ORIGINAL ARTICLE

Physicochemical properties of *Arenga pinnata* Merr. endosperm and its antidiabetic activity for nutraceutical application

Juliati Br. Tarigan, Diana A. Barus[1], Aminah Dalimunthe[2], Sabarmin Perangin-Angin, Trung T. Nguyen[3,4]

Departments of Chemistry, [1]Physics and [2]Pharmacology, Universitas Sumatera Utara, Medan, Indonesia, [3]Department of Food Technology, An Giang University, Long Xuyen City, [4]Department of Medical Biotechnology, Flinders University, Adelaide, Australia

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ABSTRACT

This study aims to provide information on physicochemical properties of *Arenga pinnata* endosperm (APE) and its antidiabetic activity for utilization in the food and pharmaceutical industries. The antidiabetic effect of APE was studied through an observational experiment on the blood glucose level of rats. The physicochemical properties of APE were determined using a texturometer, X-ray powder diffraction, Brookfield viscometer, scanning electron microscopy, Fourier-transform infrared spectroscopy, and light microscope. The APE was categorized based on its texture into three groups. The crystal structure of APE is microspore and amorf while the hydrogel has a non-Newtonian property and is stable at 50°C. The viscosity index was increased in the increasing temperature with the order of high viscosity of APE being 1, 2, and 3. The hydrogel shape of APE 1 and 3 was lameral in the concentration of 1.25%. For antidiabetic study, the findings demonstrated that the APE could reduce the blood glucose level. The APE powders 1 and 2 with the respective weight of 50 and 200 mg have significant effects on reducing rat blood glucose level compared to the diabetic rats. Based on these properties, APE could potentially be used as a natural antidiabetic food without having any side effect and in the pharmaceutical industry for some purposes.

Key words: Antidiabetic, *Arenga pinnata* endosperm, physicochemical properties

INTRODUCTION

Nowadays, several polysaccharides such as gum, mucilage, and galactomannan derived from plants have been intensively used in food and pharmaceutical industries. The abundance of natural polysaccharides makes them more attractive due to their low cost, diversification, and easy to modify as nontoxic.[1,2] Applications of these polysaccharides were mainly dependent on their physicochemical properties. Therefore, extensive researches on improving physicochemical properties of polysaccharide have been carried out for years aiming to fulfill food and pharmaceutical industry demands.[3] One of the most abundant polysaccharides in Indonesia is galactomannan, which is collected from immature *Arenga pinnata* endosperm (APE). The utilization of APE remains limited for food. APE consists of two fractions which are dissolved and undissolved fractions. The water-soluble fraction is composed of polysaccharide (62.49%) and crude fiber (1.11%). The main ingredient of polysaccharides in APE is galactomannan, which is water-soluble polysaccharide with the ratio of galactose and mannan being 1:1.33 and

Address for correspondence:
Dr. Juliati Br. Tarigan,
Jl. Bioteknologi No. 1 Kampus USU Padang Bulan,
Medan 20155, Indonesia.
E-mail: juliati@usu.ac.id

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IC_{50} value of 22.109 mg/mL. Several studies investigating the physicochemical properties of galactomannan have been carried out.[3,4] However, to the best of our knowledge, there is no literature regarding physicochemical properties' study of APE.

Based on that, it is necessary to determine the properties of APE. The physicochemical properties of APE and the chemical composition were determined based on the Indonesian standard (SNI) and the Association of Official Analytical Chemists (AOAC). Furthermore, the antidiabetic property of APE was explored through an observational experiment on blood glucose level of rats fed by the APE powder.

MATERIALS AND METHODS

The APE was purchased from a traditional market in Medan, Sumatera Utara, Indonesia. The APE was divided into three groups based on their hardness texture determined using a penetrometer precision. The preparation of APE was conducted following previous research procedure.[1] The compositional analysis of APE powder was conducted following SNI 01-2891-1992 and AOAC 1995. The X-RD pattern was recorded using an X-ray diffractometer model, Shimadzu XRD-7000. The morphological surface of the APE powder was observed using scanning electron microscope (SEM). The absorption spectra of functional groups containing in the APE powder were detected using Fourier-transform infrared (FT-IR) spectroscopy. The thermal behavior of APE was determined using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) to study the thermal effect on polysaccharide structure. The flow behavior of APE was determined using a Brookfield viscometer. The APE hydrogel shape was observed using a light microscope. Determination of the blood glucose level of rats was conducted based on previous published procedure.[5] Each rat with weight ranging from 150 to 200 g had food fasting for 24 h and was fed by hydrogel from APE group 1–3 with dosage of 50, 100, and 200 mg. For comparison, group of rats fed by glibenclamide and glucose dosage 50% also determined.

Statistical analysis

The data reported in this study were the average of duplicate measurements, and the statistical analysis was conducted using Statistica 13 software (TIBCO Software Inc., Palo Alto, CA 94304, USA). The difference between groups was assessed by Tukey's test and it was considered statistically significant if $P < 0.05$.

RESULTS

It is clear that APE has different toughness due to different maturity. Based on that, the APE was categorized into three groups. The texture of APE and chemical composition of each APE group is presented in Table 1.

FT-IR spectroscopy was used to observe the vibration of functional groups contained in APE. The absorbance of APE functional groups was recorded in the wavelength ranging from 4000 to 600 cm^{-1}. The FT-IR spectra of APE 1, 2, and 3 are presented in Figure 1. Crystallinity degree of APE was determined using an X-ray diffraction spectrophotometer. The X-ray diffraction images of APE 1, 2, and 3 are shown in Figure 2.

Scanning electron microscope was used to study the surface morphology of the APE. The SEM images of the sample are presented in Figure 3. Thermogravimetric analysis was used to study the thermal degradation mechanisms of polysaccharide.[6] TGA curve contains information regarding the pyrolysis temperature and kinetics based on weight loss of the sample in the increment of temperature providing thermal behavior of the polysaccharide.[2]

To study the cell surface of APE, light microscopy was used, in which the APE was in the hydrogel form. The results are shown in Figure 4. The correlation between shear rate and viscosity of 1% APE solutions is depicted in Figure 5.

It has been known that some polysaccharides have antidiabetic property, particularly fiber which significantly can reduce blood glucose level.[7] The effect of APE extract on blood glucose level of rats is presented in Figure 6.

Table 1: Chemical composition, yield, and texture of *Arenga pinnata* endosperm

Parameter	APE Group 1	APE Group 2	APE Group 3
Texture value range (mm^{-1})	120-100	100.1-80.1	80.0-60.2
Texture (g/mm^2)	0.2083-0.249	0.250-0.310	0.312-0.4153
Yield (%)	2.785	5.650	7.144
Water content (%)	12.1	14.8	14.5
Protein (%)	2.75	2.25	2.72
Lipid (%)	0.40	0.20	0.47
Carbohydrate (%)	63.4	64.9	66.3
Fibre (%)	46.53	47.74	51.60

APE: *Arenga pinnata* endosperm

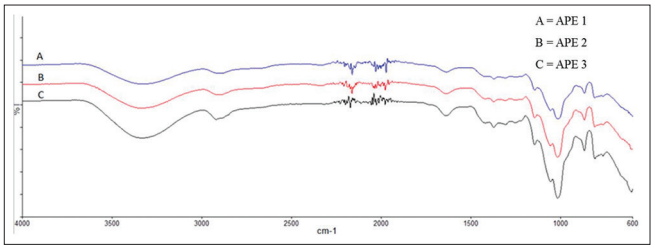

Figure 1: The Fourier-transform infrared spectrum of three different *Arenga pinnata* endosperm groups

DISCUSSION

As shown in Table 1, APE group 3 has the largest texture value ranging from 0.3117 to 0.4153 g/mm^2, which means that it is the toughest one. The most exciting finding was that the chemical composition of all APE groups is quite similar to carbohydrate and fiber. Therefore, it can be concluded that the maturity of the APE affects the toughness and was not significant to its chemical composition. The toughness of the APE is influenced by the mannan compound rendering increasing its durability against mechanical and water damage.[8]

FT-IR spectra of all the APE spectra have similar shape and vibration as shown in Figure 1. As predicted, the APE spectra match with those observed in other studies.[4,9] The specific functional group of hydroxyl from polysaccharide was appeared at the wavelength of 3333, 3339, and 3342 cm^{-1} for APE 1, 2, and 3, respectively. This peak appeared also due to the presence of water which was strengthened by the existence wavelength of 1638, 1637, and 1643 cm^{-1}. Another point to state in this study is the appearance of wavelength at 868, 868, and 857 cm^{-1}, which is characterized as β-D-mannose pyranose bond.[10] It is apparent from Figure 1 that the intensity was increased from APE 1 to APE 3. This is presumably because the structure of APE becomes more crystalline in APE 3 due to increasing OH bond. The intensity of bending vibration of CH2 appeared to decrease from APE 1 to APE 3 represents the structure changed from amorf to crystalline.

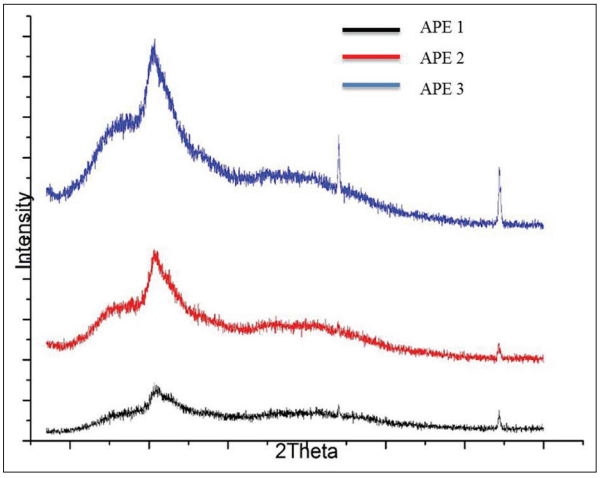

Figure 2: X-ray diffraction pattern of three different *Arenga pinnata* endosperm groups

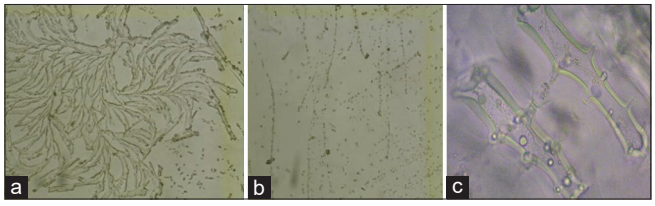

Figure 4: The light microscopy images of (a) *Arenga pinnata* endosperm 1, (b) *Arenga pinnata* endosperm 2, and (c) *Arenga pinnata* endosperm 3

As shown in Figure 2, it is apparent that all APE X-ray diffraction images have similar patterns. The scatter peaks at 2θ = 20.88°, 20.88°, and 20.58° for APE 1, APE 2, and APE 3, respectively, were observed, which showed that the APE crystal is amorf. Based on its intensity, the APE 3 has the highest crystallinity degree among three APE groups. This is presumably due to APE 3 contains more water undissolved (mannan) and fiber as shown in Table 1. The present findings seem to be similar to other studies in which guar galactomannan, food-grade guar gum, and Matador endosperm powder exhibit a sharp peak at 2θ = 20°.[11]

The SEM images revealed that the APE 1 has hollow and rough surface than APE 2 and APE 3. However, APE 3 has a more hardened surface which is due to high interaction polymer chains through van der Waal forces. Interestingly, this result corresponding with the X-ray diffraction reported above which support the conclusion that the APE 3 structure is crystal. All the SEM micrographs displayed fiber of polysaccharide from galactomannan and mannan. These findings confirm the result from the previous researches which revealed fiber of galactomannan from the endosperm of *Gleditsia japonica*.[12]

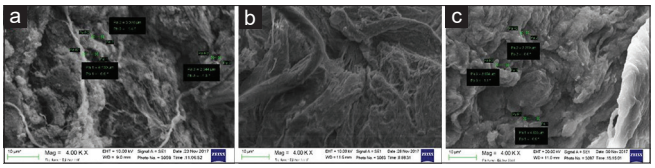

Figure 3: The SEM of different *Arenga pinnata* endosperm groups (a) *Arenga pinnata* endosperm 1, (b) *Arenga pinnata* endosperm 2, and (c) *Arenga pinnata* endosperm 3

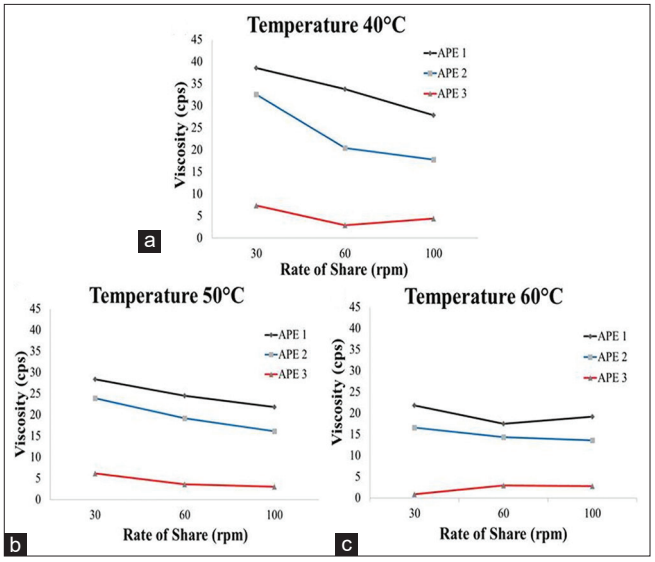

Figure 5: The effect of shear rate on viscosity of *Arenga pinnata* endosperm determined at different temperature, (a) 40°C; (b) 50°C; and (c) 60°C

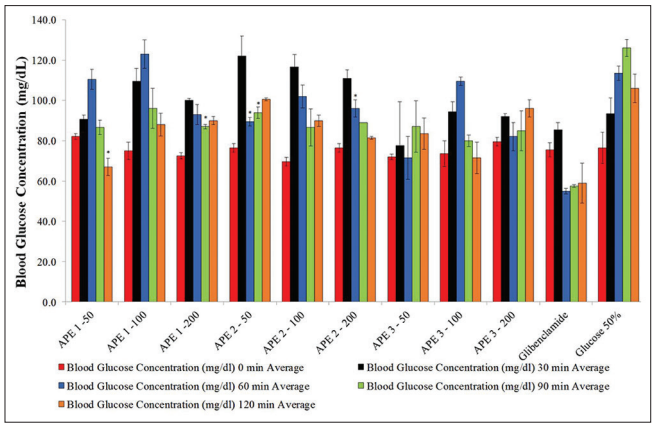

Figure 6: The effect of *Arenga pinnata* endosperm extract with different doses on the blood glucose level of rats. The symbol* indicate a significant difference in mean by the Tukey's test ($P < 0.05$)

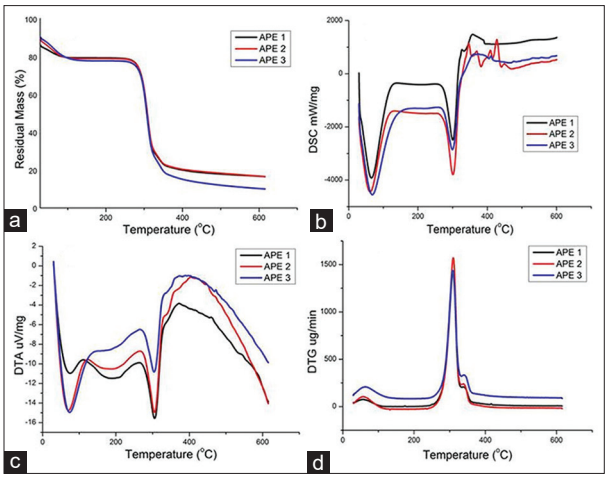

Figure 7: The thermal properties of *Arenga pinnata* endosperm groups determined with (a) thermogravimetric analysis, (b) differential scanning calorimetry, (c) Differential thermal analysis, and (d) Differential Thermogravimetric

As shown in Figure 7a, water contained in the APE was evaporated at the first stage at a temperature of 101, 109, and 104°C for APE 1, 2, and 3, respectively. The weight loss at this stage was 8%–12%, which is similar to water content describing in Table 1. The finding supports previous research which showed that water was released in the range of 11%–13% at a temperature below 150°C.[13] The TGA curves showed a sharp decrease in a weight loss of 77%–80% at the second event. At that stage, polysaccharide was decomposed with T_O (onset temperature) of 246, 251, and 252°C and T_P (peak temperature) of 357, 355, and 360°C for the APE 1, 2, and 3, respectively. These results match with those observed by another research which found that galactomannan degradation occurred at a temperature range of 200-400°C.[11]

It can be seen from Figure 7b that an endothermic peak at 64.2, 60.4, and 66.9°C for APE 1, 2, and 3, respectively, was observed which was due to water evaporation. Another endothermic event occurred at 300.2, 299.2, and 299.8°C for the APE 1, 2, and 3, respectively, which correspond to weight loss of the APE due to thermal degradation. Again, these DSC thermograms are in agreement with TGA curves. However, it is somewhat surprising that all APE thermograms did not show an exothermic event as previous researchers found. For example, Gliko-Kabir *et al*. reported an exothermic peak at 310°C for guar gum and the exothermic peak remained observed even after crosslinked reaction with glutaraldehyde.[14]

Nevertheless, galactomannan isolated from *Gleditsia triacanthos, Caesalpinia pulcherrima,* and *Adenanthera pavonina* showed two endotherm peaks[6] similar to this study result. The difference of the DSC curves presumably due to different polysaccharide compounds containing in APE and guar gum. In addition, based on the DSC analysis, the gelatinization temperature of the APE was lies in the range of 60–70°C. From Figure 7c, the endothermic peaks at 72.1, 68.7, and 74.9°C for the APE 1, 2, and 3, respectively, corresponded to water evaporation. The second endothermic event appeared at 306, 307.9, and 304.9°C due to thermal decomposition. Basically, the differential thermogravimetric (DTG) is different with TGA in which the function of mass loss with respect to time against temperature was recorded. Figure 7d shows the DTG of APE. The maximum weight loss of APE was presented at 309.1, 310.3, and 311°C for the APE 1, 2, and 3, respectively.

As shown in Figure 4, the lamellar structure was formed in the concentration APE hydrogel of 1.25%, which is due to van der Waal interaction between hydroxyl groups in polysaccharide polymer with water within lamellar planes. These tests revealed that all APE solutions performed a non-Newtonian property as their viscosity decreases with an increasing shear rate. The findings of the current study are consistent with those of other studies which concluded that heating galactomannan >60°C provides high viscosity rendering interior stability depending on their raw sources.[15] As shown in Figure 5, viscosity was decreased as the temperature increased. The constant Newtonian property occurred at a temperature of 50°C for the APE 1 and 3, while the APE 2 remained stable at 60°C. Therefore, it is suggested to heat APE at temperature 50–60°C. This finding is in agreement with the DSC result which showed that the gelatinization temperature was in the range of 60–70°C. In contrast, locust bean gum required heating at 80°C for 20–30 min and guar gum at 25–40°C for 2 h.[15]

Some researchers have demonstrated that galactomannan could decrease blood glucose level.[16] However, no study has found for APE in decreasing blood glucose level. The blood glucose level was determined in the range time of 0–120 min using a glucometer. The result shows that the APE extract could reduce blood

glucose due to its high fiber content. Most of the rats fed by the APE extract showed slightly higher blood glucose level than rats fed by the antidiabetic drug (glibenclamide). This is because the concentration of APE used is lower than glibenclamide. However, the extract containing APE 1 for concentration of 50 and 200 mg after treatment of 120 (67.0 ± 4.2 mg/dl) and 90 min (87.0 ± 1.0 mg/dl), respectively, and APE 2 with a level of 50 and 200 mg after treatment of 90 (94.0 ± 2.8 mg/dl) and 60 min (96.0 ± 4.2 mg/dl), respectively, significantly reduced the blood glucose level compared to the diabetic rats determined by Tukey's test. Therefore, it can be concluded that APE for some concentration could be used to reduce blood glucose level.

CONCLUSION

The APE could categorize on three groups based on its texture. The yield of 2.785%, 5.650%, and 7.144% was obtained for APE 1, APE 2, and APE 3, respectively. The SEM images revealed that the morphology of APE is microspore and amorf. The hydrogel of APE indicates that it has non-Newtonian property and remains constant at the temperature of 50°C. The viscosity varies from high to low for the respective APE 1, APE 2, and APE 3. The lamellar structure occurred for the APE 1 and 3 at a concentration of 1.25%. These findings suggest that in general based on its chemical composition and physicochemical properties, APE could be used in food and pharmaceutical industry. The antidiabetic experiments showed that the APE extracts of 1 and 2 with a respective dosage of 50 and 200 mg significantly reduce blood glucose concentration of rats compared to diabetic rat. It can be concluded that APE could be used as a natural antidiabetic food without any side effect or functional ingredients for nutraceutical and pharmaceutical products.

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Conflicts of interest

There are no conflicts of interest.

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