Synthesis of Carboxymethyl Polysaccharide from Arenga Pinnata Polysaccharide and Monochloroasetic

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Abstract: Synthesis of carboxymethyl polysaccharide Arenga pinnata has been prepared through etherification of Arenga pinnata polysaccharide and monochloroacetate (MCA). Firstly the polysaccharide was extracted from Arenga pinnata endosperm with different toughness (soft and hard) followed by etherification with monochloroacetate using sodium hydroxide as a catalyst with variation of molar ratio MCA : NaOH of 0.50; 0.55; and 0.60 and ethanol 96% as solvent at temperature of 60 C for 10 hours in oven. The substitution degree was determined using titration method in which the Na-carboxymethyl was hydrolysis with HCl 2M in methanol 70% to form carboxymethyl polysaccharide. The yields of carboxymethyl polysaccharide were 90% and 83% for hard and soft, respectively. The substitution degree of carboxymethyl polysaccharide from soft Arenga pinnata endosperm was 1.600; 1.068 and 0.403 and from hard endosperm was 0.688; 0.467 and 0.202. The substitution degree of carboxymethyl polysaccharide was increased in the increasing of NaOH used which polysaccharide from Arenga pinnata endosperm soft has higher substitution degree than from the hard. The appearance stretching vibration of the carbonyl group (C=O) at a wavelength of 1730 cm-1 confirmed the formation of carboxymethyl polysaccharide. The morphology surface of carboxymethyl polysaccharide showed rough surface than Arenga pinnata polysaccharide revealed that the granule of polysaccharide have been changed.

1 INTRODUCTION

Chemical modification of polysaccharides is considered as one very important pathway to improve the properties of this biopolymer. Recently research has been directed towards the functionalization of a material. Carboxymethylation of polysaccharides is one of the conversions widely studied for the development of new biomaterials with very promising applications (Parvathy et al., 2005). Carboxymethylation could improve solubility in water (increases hydrophilicity), insoluble polysaccharides or low solubility in water (Yang and Zhang, 2009). Gum polysaccharides are often not completely dispersed and commercially available gum solutions are usually cloudy and contain suspended solid particles. Such solutions are usually quite thick and cannot be filtered to separate suspended solids, for this purpose they need to be modified into carboxymethyl gum which can form bright dispersion solutions compared to untreated gum (Moe, 1949). One source of polysaccharide that

is abundant in Indonesia is the endosperm of palm seeds (Arenga pinnata Merr.) which is often known as "kolang-kaling" (Mogea et al., 1991). The utilization of this endosperm is remaining limited for food (Orwa et al., 2009, Tarigan et al., 2018b).

The polysaccharide containing in Arenga pinnata endosperm (APE) is galactomannan consisting of mannose and galactose with a ratio of galactose : mannose of 1 : 1,33. Those monosaccharide linked in a linear chain of 1,4- β-D-mannopyranosil and is water soluble if the galactose residue is >5% and is insoluble in water if <5% (Aspinall, 1959, Bento et al., 2013, Tarigan et al., 2012, Tarigan et al., 2018a).

Carboxymethylation reactions have been studied in some natural polymers such as cellulose, funegeric gum, sesbania gum flour, guar gum (Gong et al., 2012, Liyanage et al., 2015, Noleto et al., 2009, Parvathy et al., 2005). In general, the carboxymethylation method is carried out using the catalyst NaOH and NaHCO$_3$ and monochloroacetate (MCA) reagents. Reactions can take place either in with or without water in increasing temperatures

(Parvathy et al., 2005). Gong et al. (2012) have examined the synthesis and characterization of carboxymethyl guar gum with the dry method and alkalization process using NaOH catalyst and carboxymethylation using (MCA) and the degree of substitution was determined by titrimetric. The dry method is used to avoid the formation of gels that can resist the effective penetration of the catalyst and reagent rendering incomplete reaction resulting in very low substitution degrees.

Based on the description above, this study aimed to modify the polysaccharide of APE into carboxymethyl using MCA and NaOH as a catalyst through the dry method. The type of APE powder used is hard and soft which is determined by the penetrometer. The carboxymethyl obtained was determined by FT-IR spectrophotometer, surface properties with SEM, and degree of substitution by titration.

2 MATERIALS AND METHODS

2.1 Materials

The Arenga pinnata was collected from a traditional market in Medan – Indonesia. All chemicals used in this study were brought from the local chemical supplier without any purification.

2.2 Methods

2.2.1 The APE Texture Determination

The stratified random sampling method was used to collect palm seeds. Texture measurements are carried out using a penetrometer. The prepared sample was stabbed at four points using a precision penetrometer which was pressured 250 g with a scale of 1/10 mm for 10 seconds. Texture values can be read on the scale indicated by the needle instructions and the four values are averaged. The texture value was calculated with the formula as below.

$$Texture \ (g/mm) = \frac{250}{\frac{(X1+X2+X3+X4)/\ 4}{1/10}} \qquad (1)$$

2.2.2 Preparation APE Extract

The preparation of APE extract was conducted following the previous procedure (Tarigan and Purba, 2015). The APE group I (hard) was chopped as thick as 2 mm and crushed roughly with a blender at a scale speed of 1 for 2 minutes with 250 mL of water. The hydrogel obtained was immersed in 96%

ethanol for 48 hours at a ratio of 1: 2, then filtered, the residue obtained was soaked again with ethanol which was half volume from the initial ethanol volume for 48 hours and finally with pure grade ethanol for 12 hours until the residue is submerged. The residue obtained is dried in a desiccator in a vacuum until the weight is constant. The same procedure is conducted for soft palm seeds (group II). The obtained APE powder was characterized by FT-IR analysis and SEM surface morphology.

2.2.3 Preparation of Carboxymethyl APE

The preparation of carboxymethyl APE was done using a method developed by Gong et al. (2012) with slight modification. 1.65 grams of galactomannan from APE group I were put into glass beakers and then added with NaOH in varying concentration. The mole ratio of MCA : NaOH used was 1:0.5; 1:0.55 and 1:0.6. Next, the solution was added with 1 mL ethanol 96%, heated at 30°C while stirring using a magnetic stirrer for 20 minutes and then was added 1.05 grams of MCA and stirring using a magnetic stirrer for another 20 minutes, then transferred into a watch glass and heated in the oven for 10 hours with a temperature of 60°C. Carboxymethyl polysaccharide produced was dried at room temperature, crushed and stored in a desiccator. The same treatment was carried out for APE group II.

2.2.4 Determination of Substitution Degree

The determination of substitution degree according to the procedure established by the previous researcher (Gong et al., 2012). Determination of substitution degree (SD) was carried out firstly with converting Na-carboxymethyl polysaccharide into carboxymethyl polysaccharide of palm seeds. 1.5 grams of Na-carboxymethyl polysaccharide from the group I with a mole ratio of MCA : NaOH of 1:0.50 was poured into a glass beaker which already contains 2 M HCl (in methanol 70%), stirred with a magnetic stirrer for 2 hours, then filtered. The solids of carboxymethyl polysaccharide obtained were then washed using ethanol 96% until chlorine free (tested using silver nitrate / AgNO3 0.1 M solution). The resulting solid is dried in an oven at 60°C for 2 hours. 0.2 grams of carboxymethyl polysaccharide that has been dried then dissolved in 20 mL of 0.1 M NaOH, stirred for 2 hours with a magnetic strider. The carboxymethyl polysaccharide which has excess NaOH is then titrated using a standard 0.1 M HCl solution with the addition of phenolphthalein as an indicator. The same treatment was carried out for the comparison of MCA : NaOH 0.55; 0.60 and carboxymethyl galactomannan from APE group II.

The carboxymethyl polysaccharides obtained were analyzed using FT-IR spectrophotometer and SEM.

3 RESULTS AND DISCUSSIONS

3.1 Texture

The sample is prepared by grouping samples using the stratified random sampling method. The population of 100 APE is grouped into 2 groups with almost the same level of similarity. Group 1 (50 pieces) and group 2 (50 pieces). Samples were taken randomly at 2% for each group which was for groups 1 (10 pieces) and group 2 (10 pieces) and determined in 4 positions namely: up, down, right and left, then taken evenly the points, but the data is not included in this paper. The texture and yield of APE powder and yield carboxymethyl product showed in Table 1.

Tabel 1: The texture value and yield of APE powder and carboxymethyl

Parameter	Seed of *A. pinata*	
	Hard	Soft
Texture (g/mm)	0.1054–0.1342	0.0846-0.0999
Yield of APE powder (%)	4.72	4.52
Yield of carboxymethyl (%)	90	83

The difference in texture value (Table 1) is caused by the level of maturity which in soft APE is lower than that of hard APE. The more mature the sugar palm seeds, the harder and the higher the content in the endosperm, the more resistant to mechanical damage even to water (Cerqueira et al., 2009). The hard and soft texture is also affected by the treatment given to the process of making APE, for example, soaking time, cooking, stripping, etc. that allow for differences in the texture between one APE and another. Thus the texture obtained is different from the texture we have reported in our previous study, where the hard and soft texture of the value is lower than our previous research report, this is because the APE is used uniformly and are softer and rounder (not flattened) and the source is also different. Likewise, the percentage of the APE powder obtained is also different from our previous research.

3.2 FT-IR Analysis

FT-IR spectrum (Figure 1) shows the peak characteristics of galactomannan which is similar with the literature of Boual et al. (2015) and (Tarigan, 2014); there is no difference in absorption bands for hard and soft APE, respectively. The absorption peak at 3345 cm^{-1} shows the stretching vibration of the OH group from the polysaccharide and the absorption band at 2888 cm^{-1} shows the stretching vibration -CH of -CH$_2$- (Singh et al., 2009) supported by bending vibrations -CH$_2$- on the wavelength of 1372 and 1373 cm^{-1}. Absorption at 1638 and 1636 cm^{-1} indicates the presence of an OH group bound to water. The wide peaks at an area of 900 - 1200 cm^{-1} due to stretching vibrations of -C-C-O, C-OH, and C-O-C from the main polymer chain (Buriti et al., 2014). The peak at 1021 cm^{-1}, 1020 cm^{-1} shows the C-O bending vibration of the pyranose ring. The band at 869 cm^{-1} characteristics of the β-D-manopiranose bond present in the polysaccharide (Buriti et al., 2014) and the band at 811 cm-1 showed the presence of α-D-galactopiranose bonds (Buriti et al., 2014).

Carboxymethyl polysaccharide from hard and soft APE was carried out in two stages, namely the process of adding NaOH as a catalyst (alkalization) and formed Na-carboxymethyl polysaccharide which was analyzed by FT-IR (Figures 2) followed by the addition of MCA (carboxymethylation). The carboxymethyl polysaccharide of APE formed was analyzed with an FT-IR spectrophotometer (Figures 3).

In the formation of Na-Carboxymethyl polysaccharides the peak changes at wave number 1636 cm^{-1} on hard APE and wave number 1638 cm^{-1} on soft APE. The formation of carboxymethyl polysaccharide from hard and soft APE is shown by the formation of absorption bands at wave numbers around 1730 cm^{-1} which states the formation of carbonyl groups (C = O). Figure 3A shows that the peak formation of carboxymethyl carbonyl groups of hard APE at a ratio MCA : NaOH of 1 : 0.50 while for carboxymethyl soft palm seeds (figure 3B) the appearance of carbonyl peaks at mole ratio MCA : NaOH of 1 : 0.50 and 1 : 0.55. This is inseparable from the less crystalline polymer structure in soft APE. The structure is increasingly crystalline because of the presence of hydrogen bonds (Niroomand et al., 2016). The release of galactosyl residues from galactomannan polymers will provide an increase in the mannose ratio in galactomannan or the formation of mannan (Bento et al., 2013) rendering the APE get harder.
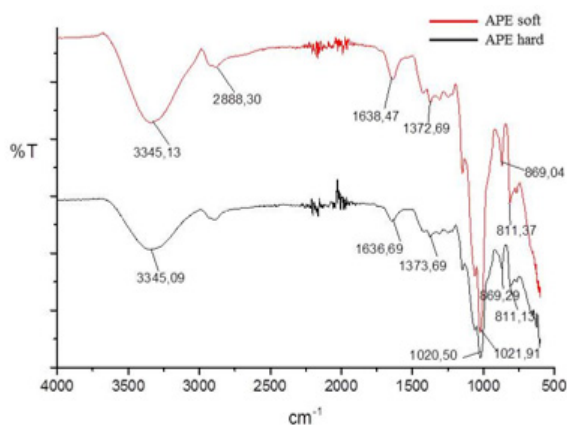
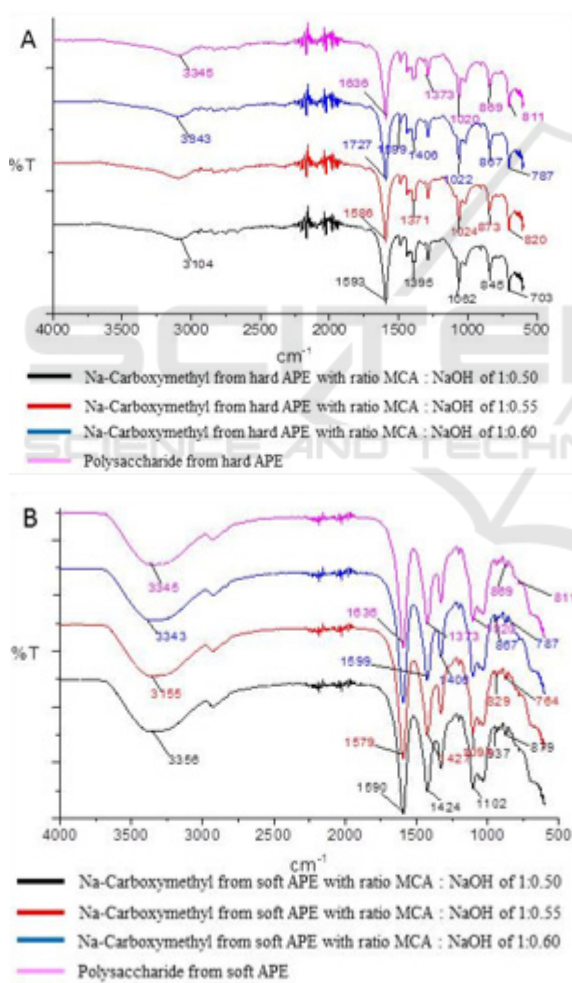Figure 1: FT-IR spectrum of APE powder hard and soft

Figure 2: FT-IR spectrum of Na-carboxymethyl with ratio MCA : NaOH of 1 : 0.50; 1 : 0.55; 1 : 0.60 from (A) hard APE and (B) soft APE.

3.3 SEM Images

The surface morphology images polysaccharide of hard and soft APE and carboxymethyl polysaccharide of hard and soft APE is shown in Figure 4. The surface morphology of hard APE is indicated by a rough and irregular surface and fused with each other while soft APE is soft and the surface is smoother. The morphology of the surface of carboxymethyl polysaccharides of hard APE becomes rough and bumpy which indicates that the interaction between the polymer chains is irregular.

3.4 The Substitution Degree

The substitution degree was determined using titrimetric method and the result is presented in table 2. The substitution degree (DS) is the average value of the hydroxyl group exchanging with the carboxymethyl group present in each anhydroglucose monomer unit. Substitution degree test was conducted to determine the number of carboxyl compounds present in each polysaccharide monomer. The substitution degree describes the quality of the carboxymethyl polysaccharide produced. Theoretically, the maximum substitution degree value is 3. The substitution degree value produced from hard APE carboxymethyl polysaccharide is 0.688 whereas in the carboxymethyl polysaccharide of soft APE produced a substitution degree value of 1,600. From the table above it can be said that the higher or more the amount of sodium hydroxide (NaOH) is used, the greater the degree of substitution possessed by the carboxymethyl polysaccharide. The increasing amount of NaOH used makes the etherification process better because the alkali polysaccharide forms reactivity which is higher in proportion to the high concentration of NaOH used. The substitution degree in carboxymethyl polysaccharide of soft APE is greater than that of carboxymethyl polysaccharide of hard APE. This is because NaOH is easier to penetrate increasing swelling properties, for which monocloroacetic compounds are easier to substitute.

Tabel 2: The substitution degree of carboxymethyl polysaccharide

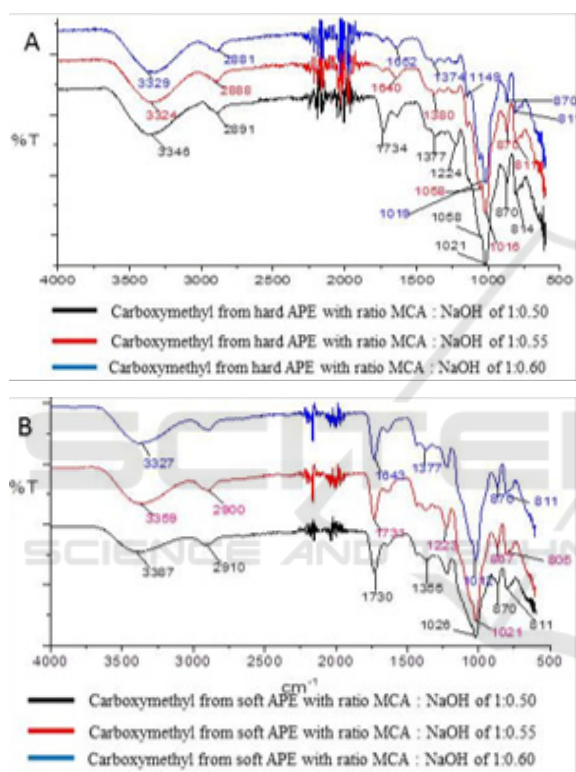| Parameter	Mole ratio of MCA : NaOH		
	0.60	0.55	0.50
SD of carboxymethyl polysaccharide from hard APE	0.202	0.467	0.688
SD of carboxymethyl polysaccharide from soft APE	0.403	1.068	1.600

Figure 3: The FT-IR spectrum of carboxymethyl polysaccharide with ratio MCA : NaOH of 1 : 0.50; 1 : 0.55; 1 : 0.60 from (A) hard APE and (B) soft APE
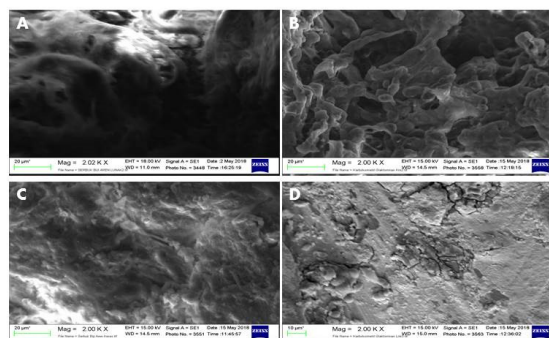

Figure 4: The SEM images polysaccharide of (A) hard APE, (B) soft APE and carboxymethyl polysaccharide from (C) hard APE, (D) soft APE

4 CONCLUSIONS

Carboxymethyl polysaccharide from hard and soft APE can be synthesized by the dry method with 2 stages, namely alkalization and carboxymethylation. Carboxymethyl formation is characterized by the appearance of C = O peaks at wave number 1730 cm-1. Percent yield for hard and soft APE was 90% and 83%, respectively. The surface morphology of polysaccharide was change from smooth to wavy in carboxymethyl. The degree of substitution of carboxymethyl hard APE is smaller than that of soft APE.

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