Separation of proanthocyanidin from red sorghum seed extract using macroporous resin

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Abstract. Proanthocyanidin has been extracted from red sorghum seed with Ultrasound-Assisted Extraction using an aqueous-based solvent. Proanthocyanidin concentration in the extract is relatively low mixing with other compounds. The adsorption method was chosen to separate proanthocyanidin compounds due to the relatively simple operation to recover targeted solutes from low concentration solutions. The purpose of this study was to find an efficient adsorbent for separating proanthocyanidin from red sorghum seed extract. Two types of macropore resin were investigated, namely macropore resin AB-8 and D101. Besides the slight difference in average pores diameter, D101 is non-polar while AB-08 is weak polar resin. The initial concentration of proanthocyanidin compounds in the extract was 0.872 mg/ml. Adsorption processes were performed to reach equilibrium. AB-8 and D101 macropore resins showed adsorption capacity of 34.65 mg/g and 30.36 mg/g with the percent recovery of 79.64% and 69.79%, respectively. Macropore resin AB-8 offers a higher adsorption capacity than macropore resin D101. Additionally, adsorption with AB-8 could reach equilibrium after 24 hours, while D101 took significantly longer to reach equilibrium after 48 hours. While both AB-8 and D101 macropore resins demonstrated reasonable performance as potential adsorbents for recovering proanthocyanidin from red sorghum extract, AB-8 showed better performance in terms of capacity and adsorption rate.

1. Introduction
In addition to having high enough macronutrients compared to rice and corn, sorghum grains also contain phenolic compounds that are useful for antioxidants[1]. One of these compounds which have many benefits in the health sector and have a high selling value is proanthocyanidin. Proanthocyanidins are also called condensed tannins, which are the condensation of flavanols. Proanthocyanidin is an antioxidant compound found in plants that have various pharmacological benefits such as anti-inflammatory, anti-cancer, antiviral [2]. The proanthocyanidin content in sorghum grains varies depending on the variety and location of planting sorghum plants [3].

Proanthocyanidin in sorghum can be taken through an extraction process. Therefore it is necessary to collect proanthocyanidin using an efficient way to increase the concentration of proanthocyanidin. Proanthocyanidin has a relatively large molecular size of 579.14 gr/mol, is very non-volatile, and is also possibly sensitive to high-temperature treatments [4].

The extraction process of proanthocyanidin compounds from sorghum grains has been maximized by various methods, including conventional extraction methods [5] and Ultrasound-Assisted Extraction (UAE)
[6]. Quantitative evaluation of the process has been carried out with both empirical and mechanistic approaches. The PA content resulting from the extraction process is generally still low. For example, it was produced from conventional extraction at 70°C at 400 rpm, resulting in maximum proanthocyanidin levels at 0.837 mg/ml [1]. It is known that unrefined raw plant extracts invariably contain carbohydrates, protein, and other impurities, which can limit further identification and even bioactive application [4].

One of the separation methods that suit the characteristics of proanthocyanidin is adsorption using macroporous resin. Adsorption using macroporous resin is one of the adsorptions classified as many effective advantages, such as high absorption results, low costs, recyclable, environmentally friendly, and suitable for large-scale industries [7]. Adsorption is the process of absorbing fluid components (adsorbates) by selectively transferring these components to the surface of an adsorbent solid (adsorbent) so that separation occurs. The mass transfer mechanism in the adsorption process generally goes through several stages. The first is the mass transfer of adsorbate from the solute to the outer surface of the adsorbent through the film layer, then the intraparticle mass transfer through the pores, and the last is the mass transfer of the adsorbate from the pores to the pore surface (adsorption)[8].

In a study conducted by Wan et al. (2014) purification of total flavonoids from Flos Populi extract using macroporous resins S-8, NKA-9, AB-8, D101, and X-5, it was shown that NKA-9 and AB resins -8 had the highest adsorption capacities, namely 150.19 and 150.10 mg / g dry resin. Furthermore, the use of macroporous resin to purify organic compounds was carried out by Chu et al. (2018); six resins were used, namely HPD600, NKA-9, AB-8, X-5, D101, and HPD300. The results showed that D101 resin had an adsorption ability of (17.8 μmol AAE / g) slightly lower than HPD600 resin (19.1 μmol AAE / g), which was the highest among the resins tested. However, the desorption ratio of D101 resin (90.4%) is higher than that of other resins.

From the literature study that has been conducted, there has been no research on the separation of proanthocyanidin from red sorghum grains using macroporous resin AB-8 and D101. The purpose of this study was to determine the performance of AB-8 and D-101 resins in the separation of proanthocyanidin from red sorghum seed extract.

2. Materials and method

2.1. Materials
Red sorghum grains (Sorghum bicolor L. Moench) were obtained from Wonogiri, Jawa Tengah, Indonesia. Aquadest, citric acid, n-butanol, and 37% HCl were obtained from Merck Co. (Darmstadt, Germany). FeNH4 (SO4) 2×12H2O was obtained from JTBaker Avantor. Inc. (Center Valley, Pennsylvania). Macroprus resin AB-8 and D101 were obtained from Bengbu Dongli Chemical Co., Ltd., Anhui, China.

| Resin  | Surface Area (m²·g⁻¹) | Average Pore Diameter (nm) | Particle Size Range (mm) | Polarity     |
|--------|------------------------|----------------------------|--------------------------|-------------|
| AB-8   | 480-550                | 13-105                     | 0.3-1,2                  | Weak Polar  |
| D101   | 600-700                | 9-10                       | 0.3-1,2                  | Non Polar   |

2.2. Ultrasound-Assisted Extraction (UAE)
The process of proanthocyanidin extraction is carried out using the Ultrasound-Assisted Extraction (UAE) method. The ultrasound wave for extraction was transmitted from the Ultrasonic Generator’s probe from Hangzhou Dowell Ultrasonic Technology, DW-SD20-1200. A total of 1000 mL of aquadest is ultrasoned until the temperature reaches 60°C. After the temperature reaches 60°C, add 100 grams of red sorghum grains. To obtain red sorghum seed extract with high proanthocyanidin content, 0.35 grams of citric acid and 0.57 grams of sucrose were added into the solvent. Then sonication was conducted for 150 minutes (80% amplitude, 19.93 kHz frequency, 0.2-ampere power).
2.3. Pretreatment macroporous resin
The specifications of the two macroporous resins used in this study are shown in table 1. All resins were first soaked in 96% ethanol for 24 hours, then washed using distilled water until clean. The resin was then soaked in 1 M NaOH for 5 hours and washed again using distilled water until clean. Then the resin was soaked with 1 M HCl for 5 hours and washed using distilled water until clean. Finally, the resin was dried in an oven at 60°C until the weight was the same. This pretreatment aims to remove monomers and porogen trapped in the pores during the synthesis process.

2.4. Determination of adsorption capacity
To determine the capacity and adsorption kinetics of each resin, it studied by mixing 50 ml of sorghum seed extract and 1 gram of treated resin into 250 ml Erlenmeyer. Then stir (130 rpm) at 25°C for 48 hours. The adsorption capacity of the resin can be calculated using equation 1,

\[ q_e = \frac{(C_0 - C_e)V_i}{m} \]  

where \( q_e \) (mg / g) is the equilibrium capacity for adsorption, \( C_0 \) (mg / mL) is the initial proanthocyanidin concentration, \( C_e \) (mg / mL) is the equilibrium concentration of proanthocyanidin, \( V_i \) is the volume of the sample solution, and \( m \) (g) is the weight of the resin dry.

The proanthocyanidin adsorption recovery can be calculated using equation 2.

\[ Recovery = \frac{(C_0 - C_e)}{C_0} \]  

2.5. Determination of proanthocyanidin content
The total proanthocyanidin content in the sample solution was determined using the butanol assay method [1]. A total of 6 mL of butanol-HCl (95: 5) was mixed with 1 mL of sample extract and added 0.2 mL of iron reagent (FeNH4 (SO4) .12H2O). After the solution was stirred until homogeneous, then the solution was incubated at 90°C for 50 minutes. Finally, the absorbance was read at 550 nm using a UV-Vis spectrophotometer.

3. Result and discussion

3.1. Preparation of raw materials
The results of the extraction of red sorghum grains using the Ultrasound-Assisted Extraction (UAE) method at an amplitude of 80% for 150 minutes produced a red sorghum seed extract with proanthocyanidins content of 0.872 mg/ml. The process of releasing proanthocyanidins from the pericarp of sorghum grains in Ultrasound-Assisted Extraction (UAE) assisted by the presence of ultrasonic waves that cause pore damage [6]. Based on the proanthocyanidin levels obtained, it is known that proanthocyanidin extraction using the Ultrasound-Assisted Extraction (UAE) method resulted in higher proanthocyanidin levels than extraction using the conventional method that had been carried out by Devi et al. (2020), which was 0.837 mg/ml [1].

3.2. Adsorption capacities on resin
It can be seen in figure 1 that AB-8 resin has a better ability to adsorb proanthocyanidin than D101 resin so that the non-polar resin is more suitable for adsorbing proanthocyanidin from sorghum seed extract. In addition, the larger mean pore diameter of AB-8 resin compared to D101 could explain the high adsorption efficiency. The total proanthocyanidin adsorption capacity of sorghum seed extract on AB-8 and D101...
resins was 34.65 mg / g dry resin and 30.36 mg / g dry resin with the percent recovery of 79.64% and 69.79%, respectively.

![Figure 1. Recovery and adsorption capacities for proanthocyanidin on different resins.](image)

Proanthocyanidin can be adsorbed on the resin through Van der Waals forces or hydrogen bonds because proanthocyanidin contains polar hydroxyl groups [9]. So that the adsorption speed parameter on AB-8 resin is better than that of D101 resin. In addition, weak polar resins have a higher balance value than non-polar ones. Thus kinetics can show the value of the quantity of the process.

3.3. Adsorption kinetics on resin

Adsorption kinetics represent the solute uptake rate based on the contact time of the solution with the adsorbent. This is one of the foremost characteristics in determining the efficiency of the adsorption [7]. Therefore, in this research, the adsorption kinetics experiments of total proanthocyanidin were carried out to understand the adsorption conduct of AB-8 and D-101 resins.

In most cases, the adsorption capacity of proanthocyanidin increases significantly before the adsorption equilibrium is reached. For the two different resins, the adsorption capacity of proanthocyanidin increased with the adsorption time; then, it reached an equilibrium of about 24 hours for AB-8 resin and 48 hours for D101 resin. In the first 300 minutes, the adsorption capacity increases swiftly, and after 300 minutes, it reaches equilibrium at different times. This shows that the adsorption capacity of each adsorbent has variations that are not much different. If the adsorption results of the two resins were compared, AB-8 resin had a more significant advantage in adsorbing proanthocyanidin. Thus, AB-8 resin was elected as the most suitable resin for proanthocyanidin enrichment and was used in ensuing tests. As illustrated in figure 2,
4. Conclusion
This study shows that adsorption with macroporous resin AB-8 and D101 is a potential method to separate proanthocyanidin from red sorghum extract. While both AB-8 and D101 resin show significant adsorption capacities which are 34.65 mg/g dry resin and 30.36 mg/g dry resin with the percent recovery of 79.64% and 69.79%, respectively, AB-8 showed significantly higher capacity. Additionally, adsorption with AB-8 could reach equilibrium time after 24 hours, while that with D101 took significantly longer to reach equilibrium after 48 hours.

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Acknowledgments
The authors would like to thank “Departemen Teknik Kimia Fakultas Teknik Universitas Gadjah Mada” for research funding through “Program Hibah Penelitian Departemen Teknik Kimia FT UGM Tahun 2020” with agreement number 417/UN1/FTK/SK/HK/2020.