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Production of 5-Hydroxymethylfurfural from cassava flour with deep eutectic solvent (DES) molar variations

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KEYWORDS

5- Hydroxymethylfurfural
Cassava flour
Deep eutectic solvent

ABSTRACT

In the past few years, 5-Hydroxymethylfurfural (5-HMF) has been used in many industrial practices as a substitution for plastics and a versatile precursor for synthesis into a variety of value-added chemicals and fuels. The potential use of glucose monomers from cassava (*Manihot uttilisma*) as a potential source for 5-HMF production needed to be considered. Deep Eutectic Solvent (DES) is natural, environmentally friendly, easy to obtain, and it was used as a solvent to increase the 5-HMF yield. Therefore, the objectives of this research are to obtain the 5-HMF from cassava flour with the suitable molar ratio of the DES solvent and to analyze the effect of the addition of DES solvent on the yield of 5-HMF. The synthesis of DES was based on choline chloride with ethylene glycol with a molar ratio of 1:2, 1:3, and 1:4 and was suitable for use as a solvent in the 5-HMF dehydration process. The ratio of glucose: DES of 1:6 affects the 5-HMF yield with the highest yield was 78.61% at a glucose concentration of 51.24% and the lowest without DES was 44.49% with the same glucose concentration.

Introduction

The synthesis of 5-Hydroxymethylfurfural (5-HMF) has been carried out in several previous studies, the materials used are derived from lignocellulosic waste containing glucose and fructose such as bagasse, rice husks, wheat husks, corn cobs, pineapple leaves, and bagasse (Septian and Sugiarti, 2019). The 5-HMF is a chemical that can be used as a versatile precursor for synthesis into various chemicals such as biofuel precursors and value-added fuels and can replace terephthalic acid in the production of chemical plastics as a substitute for making plastics (Prasetya et al., 2020).

Cassava consists of cellulose, hemicellulose, lignin, and other extractive materials. Where, hemicellulose when hydrolyzed produces xylose, arabinose, galactose, and mannose. Cellulose when hydrolyzed produces glucose monomers. Glucose in cassava can produce 5-HMF (Angela et al., 2015). Chemically, starch is a polymer of glucose, namely amylose and amylopectin. The synthesis of 5-HMF from cassava flour was carried out by a hydrolysis process to obtain glucose from cassava flour. Furthermore, the dehydration process is carried out to obtain 5-HMF results (Zuo et al., 2017). Hydrolysis in an acidic environment will break the glycosidic bond, which will take place in three stages. In the first step, the proton acting as the acid catalyst will react rapidly with the glycoside oxygen linking the two sugar units, forming the conjugate acid. This step, followed by a slow cleavage of the C–O bond, produces a cyclic carbenium cation intermediate in most cases. Protonation can also occur in the oxygen ring, resulting in ring opening and non-cyclical carbenium cations (Mardina et al., 2016).

Deep Eutectic Solvent (DES) is obtained from the complex formation process between HBA (hydrogen bond acceptor) and HBD (hydrogen bond donor) to form hydrogen bonds (Smith et al., 2014). The principle of making DES is by looking at the lower melting point components being heated first until they melt later, the higher the melting point the more compounds are added to form a eutectic mixture. Then, when the two compounds have high melting points, the two components are mixed and melted, becoming one (Brett, 2018). The advantage of using DES is that the price of raw materials used in the manufacture of DES tends to be cheap, biodegradable, has a fairly high level of purity, and is non-volatile (non-volatile) so DES is

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not flammable and easy to store (Aini, Harmidia Qurotul and Heryantoro, 2017 and Cvjetko Bubalo et al., 2016). Density is one of the properties found in DES. In general, DES has a density that tends to be higher than the density of water (Dewi et al., 2021). The addition of DES will affect the 5-HMF yield where, the higher the addition of DES, the value of the 5-HMF yield obtained will also increase.

The synthesis of the 5-HMF from durian seeds material with the addition of DES based on choline chloride and citric acid as solvents showed that there was an increase in the yield value of 5-HMF because of the addition of DES as a solvent. It reduced side reactions and reactions continuation of the dehydration process (Manurung et al., 2021). Currently, there need to be research focusing on the potential of cassava flour for 5-HMF production. Therefore, the objectives of this research are to obtain the 5-HMF from cassava flour with the suitable molar ratio of the DES solvent and to analyze the effect of the addition of DES solvent on the yield of 5-HMF.

Research Methods

Materials and equipment

Cassava flour (Double Phoenix) with a mass of 15 grams and a sulfuric acid catalyst were used in the hydrolysis process . The raw materials in DES synthesis were Choline chloride and ethylene glycol as HBA and HBD respectively, while Carrez I and Careez II solution were utilized for protein precipitation in 5-HMF analysis. The equipments used in this research were a reflux condenser unit (Pyrex)., The density of DES was analyzed using a pycnometer (Pyrex), while the DES viscosity was measured using a digital viscometer (Brookfield).

The research procedures

The research was conducted in several stages, and those were the hydrolysis of cassava flour cellulose, synthesis of DES, and dehydration reaction to obtain 5-HMF. The research design contained 2 factors. The first treatment factor was the molar ratio of the Choline chloride as HBA and ethylene glycol as HBD for the DES synthesis, which was 1:2, 1: 3 and 1:4. The second treatment factor was glucose:DES ratio, which was 1:0, 1:4 and 1:6. Each treatment was conducted in triplicate.

Hydrolysis of cassava flour

The hydrolysis reaction of cassava starch was held for 120 minutes at 100°C and used 10 % sulfuric acid as a catalyst (Manurung et al., 2021). The hydrolyzed starch was filtered through filter paper and analyzed using a UV-Vis Spectrophotometer using the DNS method.

DES synthesis

The DES synthesis was carried out on the molar ratio of the Choline chloride : ethylene glycol of 1:2 as conducted in earlier research (Manurung et al., 2021). Some modifications were applied for molar ratios of 1: 3 and 1:4 with the homogenization by using a magnetic stirrer with a stirring speed of 300 rpm for 1 hour. Then the obtained DES was cooled to room temperature, and stored for a week to check the DES synthesis if there was a change in the phase of the DES. If the DES solution became crystallized or frozen, it was repeated until a DES solution was obtained in the form of a solution.

(a) **(b)** **(c)**

Figure 1. The synthesis of 5-HMF stages: (a) the hydrolysis process of cassava flour with a sulfuric acid catalyst, (b) the synthesis of DES, and (c) the dehydration process of 5-HMF.

Dehydration process

The dehydration process was based on Manurung et al. (2021) with some modifications. The treatment sample without DES (1:0) and with DES (1:4 and 1:6) was prepared. The sample with DES was stirred with a magnetic stirrer at a speed of 300 rpm with a reaction time of 1 hour and a temperature of 80°C. The mixture from the dehydration reaction is then cooled to room temperature. The results of the dehydration reaction were analyzed using spectrophotometry using the SNI 01-3545-2004 method

Analysis of density, viscosity, and pH of the DES

The density measurement principle was based on the determination of the mass of the liquid with the pycnometer method (Fischer, 2015) at a constant temperature. Density analysis was performed on each synthesized DES molar variation. Viscosity of DES measured by Brookfield viscometer, was aim to determined the degree of consistency(Lubis, 2018).

The characteristics of DES was tended to be acidic. The DES pH analysis was perform to determine the suitability of the DES pH value with the characteristics of DES.

Analysis of 5-HMF with SNI method 01-3545-2004

The 5 ml of the sample was put into a 50 ml volumetric flask and the water was added the solution volume of 25 ml. Then 0.5 ml of Carrez I solution was added and shaken and 0.5 ml of Careez II solution was added and shaken again and diluted with water up to the line mark. Strain through filter paper, and discard the first 10 ml of the sieve. Pipette 5 ml of the solution and put each into an 18 mm x 150 mm test tube. Pipette 5 ml of water and put it into one tube (sample) and 5 ml of 0.20% sodium bisulfite into the other tube (control) and mix. After that, the absorbance of the sample was measured using UV spectrophotometry with wavelengths of 284 nm and 336 nm. The absorbance results obtained are calculated by the following formula:

Concentration 5 – HMF..................................... = (A284-A336 x 14,97 x 5)/(sample weight (ml) .(1)

$$\text{Factor} = \frac{126}{16830} \times \frac{1000}{10} \times \frac{100}{5} = 14.97 \quad (2)$$

(SNI 01- 3545-2004)

$$\text{Yield 5-HMF} = \frac{\text{The concentration of 5-HMF obtained}\left(\frac{mg}{ml}\right)}{\text{Glucose concentration}\left(\frac{mg}{ml}\right)} \times 100\ \% \quad (3)$$

Results and Discussion

The characteristics of DES

The characteristics of DES were evaluated by density, viscosity, pH, and physical appearance of DES. Table 1 summarizes the characteristics of DES obtained from the tests.

Table 1. Characteristics of DES

Characteristics of DES	**Molar Ratio (Choline chloride: Ethylene glycol)**		
	1:2	1:3	1:4
Physical appearance	Clear liquid		
Density (g/ml)	1.16	1.15	1.13
Viscosity (cP)	35.88	19.20	19.41
pH (-)	4.46	4.22	4.07

The density values of DES decrease with an increasing molar ratio. Several studies on DES synthesis found that the density value of DES is between 1.1 g/ml to 2.4 g/ml (Fischer, 2015). The density value decreases as the molar HBD increases. The 1:4 molar ratio has a lower density value than the other molar ratios, namely 1:2 and 1:3.

In addition, the viscosity of eutectic mixtures was strongly affected by hydrogen bonds, van der Waals and electrostatic interactions (Zhang et al., 2012). The earlier research showed that the mole ratio between choline chloride and ethylene glycol at a temperature of 20°C for a mole ratio of 1:2 with a viscosity of 36 cP and a mole ratio of 1:3 and 1:4, is 19 Cp (Zhang et al., 2012). Increasing the ethylene glycol ratio at 1:3 and 1:4 has made the 3D intermolecular hydrogen bond network weak and had lower viscosity.

The pH value ranges from 4.07 - 4.46, meaning that the DES solution has an acidic pH. It was found that the pH value of DES by Ethylene glycol and Choline chloride range from 4.08 to 4.36 (Skulcova et al., 2019). The resulting pH tends to decrease along with the increase in the molar value of the addition of Ethylene glycol solution.

The physical appearance of the DES is shown in Figure 1b. The results obtained in the synthesis of DES which includes pH, viscosity, density, and physical characteristics were following the existing standards. Therefore, DES solvents based on Choline chloride and Ethylene Glycol with molar variations of 1:2, 1:3, and 1:4 can be used as solvents in the dehydration reaction process to produce 5-HMF.

The 5-HMF yield value in the DES molar ratio of 1:2, 1:3 and 1:4

The dehydration reaction process was carried out with 3 different molar ratios of 1:2, 1:3, and 1:4. In this reaction, the result of the hydrolysis was in the form of glucose from cassava starch mixed with the DES.

The hydrolysis of cassava flour resulted in a glucose concentration of 51.24%. The addition of

DES to the hydrolysis results, namely glucose to see the effect of the addition of the solution on the 5-HMF yield that will be produced. Therefore, a comparison of the addition of glucose and DES was given, namely 1:0 (without DES), 1:4, and 1:6. The 5-HMF yield obtained after the dehydration reaction process at each different DES molar variation was served in Figure 2.

Based on Figure 2, all DES molar ratios (Choline chloride: Ethylene glycol), a glucose: DES ratio of 1:6 resulted in the highest yields. At the 1:2 DES molar ratio, the yield of 5-HMF reached a yield of 78.61% compared to 1:4 which is 67.76 % and the lowest is at 1:0 (without DES) with a yield of 44.49 %. Similarly, at the DES molar ratio of 1:3, the yields of 5-HMF reached the value of 77.49% compared to a 1:4 ratio of 41.52 % and lower at 1:0 (without DES) with a yield of 44.49%. Correspondingly, at the DES molar ratio of 1:4, the yields of 5-HMF reached the value of 60.10% compared to a 1:4 ratio of 52.05% and lower at 1:0 (without DES) with a yield of 44.49%. The highest result in the glucose: DES of 1:6 ratio showed that the addition of DES can increase the yield of 5-HMF.

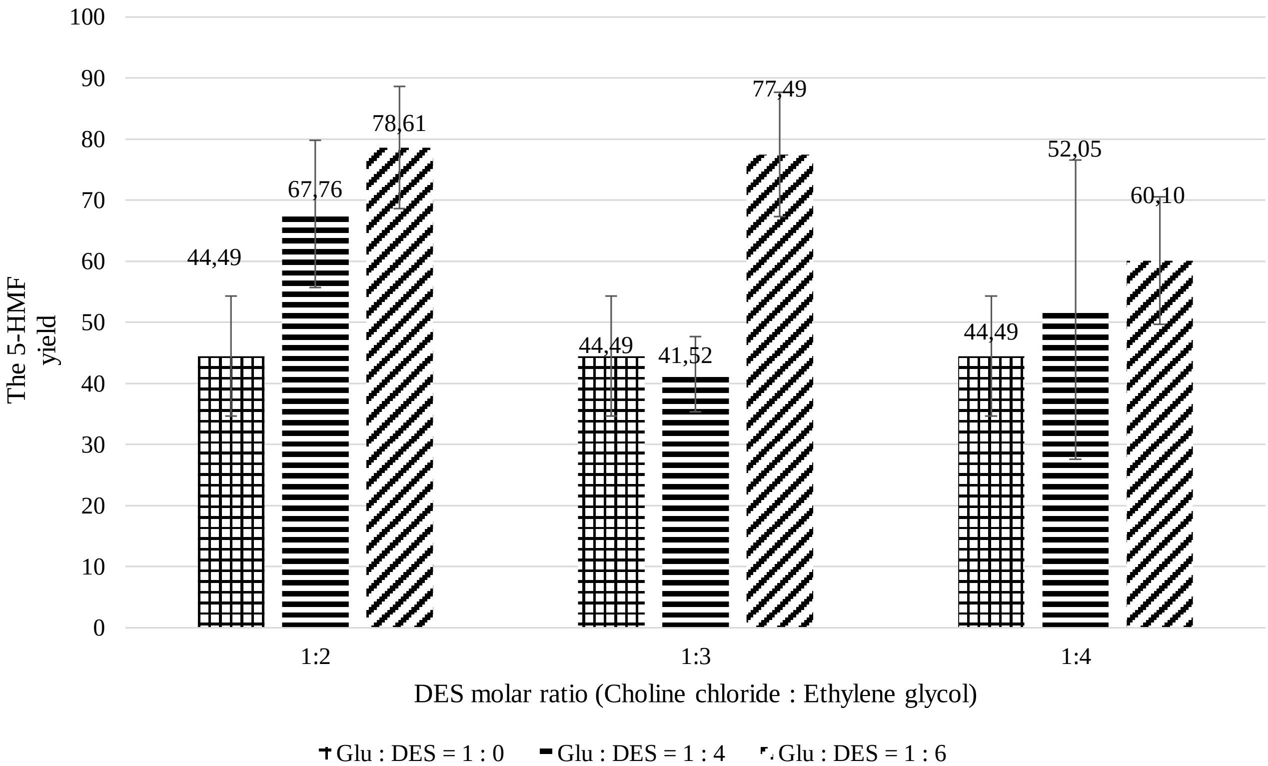

Figure 2. The yields of the dehydration reaction between the molar ratio of the DES and the ratio of glucose: DES

Comparison of the results of the dehydration reaction between the molar ratio of DES and the ratio of glucose: DES

The results of the DES synthesis in the variation of the DES molar ratio of 1:2, 1:3, and 1:4 was in accordance with the earlier research (Zhang et al., 2012). The DES result was then used in the dehydration reaction together with hydrolyzed glucose from cassava with three molar ratios, namely 1:0 (without DES), 1:4, and 1:6 (Kupiainen, 2012).

Based on the results from Figure 2, it can be seen that the dehydration of 5-HMF with DES solvent has a higher yield of 5-HMF compared to without DES. The highest result was obtained at the DES molar ratio of 1:2 and the results for the addition of glucose: DES was at a ratio of 1:6. Then, the lowest result was obtained at a molar ratio of DES 1:3 with the addition of glucose: DES, which is 1:0 or without DES. Some variables that affect the increase in yield of 5-HMF using DES solvent are viscosity, pH, density, and environmental influences.

DES generally has low viscosity and it tends to facilitate mass and heat transfer in dehydration reactions using DES-based solvents (Zuo et al., 2017). The effect of the DES viscosity on the 5-HMF yield value is that the higher the DES viscosity, the lower the yield value. This occurred because the high viscosity inhibits or slows down mass transfer due to the extensive hydrogen bonds in the DES compound so the yield value will be lower (Zainal-Abidin et al., 2017).

The density value in the 1:2 molar ratio was lower than the other molar ratios. This causes the 5-HMF yield value at a ratio of 1:2 to be higher. Meanwhile, in terms of pH, the higher the DES molarity, the more acidic it will be. The more acidic the solution, the faster the chemical reaction. The dehydration reaction time used for each comparison was the same. However, the reaction will be faster at a 1:2 molar ratio due to the lower pH and the more acidic it was. This caused the yield value at a molar ratio of 1:2 to be potentially higher. The DES can act as dehydrating agent, leading to the rapid expulsion of water from cells and hence their immediate inactivation (Smith et al., 2014). From the use of DES as an extracting solvent, sorbent, or selective binder to the analysis of trace contaminants in food and environmental samples. The 5-HMF synthesized via acid-catalyzed dehydration was lower when reacted with pure water solvent or without DES due to uncontrolled re-hydration of 5-HMF in levulinic acid and formic acid (Cunha and Fernandes, 2018).

Conclusion

The synthesis of 5-HMF from cassava flour's glucose with a solvent based on DES was successfully conducted. In comparison, the 5-HMF yield of the dehydration process without DES only reached 44.49%. While the molar ratio of 1:2 of DES and the ratio of glucose : DES at 1:6 produced the highest 5-HMF yield of 78.61%. The DES usage has potentially increased the 5-HMF yields due to its supportive characteristic in viscosity, pH, and density. The optimization of the DES molar ratio and glucose to DES ratio will be the future step to obtain the maximum 5-HMF yield.

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Declarations

Conflict of interests The authors declare no competing interests.

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