Fructose syrup production from Onggok with isomerization process by Mg/Al hydrotalcite catalyst and glucose isomerase enzyme

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Abstract. Onggok, solid waste of tapioca still contains high carbohydrate. This component can be converted to liquid sugar (glucose and fructose). Hydrolysis process convert carbohydrate become glucose syrup while isomerization process would change those glucose become fructose syrup. This study aims to produce fructose from Onggok with isomerization by Mg/Al hydrotalcite catalyst and compare the productivity 500ml Onggok substrate (10.32 percent DS) was hydrolyzed become glucose in liquefaction and saccharification process. Liquefaction took placed in 95 degree C, pH 6.5, for 1 hour, by adding 0.067 percent (v/v) α-amylase while the saccharification was start by adding 0.067 percent (v/v) glucoamylase at 60 degree C, pH 5, for 1 hour. Isomerization with Mg/Al hydrotalcite took placed in 50 ml hydrolysate sugar, 1 percent (b/v) catalyst, and 100 degree C while process with glucose isomerase was at 100 ml substrate, 60 degree C, pH 8.2, and same catalyst ratio (1 percent). Isomerization process for each catalyst would be hold in 3, 5, and 7 hours. The best result of isomerization productivity was 39.29 g/L. h for process with Mg/Al hydrotalcite catalyst, while isomerization yield was 6.18 percent for process with glucose isomerization enzyme.

1. Introduction

Cassava is abundant crops in the tropics area and it is usually used to substitute the rice consumption because of the carbohydrate content. Cassava production in Indonesia for 2015 could reach 21.8 million tons [1]. Aside from being a food substitute for rice, cassava is also used as a raw material for tapioca flour production. More than 60% solid waste of cassava is generated during the tapioca production and it is known as Onggok [2]. The carbohydrate content in Onggok reach approximately 67.935 – 68.395% and with the water content 19.70 – 20.3% [3].

Amylose and amylopectin in those carbohydrate could be converted to glucose for increasing the economic value of Onggok. Glucose could be reached from the carbohydrate by hydrolysis through the liquefaction and saccharification process [4]. Enzymatic hydrolysis had more advantages than acid hydrolysis. The process was easier to be controlled, the color damage could be minimized, efficient purification process, and the specified process of enzymatic hydrolysis obtained high quality product [5]. The previous researcher got the best result of enzymatic hydrolysis from red sorghum flour in substrate concentration 40%, enzyme concentration 0.067 ppm obtained glucose concentration up to 115.74 g/L [6].

Types of sweetener which have the relative sweetness higher than glucose is shown in Table 1. Fructose has the highest sweetness intensity in the crystalline form and so that in 10% syrup. It made fructose become more valuable than the others.

Table 1. Sweetness comparison of several sweetener [7].

1. Sugar	**2.** Sweetness intensity (crystalline)	**3.** Relative sweetness (10% syrup)	**4.** Absolute sweetness (syrups)
5. Fructose	6. 180	7. 117	8. -
9. Sucrose	10. 100	11. 100	12. 100
13. HFCS-55	14. -	15. 99	16. 97
17. Glucose	18. 74-82	19. 65	20. -

Fructose production use glucose syrup as the substrate. Isomerizing the glucose syrup by several catalyst would change the structure of glucose into fructose. The catalyst that is commonly used in is bio-catalyst enzyme, hydrotalcite, zeolite, and ion exchange resin. Isomerization of glucose syrup from red sorghum flour obtained the highest fructose concentration and conversion respectively 17.48 g/100 and 88.56% in operation with 2% of bio-catalyst glucose isomerase enzyme in pH 8, 60 °C, for 48 hours [8]. Fructose syrup from Onggok was also inspected by previous researcher in operation condition pH 8.2, 60 °C, during 43 hours with glucose isomerase, and obtained 9.3% isomerization yield with 229.30 g/l fructose [9]. Isomerizing glucose into fructose with glucose isomerase required a much time process. On the other side isomerization of pure glucose into fructose was also took placed with Mg/Al hydrotalcite catalyst in 100°C in 5 hours. It obtained fructose selectivity, yield, and conversion 53%, 25%, and 48% respectively [10]. Based on previous research, this study aims to make fructose syrup from Onggok with the Mg/Al hydrotalcite catalyst in isomerization process and compare the productivity and yield of isomerization between this catalyst and bio-catalyst glucose isomerase enzyme.

2. Methodology

2.1. Preparation of substrate

Substrate on this isomerization process was the hydrolysate from hydrolysis of Onggok. The content of Onggok was presented in Table 2 while the hydrolysis used α-amylase and glucoamylase which the characteristic was presented in Table 3, both of them based on analysis result in laboratory. Onggok solution was made by diluted the Onggok powder (125 μm) into 500 ml distilled water (10.32% DS). It was hydrolyzed become glucose by liquefaction and saccharification process. Liquefaction took placed in 95°C, pH 6.5, for 1 hour start from additional of α-amylase 0.067% (v/v). Saccharification was started from additional of 0.067% (v/v) glucoamylase at 60°C, pH 5, for 1 hour. The hydrolysis product than was purified by 0.2% (b/v) powdered activated carbon and was heated until 80 °C for 15 minutes. The solution was filtered to separate the solid. The filtrate would become the hydrolysate sugar which was used in isomerization process. Analysis of the hydrolysate sugar concentration used the DNS method.

2.2. Production of Mg/Al hydrotalcite catalyst

Mg/Al hydrotalcite for the isomerization process was generated by coprecipitation method. Magnesium nitrate (Mg (NO_3)2) and aluminum nitrate (Al (NO_3)3 in mole ratio 3:1 were the main constituent in Mg/Al hydrotalcite catalyst production. 15.4 g of Mg (NO_3)2 and 7.5 g (Al (NO_3)3 were diluted in distilled water until 200 ml for each component. Both of them were mixed and 100 ml of NaOH 1M and 100 ml of Na_2CO_3 were added into the solution as the supporting material to maintain solution pH in

9.5. The solution was stirred at 25 °C or 12 hours for the coprecipitation process. The solid formed of Mg/Al hydrotalcite was filtered and dried in 100 °C for 11 hours.

Table 2. Onggok content.

Component	Content (%)
Starch	76.06
- Amilose	15.84
- Amilopectin	60.21
Protein	2.09
Fiber	9.79
Fat	1.19
Water	13.11

Table 3. Enzyme characteristic.

Characteristic	Enzyme		
	α-amylase	Glucoamylase	Gluco isomerase
Form	Liquid	liquid	Granule
Enzyme activity (U/mL)	2.03	2.97	2.36
Density (g/mL)	1.24	1.16	-
Total viable count (/g)	< 100	< 200	-
Coliform (/g)	< 4	< 4	-

2.3. Isomerization process

Isomerization of Onggok hydrolysate sugar used Mg/Al hydrotalcite and glucose isomerase as the catalyst in a separate process. The use of each catalyst on substrate volume was 1% (b/v). Isomerization with glucose isomerase took placed on 100 ml substrate with temperature process 60 °C, pH 8.2. Meanwhile the process with Mg/Al hydrotalcite catalyst worked on 50 ml substrate solution in 100 °C. Isomerization time of each process was varied in 3, 5, and 7 hours. The fructose concentration of the samples would be observed.

2.4. Characterization and analytical method

Characteristic of Mg/Al hydrotalcite was observed in some methods. The crystallinity of the catalyst would be analysed the diffractogram by X Ray Diffractometer (XRD). The functional groups were analysed by Fourier Transform Infra-Red (FTIR) and the surface area, pore diameter, pore volume of the catalyst was investigated by Brunauer-Emmett-Teller (BET) method.

Onggok hydrolysate sugar as the substrate of isomerization process was measured by DNS method. 2 ml of diluted sample was added by 3 ml reagent 3,5 Dinitro salicylic acid and heated on the boiling water for 15 minutes than cooled in ice water suddenly. The samples were observed the absorbance by visible spectrophotometer in wave length 540 nm. While the fructose concentration from the isomerization process was analysed by Seliwanoff method. This method almost same with the DNS method but had differences on the reagent and heating time of the samples. Seliwanoff method used resorcinol as the reagent, 5 minutes of heating time, and wave length 520 nm on observation of the absorbance. The end product of fructose was characterized by the density, viscosity, pH, the total sugar value.

3. Result and discussion

3.1. Characteristic of Mg/Al hydrotalcite catalyst

Characterization of Mg/Al hydrotalcite identified the physical characteristic of the catalyst like the structure, functional groups, crystallinity, surface area, pore diameter, and pore volume. The synthesis of Mg/Al hydrotalcite generate the white crystal form. After the drying process this catalyst was grinded to increase the surface area.

XRD used to qualitative and quantitative analysis of the catalyst. Observation of qualitative aspect shown the main compound on the catalyst by comparing the d value on the analyzed data and reference data. Meanwhile quantitative analysis was used to observe amount of the hydrotalcite on the catalyst. Comparation of the diffractogram of this Mg/Al hydrotalcite catalyst and the reference [11] was presented in Figure 1 and Figure 2.

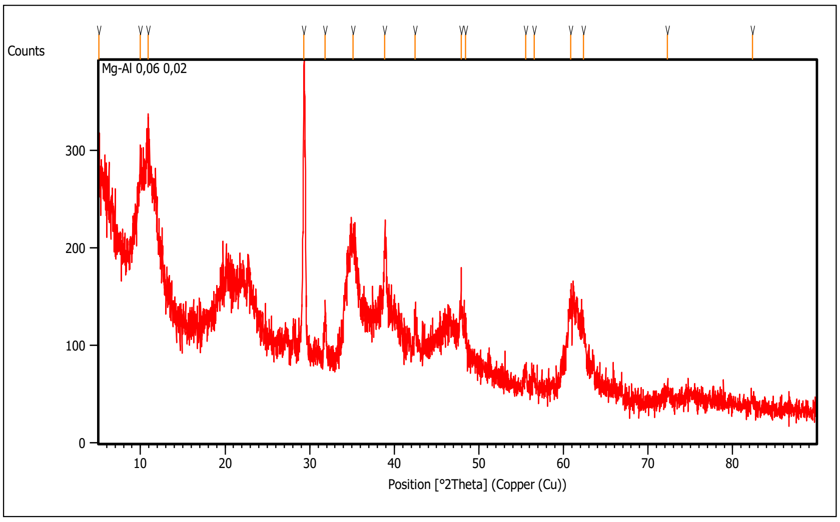

Figure 1. Diffractogram of Mg/Al hydrotalcite on mole ratio 3.

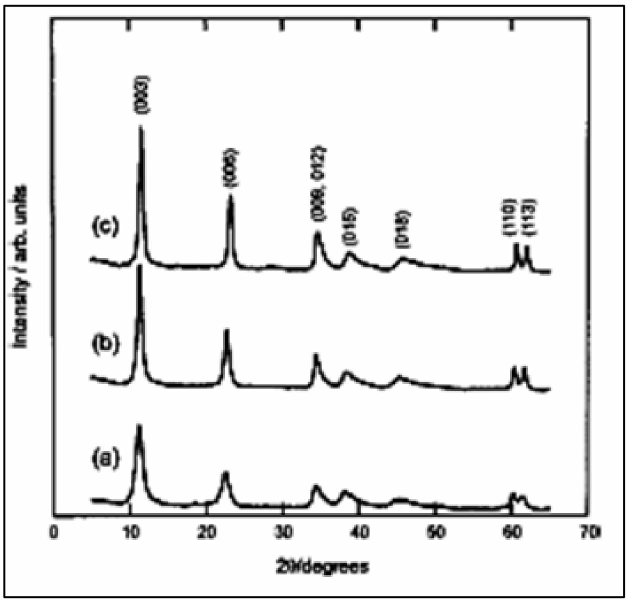

Figure 2. Diffractogram reference of Mg/Al hydrotalcite a) mole ratio 1. b) mole ratio 2. c) mole ratio 3.

Diffractogram on the Figure 1 showed that the Mg/Al hydrotalcite catalyst on the semi crystallin form based on some peak in the sharp shape. The highest intensity peak of diffractogram from Mg/Al hydrotalcite synthesis compared with the standard hydrotalcite on JCPDS (Joint Committee on Powder Diffraction Standard) [12] was presented in Table 4. The d value 7.82 Å was the characteristic peak of

Mg/Al hydrotalcite with the anion CO32- with mole ratio 3 of Mg/Al. *d value* of the peak was presented in Table 5.

Table 4. Peak of 2θ position on Mg/Al hydrotalcite.

Mg/Al hydrotalcite	2θ (°) position		
Sample	10.87	29.28	35.12
* Standard	11.27	22.7	34.86

Table 5. *d value* peak of Mg/Al hydrotalcite.

Mg/Al hydrotalcite	*d* value (Å)		
Sample	8.14	3.05	2.55
* Standard	7.84	3.91	2.61

*Standard hydrotalcite based on JCPDS.

The content of Mg/Al hydrotalcite on this catalyst determined by comparing the relative intensity of the diffractogram peak (I/I1)s with relative intensity of the whole peak (I/I1)t on the sample. Based on XRD analysis the relative content of Mg/Al hydrotalcite was 53.38 %.

As the catalyst, the surface area of Mg/Al hydrotalcite must be identified by Brunauer-Emmett-Teller (BET) while the pore diameter was analyzed with Barret-Joner-Halenda (BJH) method. Some researcher explained about the surface area of Mg/Al hydrotalcite with mole ratio 2 with CO32-interlayer approximately 210 m^2/g [13], 100 m^2/g [14], 93.705 m^2/g [15]. The characteristic of the Mg/Al hydrotalcite was presented in Table 6. Based on the result of Mg/Al hydrotalcite in this study had a similarity with the Mg/Al hydrotalcite from the other researcher.

Table 6. Characteristic of Mg/Al hydrotalcite

Parameter	Mg/Al hydrotalcite Sample		Reference
	BET	BJH	BET
Surface Area (m^2/g)	233.983	63.257	210
Pore Volume (cc/g)		0.096	
Diameter (nm)		3.051	

Fourier Transform Infra-Red (FTIR) was used to observe the functional groups of the compound. Spectrum from the Mg/Al hydrotalcite sample and the reference was presented in Figure 3 and Figure 4. There was some specific peak from the vibration of functional groups from hydrotalcite compound from the reference. Peak on the wave number 3400 cm^{-1} showed the stretch vibration of OH, 1400 cm^{-1} was asymmetric CO3, 800 cm^{-1} was outside deformation CO3, and 600-400 cm^{-1} was the stretch vibration of M-Al-O. Comparison of wave number from the Mg/Al hydrotalcite spectrum presented on Table 7. From this comparison could be concluded that the synthesis compound was the Mg/Al hydrotalcite catalyst.

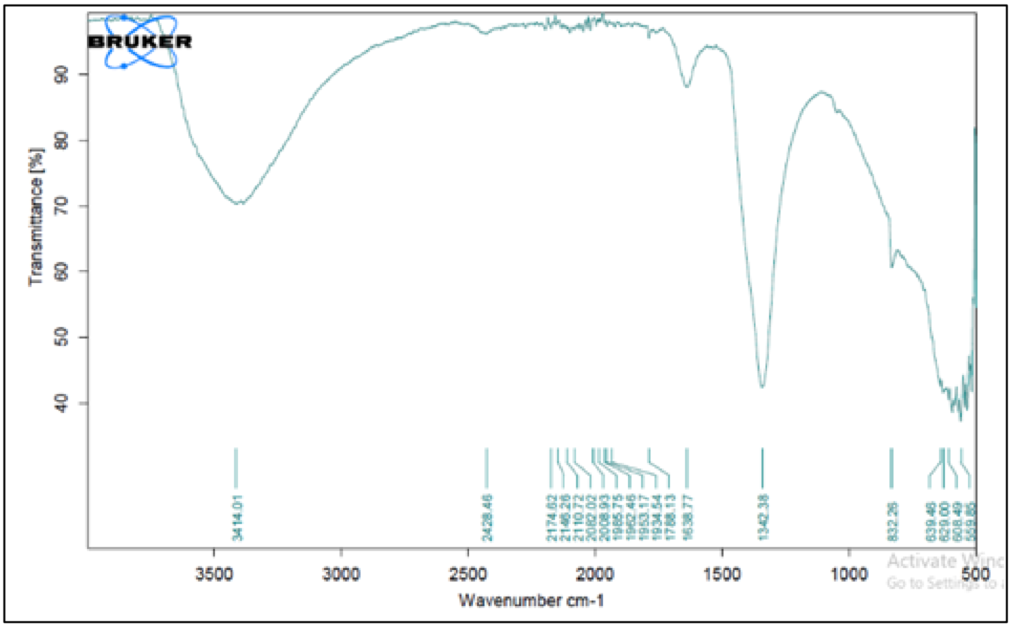

Figure 3. Spectrum of Mg/Al Sample.

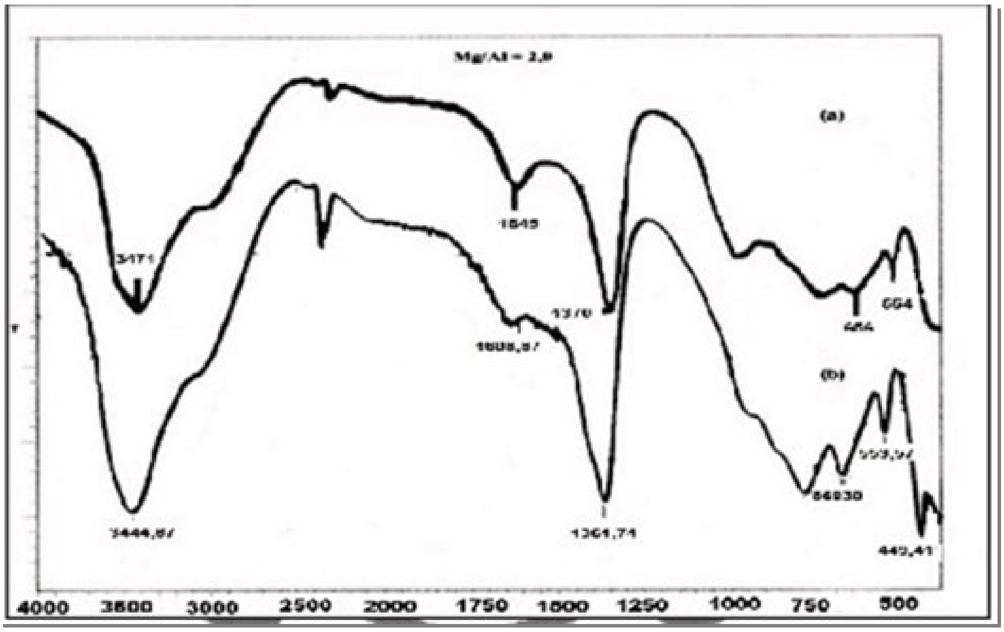

Figure 4. Spectrum of Mg/Al reference.

Table 7. Comparison of wave number FTIR spectrum between Mg/Al hydrotalcite sample and the reference.

Functional groups	Wave number (cm^{-1}) of Mg/Al hydrotalcite	
	Sample	Reference
Streching OH	3414	3400
Streching asimetris CO_3	1342	1400
Outside deformation CO_3	832	800
Streching M-Al-O	608 – 559	600 – 400

3.2. Enzymatic hydrolysis of Onggok

Hydrolysis Onggok was used to generate the glucose syrup as the substrate on the isomerization process. Onggok with 76% carbohydrate concentration would be convert to glucose by enzymatic hydrolysis by liquefaction and saccharification process. Gelatinization is the first process of the hydrolysis before the liquefaction. This process would be started on the temperature solution 58 °C - 60 °C before the α-amylase 0.067% (v/v) was added for liquefaction process. Gelatinization made bounds of amylose and amylopectin crack so that the enzyme worked properly. Viscosity of the substrate solution would increase during the gelatinization and become aqueous during the liquefaction with the darker color [16]. Liquefaction process would be held when the heating temperature in 90 °C, pH 6.5, and stirring mechanism during the process made the enzyme evenly mixed. In this process, α-amylase worked by breaking the bounds between α-(1,4)-glycoside from the inside of amylose and amylopectin and produced glucose, maltose, dextrin and the oligosaccharide [4]. Liquefaction continued by saccharification process by decrease the heating temperature until 60 °C, pH 5, with additional of glucoamylase 0.067% (v/v). This enzyme broke the bounds of α-(1,4) and α-(1,6) of amylose and amylopectin randomly and produced only molecule of glucose marked by decreasing of the solution viscosity [17]. In this study, Onggok hydrolysis produced the hydrolysate sugar (glucose) concentration up to 585.082 g/L with hydrolysis yield 52.23%.

3.3. Isomerization process

Isomerization is process to convert glucose from the hydrolysate sugar from Onggok become fructose syrup. This is a reversible reaction so that the yield process will depend on operating condition like temperature and catalyst used. In this study, isomerization was made two different catalyst, glucose isomerase at temperature 60 °C and Mg/Al hydrotalcite at 100 °C. Increasing of fructose concentration during the isomerization process was presented at Figure 5.

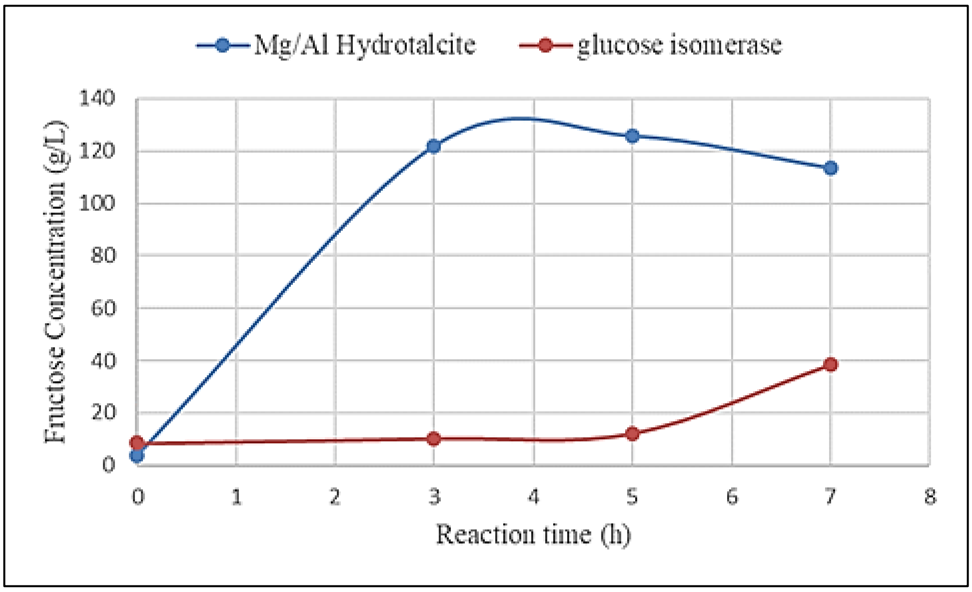

Figure 5. Fructose concentration during the isomerization process.

Figure 5 shown the speed of fructose formation from the Onggok hydrolysate sugar. Isomerization with Mg/Al hydrotalcite reached high fructose concentration in 3 hours of isomerization and slightly increased in 5 hours become 125.75 g/L. Meanwhile the process with glucose isomerase catalyst started increase on the 7 hours. Isomerization with glucose isomerase catalyst needed much time to start converting glucose into fructose because the enzyme worked on the stretch and lower temperature. Isomerization with glucose isomerase would obtain high fructose concentration over 40 hours in time operation. Operation for 43 hours would obtain fructose concentration up to 229.303 g/L [9], and isomerization of red sorghum sugar hydrolysate in 48 hours got 155 g/L. The optimal isomerization temperature of this biocatalyst working system was at 60 °C [8], setting up the lower temperature would decrease the enzyme activity [18] and higher temperature would damage the enzyme.

Isomerization process with Mg/Al hydrotalcite reached the highest fructose concentration in 5 hours reaction time. Activity of Mg/Al hydrotalcite worked optimally to convert glucose become fructose in 5 hours reaction time at temperature 100 °C [10]. During the isomerization, temperature process at 100 °C caused the water content on the Onggok hydrolysate sugar evaporated and reduced the substrate volume. The reflux system on this process did not work properly so the water evaporation still happened.

Yield of isomerization was calculated by mass fructose of the end product and mass of glucose in hydrolysate sugar. While the productivity of isomerization was fructose produced per unit volume and time process. Mass of fructose and yield of isomerization process was present on the Table 8.

Table 8. Mass of fructose syrup, yield, and productivity of isomerization process in 7 hours reaction time.

Catalyst	Substrate volume (ml)	Product volume (ml)	Fructose concentration (g/L)	Mass of fructose (g)	Isomerization yield (%)
Glucose isomerase	100	94	38.49	3.62	6.18
Mg/Al hydrotalcite	50	5	113.53	0.57	1.94

Productivity of isomerization with Mg/Al hydrotalcite (39.29 g/L.h) was higher than glucose isomerase (3.47 g/l. h) because in short reaction time (3h) fructose concentration increased significantly and slightly decreased in 5h and 7h. While the yield of isomerization for process with glucose isomerase was higher than Mg/Al hydrotalcite catalyst because the temperature process at 60 °C make the volume product stable and did not occur water evaporation. Yield of isomerization with Mg/Al hydrotalcite was 1.94% while on previous research isomerization of pure glucose into fructose with Mg/Al hydrotalcite catalyst reached yield up to 25% [10]. Low yield of isomerization in this study was caused by caramelized of product in long time operation at 100 °C and the impurity of the Onggok hydrolysate sugar. On the other side, the synthesis or coprecipitation process of the Mg/Al hydrotalcite catalyst need more longer time up to 18 hours to make sure the catalyst in crystallin form. Short time process in coprecipitation and calcination made the catalyst formed in semi crystallin form.

The end product of fructose syrup by isomerization with Mg/Al hydrotalcite catalyst was thicker (viscosity 5.5 cP) than the fructose from glucose isomerase isomerization (viscosity 1.6). It would decrease the energy consumption for separations process to remove water from the end product [18].

4. Conclusion

Onggok has high content of amylose and amylopectin which can convert to fructose syrup by isomerization process. This process needs a suitable catalyst to get high yield and productivity. The best result for the highest isomerization productivity was 39.29 g/L. h for process with Mg/Al hydrotalcite catalyst, while the highest isomerization yield was 6.18% for process with glucose isomerization enzyme.

5. References

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