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Extraction and Characterization of Galactomannan from Nipa Palm Fruit (*Nypa fruticans* W.)

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Abstract The main objective of this research was to characterize the galactomannan from Nipa palm fruit. The galactomannan, a water-soluble heteropolysaccharide was extracted from nipa palm fruit with the different condition. Different condition referred to used nipa palm fruit without drying as fresh nipa palm fruit (GalFN) and nipa palm which is done by drying as dry nipa palm fruit (GalDN). Extraction method used is aqueous extraction followed by precipitation with ethanol. Polysaccharide was studied in detail for its chemical and functional characterization. The result suggests that the different condition of nipa palm fruit (GalFN and GalDN) does not have a significant impact ($p>0.05$) on the extraction yield, solubility (25^{o}C and 80^{o}C) and oil-holding capacity (OHC), yet it has a significant effect ($p<0.05$) on the whiteness degree and water-holding capacity (WHC). The physicochemical properties of GalFN and GalDN obtained respectively are as follows: extraction yield (6.34% and 6.13%), whiteness degree (78.20 and 70.10), solubility (34.93% and 35.73%) at 25^{o}C (71.13% and 72.73%) at 80^{o}C, WHC (295.03 and 316.97 g water/100 g gum), OHC (81.70 and 86.20 g oil/100 g gum). It was concluded that between GalFN and GalDN there were significantly differences in whiteness degree, water holding capacity, viscosity on Concentration of Nypa gum 1% and 2%.

Keywords Galactomannan, Nipa Palm Fruit, Nypa Gum, Physicochemical

1. Introduction

Galactomannans are natural polysaccharides, which commonly obtained from the endosperm of seeds of some *Leguminosae* plants [1]. Galactomannans are water-soluble polysaccharides built up of a β-(1–4)-D-mannan backbone with single D-galactose branches linked α-(1–6). The four major galactomannans which commercially important in food and non-food industries are guar gum (GG, *Cyamopsis tetragonolobo*, M/G ratio = 2:1), tara gum (TG, *Caesalpinia spinosa*, M/G ratio = 3:1), locust bean gum (LBG, *Ceratonia siliqua*, M/G ratio = 4:1) and fenugreek (*Trigonella foenum-graecum* L., M/G ratio = 1:1) [2]. This multipurpose material can be applied as: emulsifier for stiffener and stabilizer in textile, pharmacy, biomedical, cosmetic and food industries [3]. Galactomannan is also known for having antioxidant and antimicrobial properties [4]. Currently, international trends demand alternative sources of galactomannans [5].

Nipa palm (*Nypa fruticans* Wurmb.) is an *Arecaceae* family adapted in mangrove forests or high tide areas [6]. Nipa palm can be found in almost all mangrove forest areas in Asia and the West Pacific [7]. Nipa palm is a tropical plant, it can grow optimally at a temperature of 20-35 ℃ [8]. Distribution growth area of nipa palm in Indonesia reaches up to 700,000 ha, which is the largest area when compared to Papua New Guinea (500,000 ha), Malaysia (20,000 ha), and the Philippines (8,000 ha) [9]. The existence of the nipa palm has ecological benefits, such as: protecting abrasion or erosion of beaches and river banks, keeping

stability of the coastline, resisting strong winds and sea waves. Sea water intrusion control, seawater is filtered into fresh land water to serve as a buffer for life on land [10].

Nipa palm fruit contains 41.96% water and 51.08% carbohydrates [11]. It is vulnerable to microbiological damage, which causes changing characteristics and compositions. Carbohydrate is degraded into certain organic acids and CO_2 by bacteria, mould, and yeast. This condition is also supported by the natural existence of nipa palm fruit growing in remote areas. Therefore, it is needed to apply material handling measures to ensure that the chemical compositions of nipa palm fruit do not deteriorate. One of the ways is drying. Nipa palm fruit was dried before being extracted. Drying or dehydration is a food preservation method that removes water from the food to inhibit bacterial growth during storage and transportation process [12].

In this study, *Nypa gum* was extracted using an aqueous extraction method followed by precipitation with alcohol [13]. Galactomannan from fresh nipa palm fruit (GalFN) is compared with galactomannan from dried nipa palm fruit (GalDN). The physicochemical properties of both were also determined.

2. Materials and Methods

2.1. Materials

Main material for this research was fresh nipa palm fruit aged around 4 months. Nipa palm fruit was collected in the courtyard of the Tundo River, Sipelot Hamlet, Pujiharjo Village, Malang Regency, Indonesia. Nipa palm fruit that has been cut from the stem of the tree was peeled directly at the plantation site. The proximate compositions of nipa palm fruit are shown in Table 1 [1]. An overview of nipa palm fruit can be seen in Figure 1.

Table 1. Proximate Compositions of Nipa palm fruit

Nutrient	Compositions (%)
Moisture	41.96
Crude Protein (CP)	2.27
Crude Fat	0.94
Ash	2.70
Crude Fibre (CF)	2.50
Carbohydrate (CHO)	51.08
Nitrogen Free Extract (NFE)	91.59

2.2. Polysaccharide Extraction

Galactomannan extraction procedure from *Nypa fruticans* W. was adopted and slightly modified from Li [14]. Figure 2 shows the flow chart representing the extraction processes of *Nypa gum* considered in this work.

(a) Extraction Procedure of GalFN

450 mL of distilled water was added to 300 g of nipa palm fruit, mixed and grounded using a blender for around 5 minutes, stored in the refrigerator for 24 hours and filtered. A mix of filtrate and 95% ethanol, with a volume comparison of 1:2 (v/v), was made and stored in the refrigerator for 24 hours. Any formed precipitation was used and washed with 95% ethanol. Wet galactomannan was dried using a *vacuum dryer* (760 mmHg, 24 hours, 40^{o}C). Dry galactomannan was grounded with a mortar and stored in a desiccator.

(b) Extraction Procedure of GalDN

300 g of nipa palm fruit was dried (50^{o}C, 12 hours) and soaked within 650 mL of distilled water. Then, it was retained in the refrigerator for 24 hours and crushed with a blender for 5 minutes. The following steps were the same as the extraction procedures from GalFN.

(A) (B) (C)

Figure 1. Nipa Fruit, Fruit Bunch (A), Unit Fruit (B), Flesh of Fruit (C)

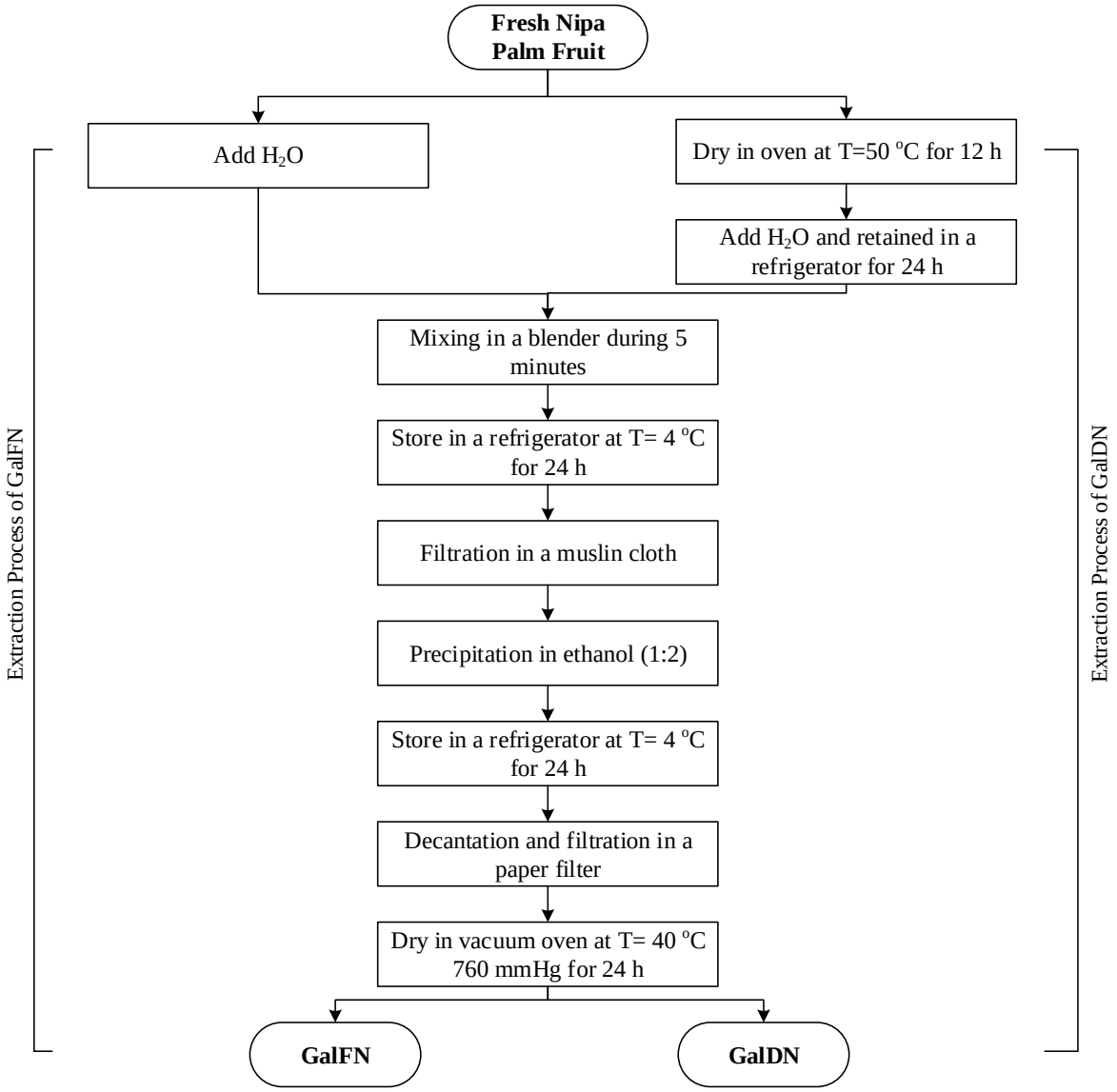


Figure 2. Flowchart of the Extraction Processes of Nipa Palm Fruit

2.3. Determination of Extraction Yield

The calculation formula for the extraction yield of *Nypa gum* can be seen as below.

$$\% \text{ Extraction yield} = \frac{\text{mass of extracted dried } nypa\ gum \text{ (g)}}{\text{mass of nipa palm fruit (g)}} \text{x } 100\ \% \quad (1)$$

2.4. Physicochemical Characteristics of Nypa Gum

2.4.1. Whiteness Degree

The whiteness degree was measured by colorimeter (PCE -CSM 1) based on Lanier [12].

$$W = 100 - [(100 - L)^2 + a^2 + b^2]^{0.5} \quad (2)$$

where,

W : Whiteness
L : Lightness
a : Reddish chroma
b : Greenish chroma

2.4.2. Solubility

The solubility test was measured based on Dakia [15]. Galactomannan powder (1%, b/v) was dissolved in distilled water, stirred at 25 and 80 ℃ for 30 minutes, and centrifuged (1800 g for 30 minutes at 25 ℃). The supernatant was dried at 105 ℃ for around 24 hours. The weight of the dry supernatant shall be represented as a dissolved fraction.

$$\text{Solubility (\%)} = \frac{\text{Supernatant concentration (mg/ml)}}{\text{initial concentration of the sample (mg/ml)}} \quad (3)$$

2.4.3. Water Holding Capacity (WHC)

WHC calculation was based on Galla and Dubasi [16]. One g of sample was dissolved into 10 mL distilled water, stirred for 2 minutes and centrifuged at 3,000 g at 4 ℃ for

30 minutes. Any water absorbed by the sample was stated as the weight of absorbed water per 100 galactomannans.

2.4.4. Oil Holding Capacity (OHC)

OHC calculation was based on Galla and Dubasi [14]. One g sample was mixed with 10 mL sunflower oil and stirred for 2 minutes. Then, this mixture was centrifuged at 3,000 g at 4^{o}C for 30 minutes. The free palm oil was decanted and any oil absorbed by the sample was stated as oil weight absorbed per 100 g galactomannan.

2.4.5. Viscosity

Viscosity test was carried out to determine the difference viscosity of galactomannan nipa solution at a concentration of 0.5%; 1%, and 2%. The preparation was carried out by weighing nipa galactomannan according to concentration, put in a 100 mL volumetric flask, added 45 ˚C water, transferred to 100 mL Erlenmeyer, then stirred with a shaker for 2 hours. The viscosity of the sample was determined using a Brookfield viscometer at 30 ℃ with a speed of 2.5 rpm.

2.4.6. Scanning Electron Microscope (SEM)

The microstructure of the sample was characterized by a scanning electron microscope (HITACHI FLEXSEM 1000).

2.4.7. Fourier transform infrared (FTIR) spectroscopy

An IR spectrum of the polysaccharide was taken using a Fourier-transform infrared spectrometer (FTIR, SHIMADZU). The powders were mixed with KBr and a pellet was compressed. The frequency range used to scan the samples is in the range of 4000 and 400 cm^{-1}.

2.4.8. Statistical Analysis

All the analysis was performed in triplicate. Means of the data were analyzed using Minitab (Version 19.0) using one-way ANOVA and Tukey's test was performed to compare significant differences between samples at $p<0.05$. All the data of the calculation results were presented as a mean ± standard deviation.

3. Results and Discussion

3.1. Nipa Palm Fruit Biomass

Any materials used included 23 bunches of nipa palm fruits, which consisted of 1,043 fruits and 859 empty fruits with a total weight of 234,900 g. Peeled nipa palm fruits produced around 15,489 g of fresh flesh (6.6%), 203,867 g of shell and fibre (86.8%), 14,133 g of cob (6%) and 1,411 g of husk (0.6%). The by-product of the nipa palm fruit peeling process was around 93.4%. This biomass is potentially processed into briquette and activated carbon.

3.2. Extraction Yield

Polysaccharides extraction yield is generally represented as the percentage of dry mass after the extraction process compared to the weight of dry materials [17]. In this study, GalFN and GalDN yields are not significantly different ($p>0.05$). It occurred as the condition of the extractions was almost the same (Figure 3). *Nypa gum* yield is slightly higher than *arenga gum* 4.99% [18], 4.15% [19], 5.50% [20], *Yanang gum* 4.54% [21], *Lepidium perfoliatum seed gum* 10.83-28.60% [22], *durian seed gum* 34.3-72.8% [23], *fenugreek gum* 10% [24]. Several factors affected the extraction yield, such as solvent: materials ratio, pH and temperature. It is in accordance with the reported conditions that the higher the solvent ratio of the materials, the higher yield will be obtained [22, 25, 23, 20]. Koocheki *et al.* [22] reported that the extraction yield increases along with the temperature increase. In addition, it is also mentioned that the extraction in alkaline conditions significantly increases the extraction results. Characteristics of Nypa gum can be seen in Table 2.

Table 2. Characteristics of *Nypa gum*

Component	Galactomannan	
	GalFN	GalDN
Extraction yield (%)	6.34^{a} ± 0.17	6.13^{a} ± 0.09
Whiteness degree*	78.20^{a} ± .002	70.10^{b} ± 0.001
Solubility (%) at25 ℃	34.93^{a} ± 2.15	35.73^{a} ± 2.24
Solubility (%) at80 ℃	71.13^{a} ± 1.97	72.73^{a} ± 2.12
Water holding capacity (g water/100 g gum)	295.03^{b} ± 4.11	316.97^{a} ± 2.54
Oil holding capacity (g oil/100 g gum)	81.70^{a} ± 2.36	86.20^{a} ± 2.82

Description: * without unit, where 100 is assumed as white color

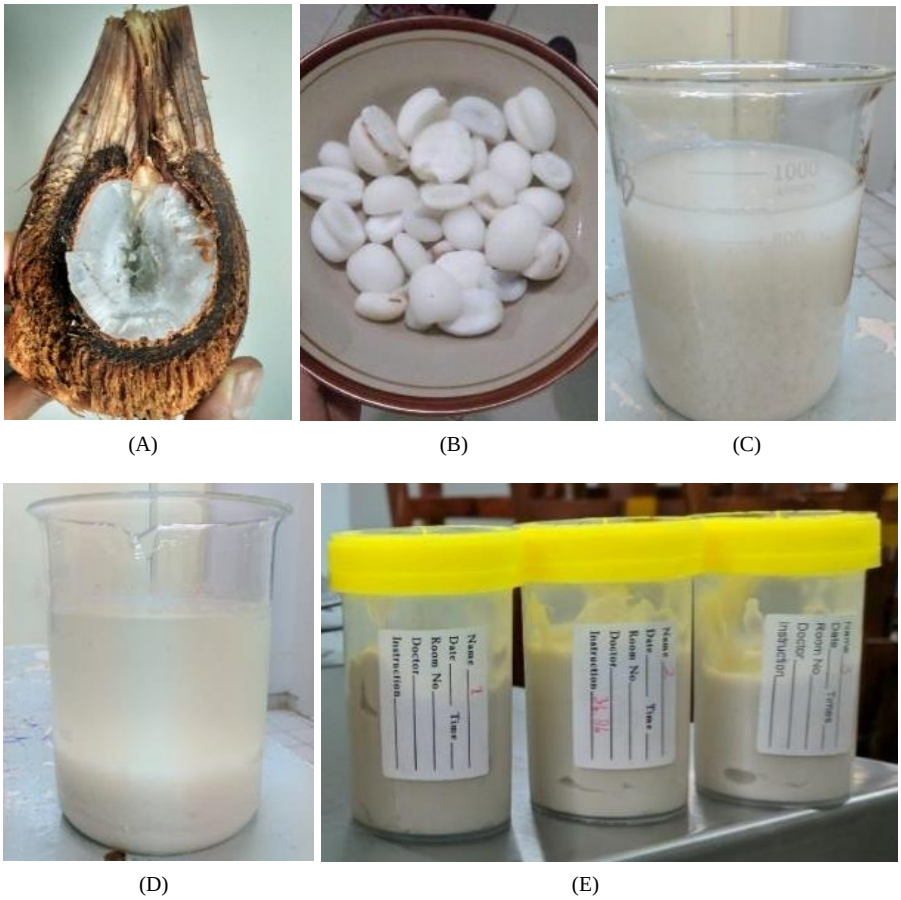


Figure 3. Overview of Nypa Gum Extraction Procedure: (A) Husk and Seed of *Nypa fruticans* (B) Nipa palm Fruit (C) Water Extraction (D) Precipitation with Ethanol (E) Filtration of Nypa Gum

Figure 4. Color of *Nypa gum*, GalFN (A) and GalDN (B)

3.3. Physicochemical Characteristics of Nypa Gum

3.3.1. Whiteness Degree

Color and appearance are crucial in product selection and acceptance in the food industry [26]. In this study, the whiteness degree of GalFN $p<0.05$) is significantly higher than GalDN. As in Figure 4, GalFN is brighter than GalDN. The whiteness degree of *Nypa gum* is lower than *Cassia grandis gum* (88.73-91.74) [27]. During the drying and heating process, there will be a reducing in sugar levels that increases significantly [28], which affects to the occurrence of browning reactions [29]. This browning reaction occurs between the carboxyl group in reducing sugar and the primary amine group in amino acids [23]. Browning reactions can also be caused by polyphenoloxidase (PPO) enzyme that oxidize phenolic

compounds in materials due to the presence of molecular oxygen [30].

3.3.2. Solubility

One of the main criteria for galactomannan selection is galactomannan solubility at various temperatures [31]. Some *arenga gum* dissolves in cold and hot water, and its characteristics are similar to the commercial gums such as *guar gum*, *tragacanth gum* and *ghatti gum* [32,20]. The result of the study shows that GalFN and GalDN solubility is not significantly different (p>0.05) at room temperature and high temperature. GalDN had a slightly higher solubility than GalFN. *Nypa gum* solubility increased along with the temperature increase, where the sample was dissolved easier at high temperature rather than at room temperature.

The value of sample solubility (35.79%) at room temperature is much lower than the solubility of *durian seed gum* (40%) [23], *locust bean gum* (45%) [15], *arenga gum* (50.93%) [20], *Propopis spp*. (69%) [33]. Samples solubility at high temperature (80 ^{o}C) is similar to the solubility of *locust bean gum* (70%) [15] and higher than *durian seed gum* (60%) [23]. At high temperatures, the hydrogen bond (H) among polysaccharides chain is broken and has caused the hydroxyl group to be exposed to water, increasing its solubility [34]. Different solubility of galactomannan is closely related to the divarication levels or galactose content along mannan chains [32]. Galactomannan with higher galactose content will dissolve easier [35, 36, 37].

3.3.3. Water Holding Capacity (WHC)

Water holding capacity (WHC) is a gum ability to bind water molecules. The result of the study shows that WHC GalFN (p<0.05) is significantly lower than GalDN. WHC of *Nypa gum* is twice as large as WHC of *arenga gum* which is around 116-150 (g water/ 100 g gum) [20]. However, the WHC of this *Nypa gum* is much lower than *fenugreek gum* (2087,8) and *guar gum* (2412) [24].

WHC depends on particle and pore size [23, 24]. WHC also depends on the hydroxyl groups' availability in the galactomannan branch structure [38, 39, 24]. The previous research also reported that WHC is affected by the drying process that can cause a change in the chemical composition of *gum* [23, 40, 29].

3.3.4. Oil Holding Capacity (OHC)

OHC is one of the most important functional characteristics of hydrocolloid that represent oil-absorbing capacity [41]. The result of the study shows that the OHC of GalFN and GalDN is not significantly different (p>0.05). *Nypa gum* has a slightly smaller capacity to hold oil than *arenga gum*, which ranges around 86-103 (g oil/100 g gum) [8], but it is much lower when compared with *fenugreek gum* (626.46) and *guar gum* (415.22) [24].

Gum ability to absorb oil depends on the existence of non-polar side chains and hydrophobic fractions, which can bind hydrocarbon units from oil, so that it can boost higher oil holding capacity [42]. Any material which has been processed to eliminate its fat content (*dehulled-defatted seed*) before extraction has higher OHC. Low oil absorption capacity is caused by fixed oil contained within the *gum*, so it is impossible to absorb the oil any further [16]. The high oil absorption level is associated with *gum* non-polar molecules, which can hold oil droplets [43].

3.3.5. Viscosity

Viscosity is highly associated with the use of galactomannan as a stabilizer as well as an emulsifier in the industry. The result of the study shows that GalFN and GalDN viscosities are not significantly different (p>0.05) at a concentration of 0.5%. Nonetheless, significant GalFN viscosity (p<0.05) is higher than GalDN at 1% and 2% concentrations. *Arenga gum* viscosity in concentration of 0.5% (117-146 cP), 1% (139-174 cP), 2% (1567-1711 cP) [20]. Viscosity of Nypa gum can be seen on Table 3.

Table 3. Viscosity of *Nypa gum*

Concentration of *Nypa* gum (%)	Viscosity (cP)	
	GalFN	GalDN
0.5%	$39.05^{a} \pm 1.01$	$36.96^{a} \pm 0.44$
1%	$74.22^{a} \pm 0.35$	$70.76^{b} \pm 0.62$
2%	$158.58^{a} \pm 0.18$	$153.8^{b} \pm 0.24$

Viscosity differences between *Nypa gum* and other gum sources are caused by different mannose ratios: galactose in various plants, which then affects its functional characteristics [44, 45]. Commonly, galactomannans with a greater galactose ratio are more soluble in the water but tend to be difficult to form a gel [36].

3.3.6. FTIR Spectroscopy

Galactomannan, as a biopolymer, has been characterized using FTIR in the previous literature [33, 46, 47, 24]. The analysis result shows that the peak of spectra is 3,389 cm^{-1}, which according to Pollard [17], spectra over a range of 3,400-3,200 cm^{-1} are identified as stretching vibration from the O-H bond that indicated the presence of H-bonding between hydroxyl from galactomannan and surrounding water content [13]. 2927 cm^{-1} correspond to the absorption peak of C-H stretching vibration in range 3000-2800 cm^{-1} [13]. The absorption peak at 1654 cm^{-1} indicated the presence of amides [24]. This condition is one of the characteristics of polysaccharide macromolecules. The peak of 1300-1000 cm^{-1} is interpreted as stretching vibration from the C-O bond in the carbonyl bond, both in C-OH and C-O-C. The amount of the resulted peak shows a great number of carbonyl groups. This condition indicates the existence of the pyranose ring within the material. The presence of O-H stretching vibration from OH groups, stretching vibration C-O from C-OH and C-O-C groups on *Nypa gum* shows that the extract has the

component of polysaccharide macromolecules.

Infrared spectra have shown in Figure 5 and its peak at 813 and 874 $cm^{-1,}$ which is related to the anomer configuration (conformer of α and β) and glycosidic bond and indicates the existence of α-D-galactopyranose and β-D-mannopyranose unit [48]. Generally, the galactomannan structure consists of β-(1-4)-D-Mannose as the main chain and it has one branch unit α-D-Galactose bound in the α-(1-6) position [49]. The peak at 3,389 cm^{-1}, 813 cm^{-1}, and 874 cm^{-1} indicates that the identified compound is galactomannan.

3.3.7. Scanning Electron Microscope (SEM)

A morphological investigation of *Nypa gum* is performed to observe the impact of nipa palm fruit condition on its physical characteristics. At magnification of 10000, known that both GalFN and GalDN show discrete and irregular granular, irregular and sharp surfaces, and only a few pores are formed in Figure 5. The results of SEM analysis indicated that in accordance in previous literature which stated that galactomannan structure has an irregular shape [50]. GalFN (Figure 6A) has a tighter and more assembled structure, while GalDN (Figure 6B) relatively more spreading structure. This condition is in accordance with WHC and OHC results which present a relatively low capacity for absorbing water and oil. The shape of its granules is similar to guar gum and *Leucaena leucocephala gum* [51,48]. It is known that the heating or drying process does not damage the galactomannan structure, as seen in GalDN structure. The galactomannan structure is not damaged when drying at less than 75°C. Secure temperature for processing the agricultural products is in range of 45-75°C [52].

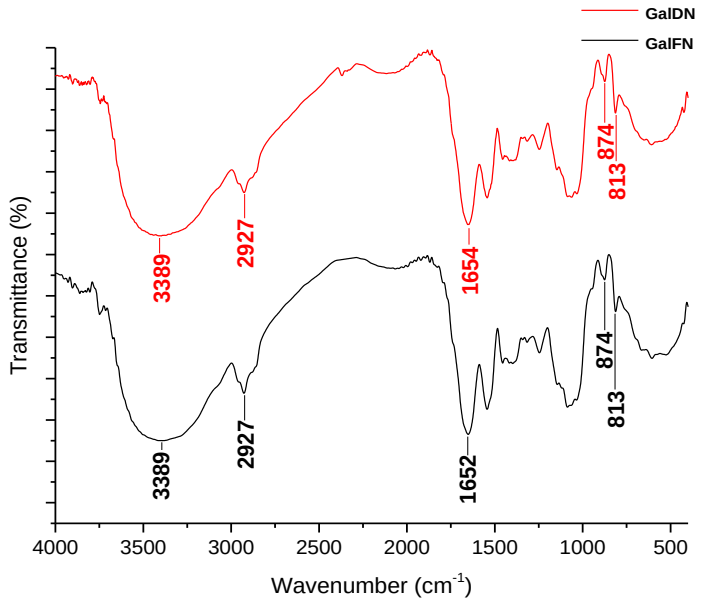
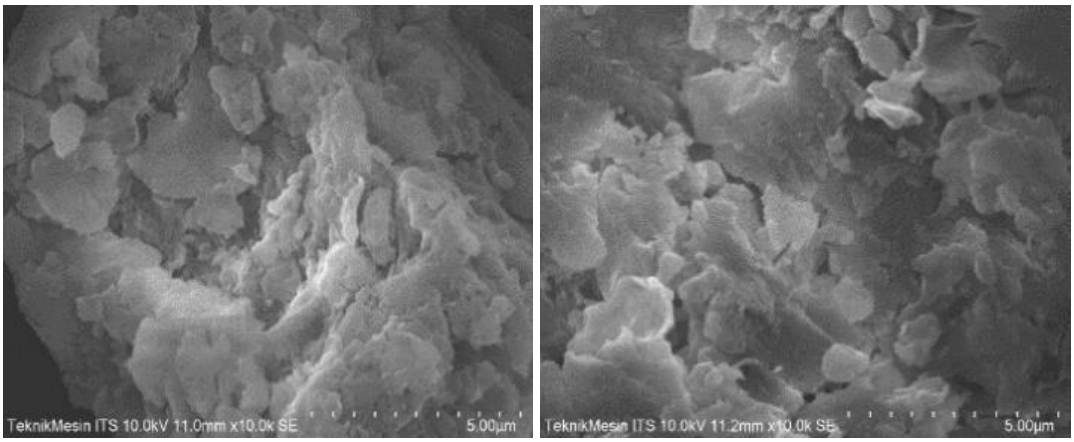


Figure 5. FTIR Spectra of Nypa Gum



(A) (B)

Figure 6. SEM Images of *Nypa gum*: (A) GalFN (10000x), (B) GalDN (10000x)

4. Conclusions

Nypa gum yield obtained from fresh and dry nipa palm fruits is not significantly different. *Nypa gum* which is extracted from fresh (GalFN) and dry (GalDN) nipa palm fruit, does not have a significant impact on the solubility (25^{o}C and 80^{o}C) and OHC. But, it significantly impacts the whiteness degree, WHC, and viscosity on 1% and 2% concentrations. From the results of the study, it was concluded that drying can be used as a method of pre-treatment of nipa fruit to minimize damage of nipa fruit before being processed into galactomanan.

Acknowledgements

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