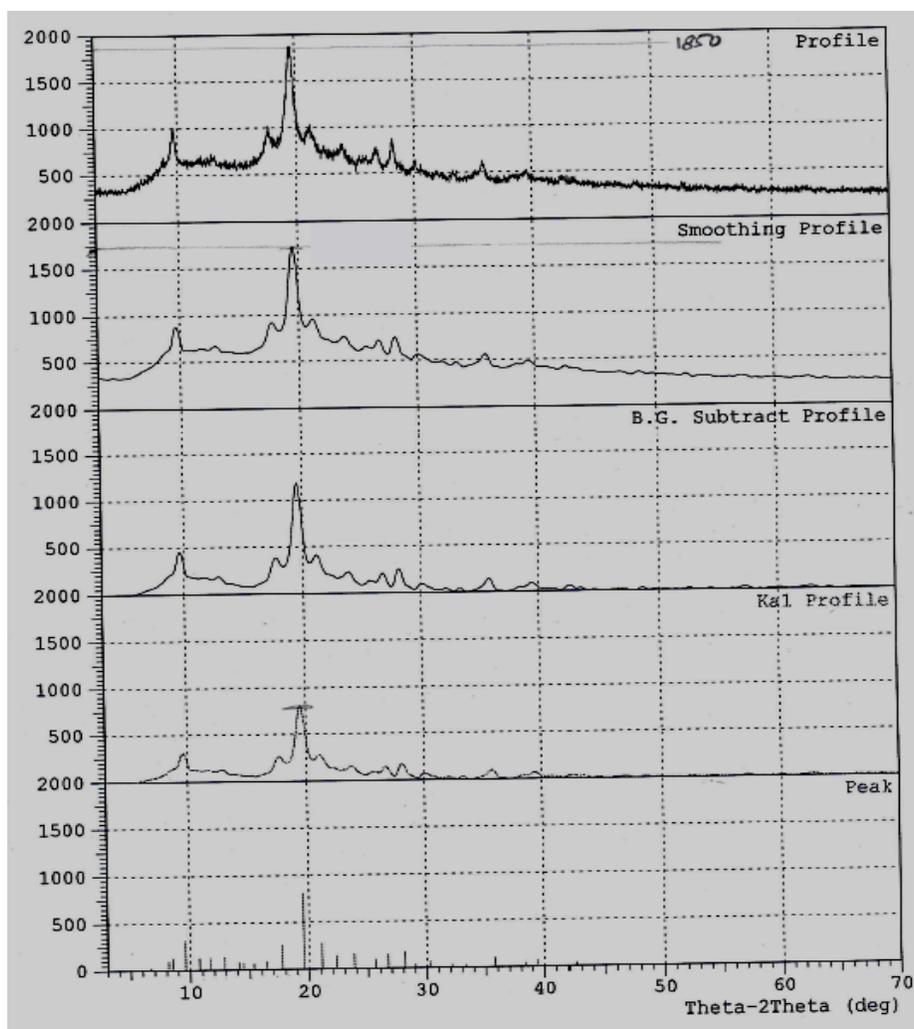


Fig. 2: XRD diffractogram of rotifer chitosan

Infrared spectroscopic analysis was adopted from Khan *et al.* (2002) and Xu *et al.* (2005). Chitin and chitosan 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 sec. The disk was conditioned in a desiccator placed in an oven at 80°C for 16 h before analysis. Sample was then inserted into ZnSe ATR cell.

RESULTS AND DISCUSSION

Crystallinity of chitin and chitosan was generated from hydrogen bond between corresponding hydroxyl and N-acetyl groups (Bartnicki-Garcia, 1988). Each crystalline peak characterizes crystallographic structure, which is generated from parallel and antiparallel alignments of polymeric chains or sheets. Semi-

crystalline chitin and chitosan have amorphous and crystalline regions (Jung, 2013). The XRD pattern of rotifer chitin exhibited diffraction peaks in the scattering range (2θ) at 8.1, 9.2 and 19 (Fig. 1) and chitosan at peaks, 9.60, 19.52 and 21.12 (Fig. 2). This is comparable with the pattern of crab chitins, with peaks at 9.1-9.2° and 19.1-19.2° 2θ and the pattern of chitosan with two peaks at 10.1° and 19.8° (Yen and Mau, 2007), similar to the pattern of shrimp chitosan with peaks at 10° and 21° 2θ (Islam *et al.*, 2011), but Prashanth *et al.* (2002) previously found that the patterns of shrimp chitosan with two major characteristic peaks at 9.9-10.7 and 19.8-20.7° 2θ . The typical chitin and chitosan diffraction pattern, showed strong reflections at 9-10° and 20-21° and minor reflections at 26.4° (Kumirska *et al.*, 2010). XRD pattern is applicable for assessing chitosan as a potent inhibitor of tumor-induced angiogenesis, as developed by Prashant and Tharanathan (2005) who depolymerized commercial shrimp chitosan, resulted in

Table 1: Crystallinity of rotifer chitin and chitosan

Product	Intensity smoothing profile	Intensity kal profile	Crystallinity index (%)	Peak 2θ
Chitin	940	319	33.94	19°, 9°, 8°
Chitosan	1700	800	47.06	21°, 19°, 9°

Table 2: Wave length of the main bands obtained for the α-chitin standard (Palpandi *et al.*, 2009) and the rotifer *B. rotundiformis* chitin

Vibration modes	Wave length (cm ⁻¹)	
	α-chitin standard	Rotifer chitin
OH out-of-plane bending	690	694
NH out-of-plane bending	752	-
Ring stretching	896	894
CH ₃ wagging along chain	952	948
CO stretching	1026	1026
CO stretching	1073	1072
Asymmetric in-phase ring stretching mode	1116	1157
CH ₂ bending and CH ₃ deformation	1418	1427
Amide II band amide II band	1563	1558
Amide I band	1661	1627
CH stretching	2878	-
Symmetric CH ₃ stretching and asymmetric CH ₂ stretching	2930	2885
NH stretching	3268	3271
OH stretching	3439	3448

appearance of diffractogram peaks at 9.46°, 11.9°, 16.7°, 20.76° and 21, 86°. Two diffraction peaks (9.60° and 21.12°) exhibited by rotifer chitosan with highest peak at 19.1° (Fig. 2) indicating a partial structural modification.

As shown in Table 1, the CI for chitin and chitosan of rotifer were 33.94 and 47.06%, respectively. In most cases, crystallinity index provides information about the crystal state, but it is also very useful for distinguishing α-chitin from β-chitin. Many other studies of chitin polymorphs have revealed differences in crystallinity peaks between α-, β- and γ-chitins obtained from various sources (Jang *et al.*, 2004; Cárdenas *et al.*, 2004; Kim *et al.*, 1996). The differences among the α-, β- and γ-forms of chitin are due to the arrangement of the chains in the crystalline regions: α-chitin has a structure of antiparallel chains, β-chitin has intra sheet hydrogen-bonding by parallel chains and γ-chitin, being a combination of α- and β-chitin, has both parallel and antiparallel structures (Jang *et al.*, 2004). Based on vibration modes of different functional groups as characterized by the wave length, the rotifer chitin is comparable to the α-chitin standard as shown in Table 2.

The results of FTIR spectra of rotifer chitin and chitosan are shown in Fig. 3 and 4, respectively. The IR

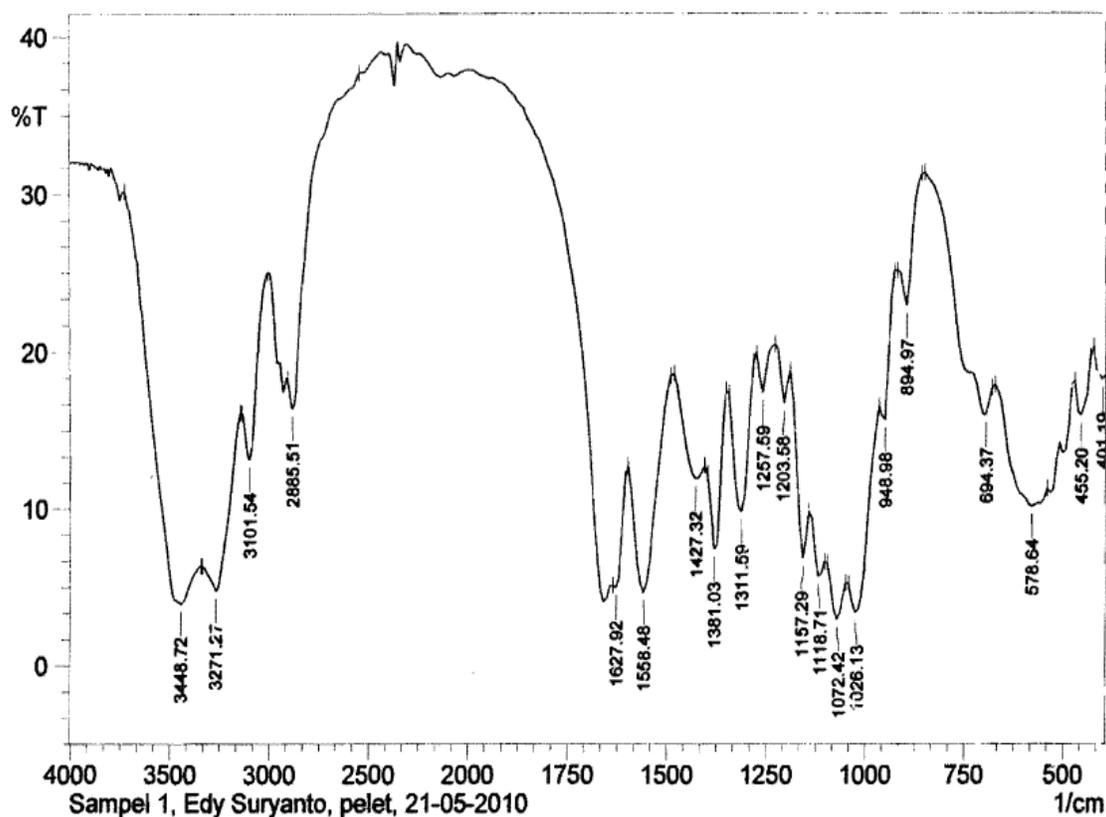


Fig. 3: Infrared spectra as a wave length function displayed by rotifer chitin

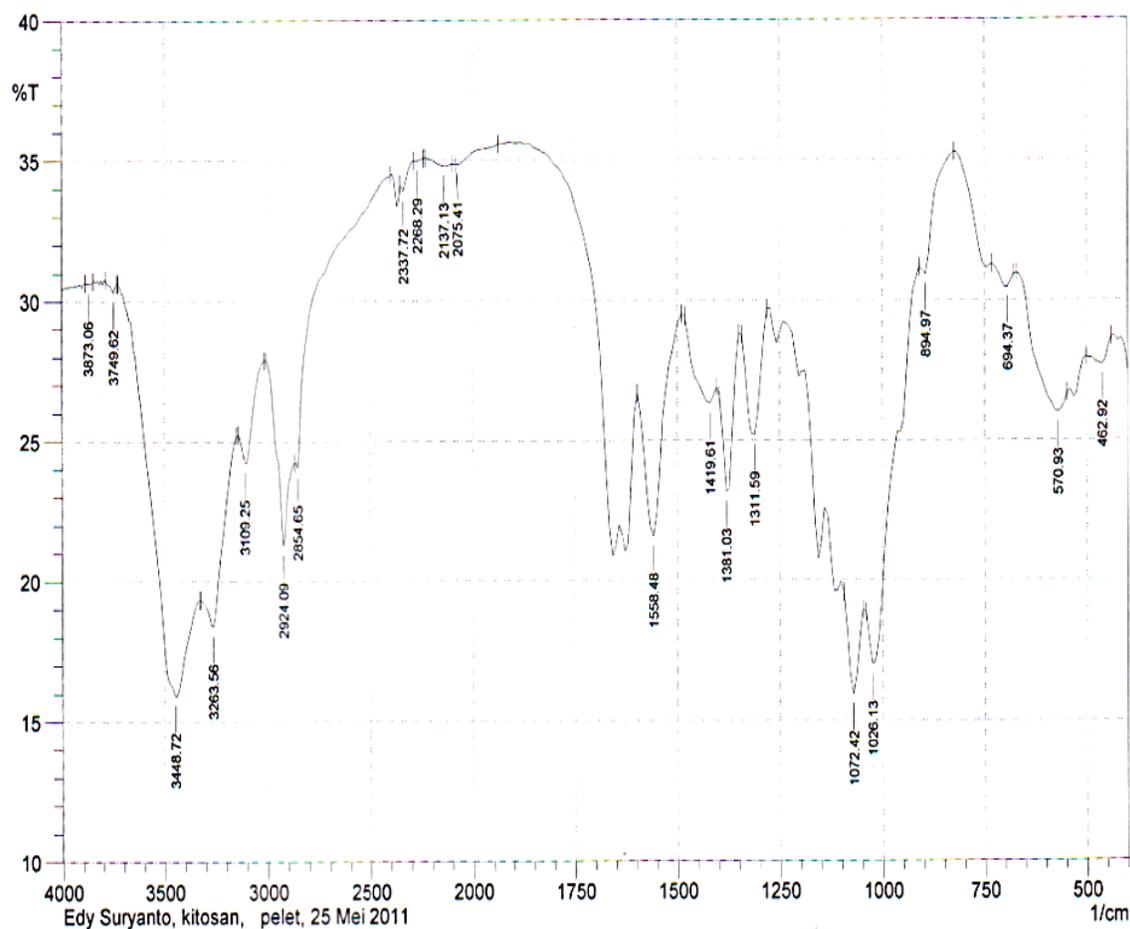


Fig. 4: Infrared spectra as a wave length function displayed by rotifer chitosan

Table 3: Functional groups of rotifer chitin compared to commercial standard chitin (Puspawati and Dan Simpen, 2010)

Group	Wave length (cm ⁻¹)	
	Standard chitin	Rotifer chitin
OH	3448	3448
N-H stretching	3300-3250	3271
C-H stretching	2891, 1	2885
C = O stretching	1680-1660	1627
N-H bending	1560-1530	1558-1562
CH ₃	1419, 5	1427
C-O-C	1072, 3	1072
N-H	750-650	694

Table 4: Functional groups of rotifer chitosan compared to commercial standard chitin (Puspawati and Dan Simpen, 2010)

Group	Wave length (cm ⁻¹)	
	Standard chitosan	Rotifer chitosan
OH	3450.0	3448
N-H stretching	3335.0	3263
C-H stretching	2891.1	2854
NH ₂ cutting, N-H bending	1655.0	1558
CH ₃	1419.5	1419
C-O-C	1072.3	1072
NH ₂	850.0-750.0	894
N-H	715.0	694

spectra of the rotifer samples of chitin and chitosan were compared to the IR spectrum of commercial (literature) on Table 3 and 4. The rotifer samples displayed similar IR spectra to that of the commercial chitin and chitosan.

The FTIR spectra of rotifer chitin exhibited a characteristic band at 3448 cm⁻¹ is attributed to -NH and -OH groups stretching vibration and the band 2885 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 1427 cm⁻¹ corresponds to a symmetrical deformation of the CH₃ group and at 1558 cm⁻¹ corresponds to the N-H deformation of amide II. The vibrations bands at 1072 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 (Puspawati and Dan Simpen, 2010).

Characteristic of the rotifer chitosan was shown by a broad absorption band in the range 3000 to 3500 cm⁻¹ which is attributed to O-H stretching vibrations and the 3263 cm⁻¹ to vibration of NH. The stretching vibrations

of methylene C-H at 2854 cm⁻¹, absorption peak at 1558 cm⁻¹ correspond to the NH₂. The amide II band is used as the characteristic band of N-acetylation (Islam *et al.*, 2011). The spectra of chitosan showed the different vibration that occurs after deacetylation process, which was not the emergence of vibration C = O at 1627 cm⁻¹ region, which indicates the vibration of C = O has been reduced on chitosan, as well as the emergence of absorption at 894 cm⁻¹ on chitosan rotifer which was the vibration of NH₂.

Chitin C₁₈H₂₆N₂O₁₀, 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 (Sanford, 1988). The marked difference between chitin and chitosan IR spectra (Fig. 3 and 4) in the interval 3271-3448 cm⁻¹ could be connected with the larger amount of N-H and O-H groups in chitosan, the larger absorption in chitin at 2885 cm⁻¹ is the consequence of larger amount of C-H bonds. In the infrared spectra, the rotifer chitin indicated the amide II band at 1558.48 cm⁻¹, a typical one for marine chitin. A typical amide I band was found at 1651.07 cm⁻¹. Chitosan showed no amide band, but hydroxyl and, amino bands at the ranged spectra up to 3500 cm⁻¹.

In terms of chemical properties, chitosan is more applicable, due to its molecular structure as a high molecular weight polymer, being a linear polyamine whose amino groups are readily available for chemical reactions and salt formation with acids (Sanford, 1988).

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