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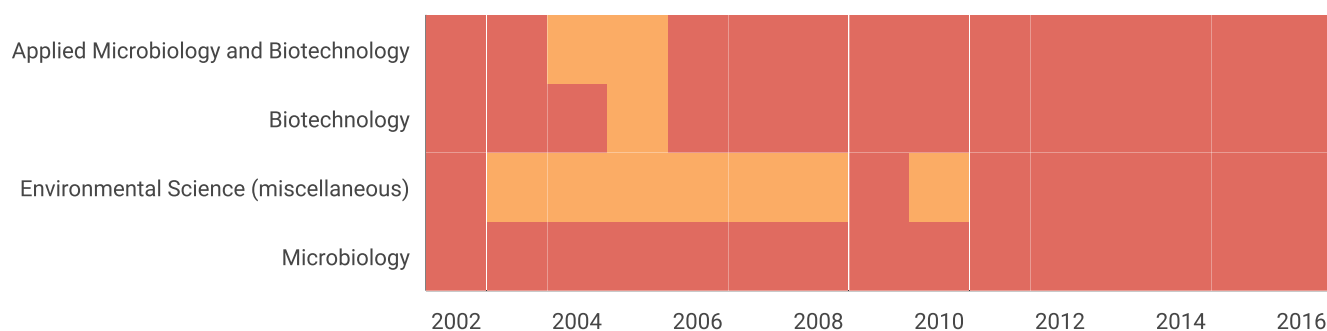
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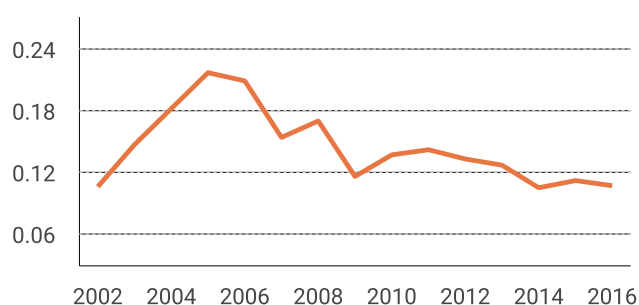
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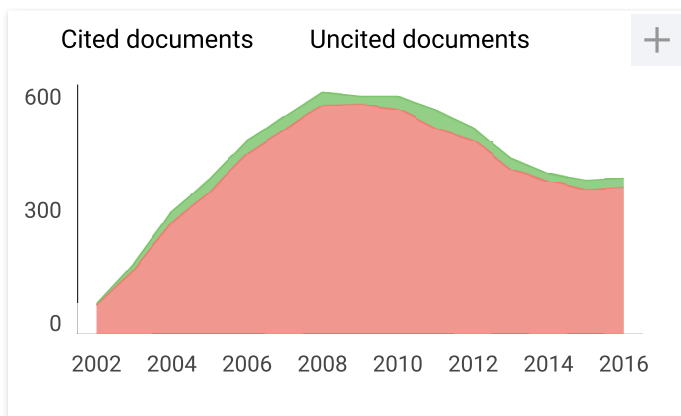
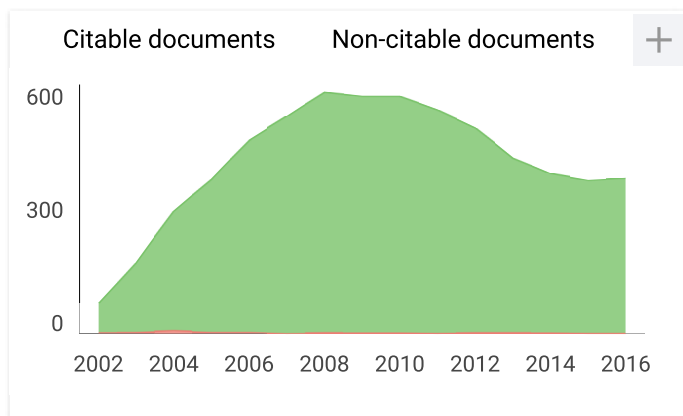
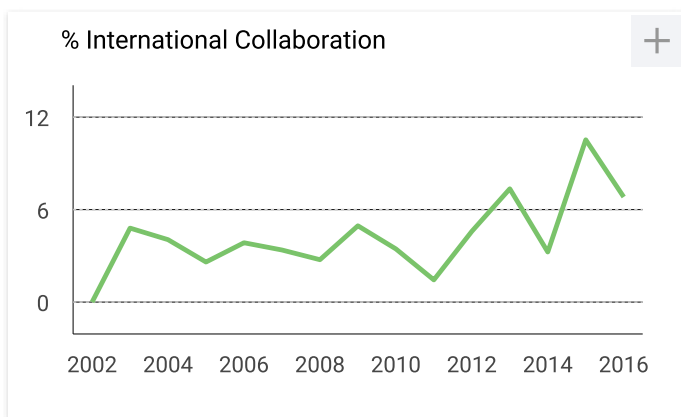
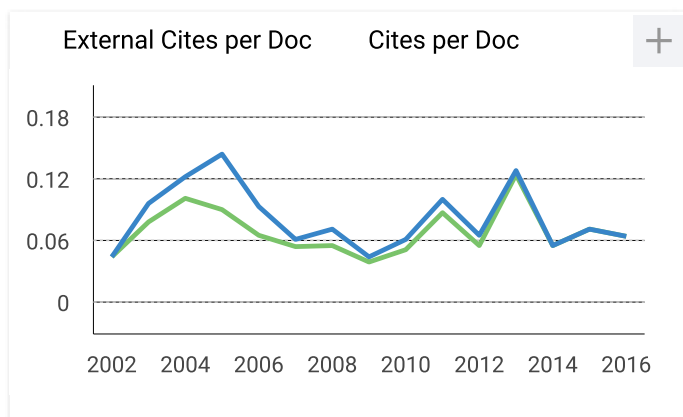
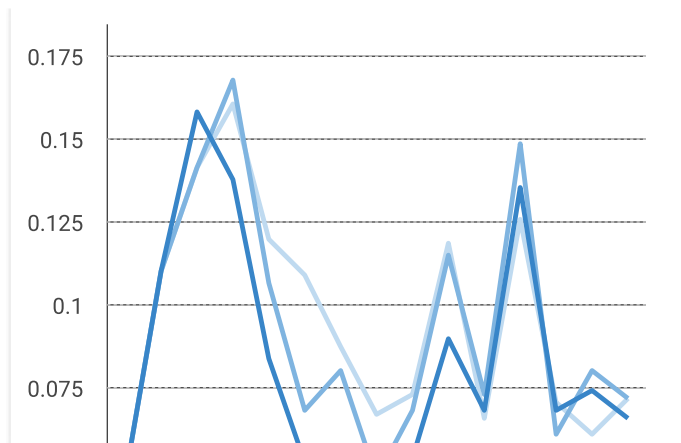
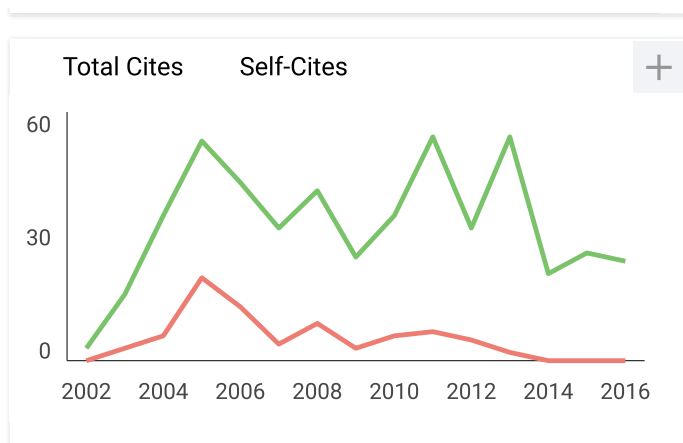
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ISOLATION AND CHARACTERIZATION OF CHITOSAN FROM LOCAL MUSSELS' SHELL (*PILSBRYOCONCHA* SP.)

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Key words : Isolation, Characterization, chitosan, Shell, local mussels (*Pilsbryconcha* sp.)

Abstract - This research is aimed to utilize local mussels' shells in order to have economic value and to minimize the environmental pollutant through isolating and characterizing the chitosan of local shells (*Pilsbryconcha* sp.) from Gorontalo, Indonesia. This exercise was conducted through preparing the sample, deproteinization process, demineralization, depigmentation, deacetylation and characterization of chitosan chitin. The isolation stage of 390 g of local mussels' shells produced 70.78 g of chitosan. The characteristics of chitosan from this process were white, powdery with 1% of moisture, ash content 0.09%, deacetylation degree 99%, soluble in CH₃COOH 2%, insoluble in water, slightly soluble in condensed HCl and HNO₃, insoluble in condensed NH₃ and insoluble in Na₂SO₄ 2%.

INTRODUCTION

Indonesian waters have various types of mussels, one of them is in Lake Teratai, Boalemo, Gorontalo province. This local mussel (*Pilsbryconcha* sp.) is categorized as mollusk, with bilateral symmetrical shape that consists of two shells. This local mussel has not been optimally utilized. The utilization process currently limited to its meat, meanwhile the shells are thrown away as waste. The waste from these mussels has become an environmental problem, therefore, it is expected that the waste can be recycled to become something useful.

Various research on shells of the arthropods, annelids, mollusks, coelenterates, and nematodes for their potentials to produce the chitin (Lesbani *et al.*, 2011). Chitin is a biopolymer with varied characteristics or chemical composition based on the source and the isolation process. More than 80% distilled chitin is called chitosan. Chitin and chitosan have high economic values. Chitosan is widely used in food inashry, medical application, cosmetics, water processing, detergent, paper, textile, antimicrobial agent, antioxidant, edible film inashry, and other biotechnology application (Ahmed *et al.*, 2010; Lee *et al.*, 2010; Bourbon *et al.*

2011; Ahmed *et al.*, 2014; Trung and Bao, 2015). Chitin isolation can be done through demineralization process, deproteination, and depigmentation, meanwhile chitin transformation into chitosan can be conducted through deacetylation (No *et al.*, 1989; Sofia *et al.*, 2010; Sarwar *et al.*, 2014). Characteristics of the chitosan product highly depend on the source of the animal and its production method. This research aims to utilize the local mussels' shells into chitosan hence it has economic values and it would no longer be environmental pollutant, isolating and finding out the characteristics of chitosan of the local mussels' shells (*Pilsbryconcha* sp.) from Gorontalo waters.

METHODS

The tools used in this research are oven, grinder, 100 mesh sieve, mixer, biker, thermometer, pH meter, analytical balance, sieve, heater, FTIR (Fourier Transform Infrared). The ingredients used in this research are the shells of the local mussels obtained from the lake Teratai at Pontolo village of Boalemo district, Gorontalo), acetate acid (Merck), NaOH (Merck), HCl (Merck), HNO₃, NH₃, Na₂SO₄, aquades, H₂O₂ 2%.

Sample Preparation

The mussels' shells taken from the lake Teratai of Pontolo village, Boalemo, Gorontalo washed with water (scrubbed when needed in order to get rid of the remaining dirt. Further, the shells were dried in an oven with 60 °C temperature for 6 hours. When the shells were dried, they were grinded and sieved to produce shells' powder with the diameter of 0.25 mm. This product is then used for extraction process.

Deproteinization

Sieved sample of the local mussels' shell were taken 390 g then put into the beaker with stirrer and thermometer. Next, 3.5% (w/v) of NaOH with 1:10 ratio was put into the beaker. Then, the mixture was heated in 90 °C temperature while stirred for 4 hours. A sieving process followed this stage. The product of this process then washed using aquades up to a neutral pH was reached. The residue from this process then dried in an oven with 60 °C temperature for 24 hours.

Demineralization

The result from deprotenization process was put into a beaker and added with HCl 1 N solution with the ratio of 1:7. Further, a room temperature heating was done while stirring it for 1 hour. The mixture then is sieved, the sediment then is washed using aquades. The residue (chitin) produced from this process then dried in an oven with 60 °C temperature for 24 hours.

Depigmentation

The mineral-free residue (chitin) was then added with the 2% of H₂O₂ solution with the ratio of 1:10. This mixture then sieved. The sediment then washed with aquades to produce a neutral pH. The residue from this process then dried in an oven with 60 °C temperature for 24 hours.

Deacetylation

The residue powder was put into beaker with stirrer and thermometer, then 50% NaOH solution with 1:10 ratio was added into the beaker and stirred for 1 hour in 120 °C temperature, then, sieved. The residue from this process then washed with aquades until a neutral pH was obtained. The product of this process then heated in an oven with 60 °C temperature for 24 hours.

These deproteinization, demineralization,

depigmentation, and deacetylation process referred to the result of Lukum's (2010) research.

Characterization of chitin-chitosan

Characterization used to differentiate chitin and chitosan stoichiometrically are the moisture level, ash level, and deacetylation degree using the spectrophotometric infrared. Moisture level testing (AOAC 2007), ash level testing (Sudarmadji et al 1994; AOAC 2007), chitosan solubility test. Several solvents used in chitosan solubility test are 2% of CH₃COOH, water, HCl, HNO₃, NH₃, and 2% Na₂SO₄ (modified of Lukum, 2010). Further, deacetylation degree was determined using the FTIR method (Puspawati and Simpen, 2010).

RESULTS AND DISCUSSION

Isolation and characterization process of chitosan from local mussels' shells (*Pilsbryoconcha* sp.) from Gorontalo water was conducted several steps of deproteinization, demineralization, depigmentation, deacetylation, followed by moisture level test, ash level test, and determination of deacetylation degree and solubility level (by means of 2% of CH₃COOH, water, condensed HCl, condensed HNO₃, condensed NH₃, and 2% of Na₂SO₄). Isolation process of 390 g of samples of local mussels' shells produced 70.78 g of white colored chitosan. The deproteinization process and demineralization process were conducted to lower the yield produced, and after the depigmentation process a whiter color was obtained.

Deproteinization is aimed to eliminate the protein in the sample by means of sodium hydroxide. Efficacy of protein elimination from chitin is influenced by alkaline level and temperature used. According to Martati *et al.*, (2002) that the level of nitrogen residue in chitin significantly lowered when the temperature and deproteinization time are increased.

Demineralization process is intended to eliminate the minerals content in the chitin such as, CaCO₃ and CaPO₄. On the other hand, depigmentation by using the H₂O₂ is intended to get rid of any color substance using the solvent. The solvents used in this process are mixture of ethanol-ether, sodium hypochlorite, chloroform, H₂O₂, ethyl acetate, and acetone.

Deacetylation is a process of chitosan formulation from chitin by using NaOH to substitute the acetamide structure with amine structure. The

elimination of acetyl structure using the 50% NaOH will influenced the level of deacetylation process of chitosan. The success rate of chitin-chitosan deacetylation process is influenced by various factors, such as, NaOH concentrate, temperature, time, stirring in deacetylation process and the origin of sample. Hargono *et al.*, (2008) said that deacetylation process using the 50% of NaOH yields best quality chitosan. Within the solution, NaOH decomposed into Na^+ and OH^- . Hydroxyl ion invades the electropositive carbon carbonyl. The final products are chitosan and sodium acetate (shown in Figure). In Each steps from deproteinization to deacetylation, the residue were washed using the aquades in order to neutralize its pH.

The characterization result of chitosan from

local mussels' (*Pilsbryconcha* sp.) shells can be seen in the following Table.

Moisture level

Moisture level is one of the important parameter within the standard of qualified chitosan and will influence the storage time of the chitosan itself. The analysis result showed a relatively low moisture level (1%). Standard quality of chitosan dictates that the moisture level is no more than 10%, due to the higher the moisture level, the faster the damage process happened to the product. According to Sofia *et al.*, (2010) commercial chitosan produced by Sigma has 3.5% level of moisture. The usage of NaOH in deproteinization process caused the softening of the cell's wall that will increase

Table. 1 Chitosan characterization of local mussels' shell (*Pilsbryconcha* sp.) from Gorontalo water

Parameter	Chitosan sample	Chitosan standard
Particle size	Powder	-Bubuk
Moisture level	(% w/w)	$1 < 10$
Ash level	(% w/w)	$0,09 < 2$
Deacetylation degree	(%)	$99 > 70$
Solubility	<ul style="list-style-type: none"> • 2% CH_3COOH • Water • Condensed HCl • Condensed HNO_3 • Condensed NH_3 • 2% Na_2SO_4 	Soluble Insoluble Slightly soluble Slightly soluble Insoluble Insoluble

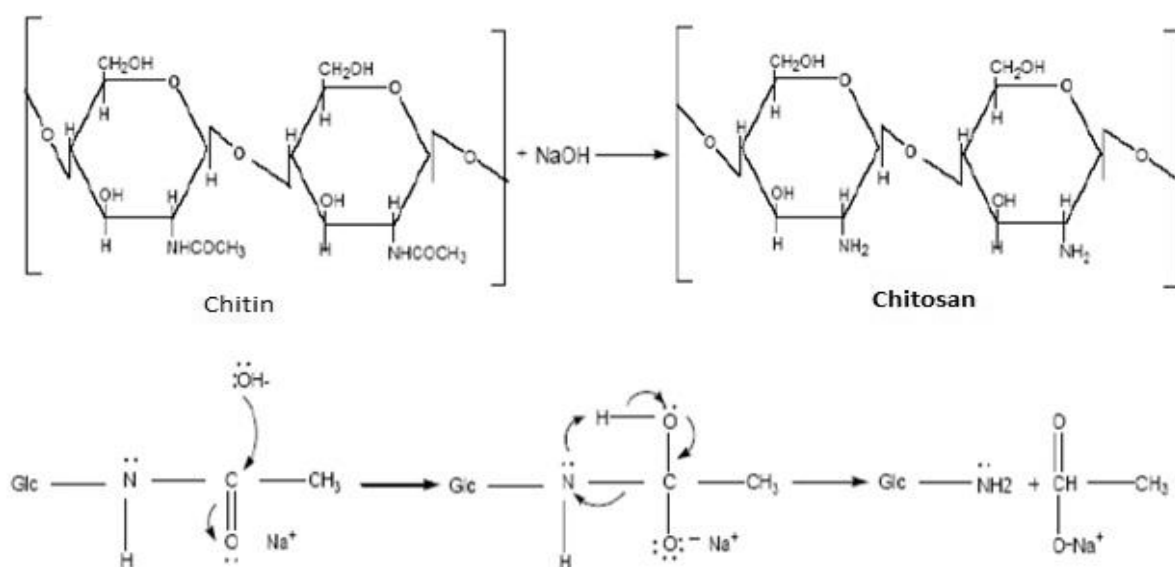


Fig. 1 Chitin transformation to Chitosan

permeability of the cell's wall, hence, it enables moisture to be released from the cell's wall and help the drying process. Appropriate packaging and storage will maintain the low moisture level of chitosan (Martati *et al.*, 2002).

Ash Level

The ash level in this research was lower (0.09%) than the ash level in chitosan from the shells of Javanese crabs (8.01%) (Saputro *et al.*, 2011), and has met the standard of chitosan. Setting up the ash level aims to find out the extent of minerals content that have not been eliminated during the demineralization process. The usage of HCl was effective in eliminating inorganic minerals like CaCO_3 and CaPO_4 . No and Meyers (1995) said that the standard requirement for high quality chitosan has to have less than 1% of ash level. According to Martati *et al.*, (2002) numerous dirt and residual mineral causing the ash level to be low is due to the temperature and length of deproteinization time.

Deacetylation degree

Deacetylation degree of chitosan from the samples of local mussels' (*Pilsbryconcha* sp.) shell was 99%. Deacetylation degree in this research is higher than deacetylation degree (DD) of shrimp waste in Camacho *et al.*, (2010). The high level of deacetylation degree shows that the chitosan produced from this research are pure chitosan. Deacetylation degree is one of the quality parameter of chitosan product that shows the percentage of acetyl structure that can be rid of both from the chitin and chitosan. The higher the deacetylation degree of a chitosan, the lower the acetyl structure of that chitosan, hence, the interaction among ion and hydrogen chain will be stronger. Deacetylation degree of chitosan is influenced by the concentration of sodium hydroxide (NaOH) and the temperature during the processing. The bigger the concentration of NaOH solution, the higher the value of deacetylation degree (Apriani *et al.*, 2012; Hossain and Iqbal, 2014).

Solubility test

The parameter that can also be used as standard in measuring the quality of chitosan is the solubility of that chitosan. The result of solubility test on 2% CH_3COOH , water, condensed HCl, condensed

HNO_3 , condensed NH_3 , 2% Na_2SO_4 are matched with the chitosan standard. Chitosan is soluble in acetate acid solution (2% CH_3COOH), insoluble in water, condensed NH_3 and 2% Na_2SO_4 , slightly soluble in HCl and condensed HNO_3 . Chitosan solubility in CH_3COOH is due to the high temperature during the deacetylation process. Savitri *et al.*, (2010) stated that the higher the concentration of NaOH and the temperature during deacetylation process, the higher the solubility of chitosan within the CH_3COOH and the more the acetyl structure being replaced, hence the value of deacetylation degree increased. Chitosan is insoluble in water, strong alkaline solution, and slightly soluble in HCl and HNO_3 , and soluble in acetate acid and formate (Apriani *et al.*, 2012).

CONCLUSION

Based on the findings of this research on the isolation and characterization of chitosan from local mussels' shells (*Pilsbryconcha* sp.) of the Gorontalo lake it is concluded that the local mussels' shells can be utilized as chitosan. 70.78 grams of white colored powder chitosan was produced from deproteinization stage, demineralization, depigmentation, and deacetylation stage. This chitosan has 1% of moisture level, ash level of 0.09%, deacetylation degree 99%, soluble in 2% of CH_3COOH , insoluble in water, condensed NH_3 and 2% of Na_2SO_4 , slightly soluble in condensed HCl and condensed HNO_3 .

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