Chempublish Journal
Journal Homepage: online-journal.unja.ac.id/chp
Department of Chemistry
Faculty of Science and Technology
Universitas Jambi
ISSN (Online): ISSN 2503-4588
No. 23/E/KPT/2019

Article

Optimization of Liquid Fructose Sugar Production from Cassava Peel Waste using the Isomerization Process

Ratu Mayoreta Debora[1*], Dicky Chandra Hermawan[2], Isni Utami[3], Fachrul Nurcholis[4]

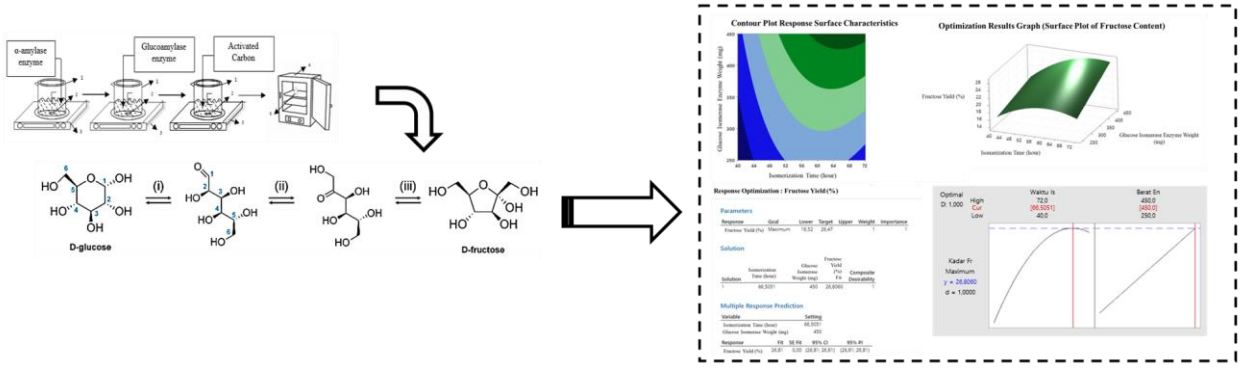
[1,2,3]Department of Chemical Engineering, Univeristy of Pembangunan Nasional Veteran Jawa Timur, Surabaya 60294, Indonesia
[4]Department of Chemical Engineering, National Taipei University of Technology, Taipei City 10608, Taiwan

Abstract

Cassava is a type of tuber that is commonly consumed in Indonesia. The peel is one of the parts of cassava that is occasionally used. Cassava peel has a carbohydrate content of 74.73%. Cassava peel has the potential to be processed into liquid fructose sugar through an isomerization process. This research aims to determine optimal results based on results and equations through Response Surface Methodology (RSM). The research was carried out in the stages of making cassava peel flour and saccharification, then through a hydrolysis process to break down the starch with the help of 3.4 ml of α-amylase enzyme and 3.4 ml of glucoamylase enzyme. The final stage is isomerization, which converts glucose into fructose by adding the enzyme glucose isomerase. Variations in adding the glucose isomerase enzyme were 250, 300, 350, 400, and 450 mg at 60ºC for 40, 44, 48, 52, 56, 64, and 72 hours. The best research results were under an isomerization time of 72 hours and a glucose isomerase enzyme weight of 450 mg with a fructose content of 26.05%. Meanwhile, the optimization results using the RSM method were under conditions of an isomerization time of 66.5 hours and a glucose isomerase enzyme weight of 450 mg with a fructose content of 26.8%

Keywords: *Cassava peel, Fructose, Isomerization, RSM*

Graphical Abstract



* Corresponding author
Email addresses: ratumayoretadebora25@gmail.com

DOI: https://doi.org/10.22437/chp.v8i1.33117
Received May 11th 2024; **Accepted** June 26th 2024; **Available online** June 30th 2024

Introduction

The cassava plant is a perennial woody shrub that can grow up to three meters in tropical regions. Cassava has a big role in meeting people's food needs compared to other types of tuber plants. Usually, the edible parts of the cassava plant are the tubers and leaves. Meanwhile, cassava peel is generally considered a useless by-product, so it is not used and is often wasted. This waste can cause severe problems if not treated. Every 1 kg of cassava produces 0.2 kg of skin[1,2].

Cassava peel has quite large economic potential, the carbohydrate content in cassava peel is higher than the flesh itself, namely around 74.73% (cassava peel) compared to 36.8% (flesh)[3]. Cassava peel is something that can be useful, especially when used for the production of liquid fructose sugar. By using the method of starch hydrolysis and glucose isomerization, cassava peel waste can be converted into liquid fructose sugar[4]. According to research that has been carried out, the carbohydrate content in corn can be used as high fructose corn syrup using a catalyst[5].

The conversion of glucose to fructose expands the range of biomass utilization. Not only is it widely used as a sweetener in food and drinks, fructose is more easily changed and converted into other compounds than glucose itself. Examples are the compounds 5-HMF and levulinic acid which are materials used to produce medicines, polymers and biofuels[6].

Starch is a type of polysaccharide consisting of glucose linked together via α-glycosidic bonds. Starch consists of two parts, soluble (amylose) and insoluble (amylopectin)[7]. Glucose ($C_6H_{12}O_6$) is a monosaccharide that has six carbon chains with an aldehyde group. Glucose generally comes from starch and is often used as a food sweetener[8]. Glucose and fructose have the same chemical formula, the difference is the different arrangement of their atoms. Glucose has a six-carbon ring structure, while fructose has a carbon ring. Fructose is also often referred to as fruit sugar. In general, fructose is marketed in crystal and syrup form. Fructose is about 1.8 times sweeter than glucose. Fructose as a sweetener can be produced from cassava peel[9]. Hydrolysis of starch into glucose syrup involves the stages of gelatinization, breakdown of starch molecules, and saccharification[10]. Gelatinization is the expansion of starch granules caused by heat, by disrupting the hydrogen bonds in starch glycosidic bonds. Breaking down starch molecules without going through the gelatinization process will take longer[11]. Enzymatic starch hydrolysis refers to the breakdown of starch molecules (polysaccharides) into smaller elements, including dextrin, maltotriose, maltose and glucose. The speed of the process of breaking down starch molecules is determined by the enzymatic activity of α-amylase. The rate of enzymatic activity can be increased by utilizing substrates that have previously undergone gelatinization[12]. Generally, the enzymes that are often used are the α-amylase enzyme and the glucoamylase enzyme. The α-amylase enzyme plays a role in breaking down the α-(1,4) glycosidic bonds of starch into glucose and maltose. The glucoamylase enzyme plays a role in converting maltose produced by α-amylase into glucose[13]. Optimal conditions for the α-amylase enzyme are between 90 and 105°C and pH 6. The enzyme will be disrupted and damaged by temperatures that are too high, and partial gelatinization of starch will occur due to temperatures that are too low[14].

Saccharification is continued hydrolysis after the process of breaking down starch molecules. The glycoamylase ecoenzyme will hydrolyze α-1,4 bonds and a small amount of α-1,6 bonds at the branching point. This enzyme will hydrolyze glucose from maltose and starch from maltose. Saccharification takes two hours and can be carried out at temperatures between 60°C and pH 4[12]].

The isomerization process is used to convert glucose into fructose. The isomerization process is assisted by the enzyme glucose isomerase and the process is carried out in an incubator for 48 hours[15]. The process of glucose isomerase enzyme activity begins with the opening of the carbon chain ring, isomerization using a hybrid

shift mechanism, and the closing of the carbon chain ring[16].

Optimum conditions for the productivity of the glucose isomerase enzyme are influenced by several factors. Higher operating temperatures will increase enzyme activity while reducing the level of enzyme stability. The isomerization process of glucose to fructose is generally carried out at a neutral pH solution. Glucose cannot be completely converted into fructose in one step, because of its thermodynamic properties, the equilibrium ratio between glucose and fructose at a normal temperature of 25^0C is 54:46. Meanwhile, at higher temperatures, the equilibrium shifts towards fructose. Therefore, most of the temperatures used for the isomerization process are high temperatures [17]. According to Mahreni's research, the right conditions for the glucose isomerase enzyme to work optimally are at a temperature of around 60°C and the pH is set at 7.8 to 8.3 [15]. In addition, the contact time was set at 48 hours to minimize the formation of by-products such as dyes [18].

Based on similar research that has been carried out, the process of making fructose sugar from cassava peel can be varied in various operating conditions, such as type of enzyme, amount of enzyme, pH, temperature and operating time. The results of the fructose sugar content were a yield value of 9.298% or a fructose concentration of 229.3 g/L conditioned on a substrate concentration of 35% with a solution volume of 300 ml and a pH of 5.5 [19]. Making fructose using 1.5 mL of *Bacillus licheniformis* microbes yielded a yield of 6.07% [20]. Meanwhile, research using a raw material of onggok flour solution with a concentration of 12% at pH 7 and a temperature of 120℃ obtained a yield value of 36.19% [21].

The existing data encourages the development of a method for making liquid fructose sugar from cassava peel waste. This is done with the consideration that cassava peel has a higher starch content so it will be used as a more useful ingredient, namely fructose liquid sugar. Apart from that, to support research, it is necessary to optimize the results so that they are more consistent and the research carried out gets more ideal results. The optimization process can be carried out in two ways, namely empirical and statistical methods. In empirical methods, each factor is usually tested and optimized once, resulting in a whole process but it is very time consuming and ignores the interactions between factors that influence the research. The statistical method is not too time consuming and is able to process interactions between influential factors. Statistical methods are more reflected in the Response Surface Methodology (RSM) method used to evaluate various variables in this research [22].

Optimization is carried out using the response surface method (RSM) to identify ideal conditions for building an experiment. RSM analysis uses a response function parameter estimation process based on a least squares approach, which is basically comparable to regression analysis. Simply put, the optimal point is determined by combining RSM studies with mathematical techniques to obtain maximum response [23].

Materials and Methods

Chemical and Equipments

Some of the materials used in this research include cassava peel obtained from waste from the Cap Lumba Lumba cassava chips industry in Turen, Malang Regency. The analysis results published by the Nutrition Laboratory, Airlangga University stated that the starch content in cassava peel was 69.73%. The next ingredients are the α-amylase enzyme and the glucoamylase enzyme as a bond-breaking agent in starch into glucose, both enzymes were purchased from the Nanobio Laboratory. Glucose isomerase enzyme purchased online via the Tokopedia application in the Agrotekno store. The glucose isomerase enzyme functions to convert glucose into fructose and distilled water as a solvent. The rest of the materials are purchased at Ngagel Jaya Kimia Store, Surabaya, such as 0.06 M citric acid and 0.2 M calcium hydroxide as a pH regulator, and activated carbon to bind impurity compounds.

The tools used in this research are as shown in Figure 1, with the numbers (1) beaker glass, (2)

magnetic bar, (3) magnetic stirrer hotplate, (4) incubator, (5) erlenmeyer.

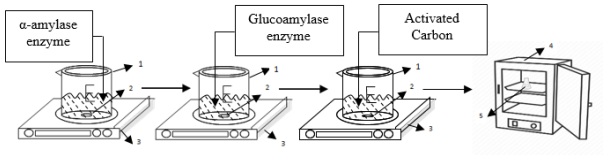


Figure 1. Equipment Schematic Process

Hydrolysis and Saccharification

Hydrolysis and Saccharification Process. Preparation of raw materials is done by preparing 15 grams of cassava peel flour and dissolving it in distilled water to a volume of 100 mL. The suspension solution formed is then heated and stirred using a magnetic stirrer at a temperature of 60℃ for the gelatinization process until the solution thickens. Followed by the liquefaction process by adding 3.4 mL of α-amylase enzyme at a temperature of 90℃ and pH 6. Then the saccharification process by adding 3.4 mL of glucoamylase enzyme at a temperature of 90℃ and pH 4.5 for 2 hours [24].

Isomerization Process

Isomerization Process. The hydrolysis and saccharification process produces liquid glucose. The resulting liquid glucose must first be purified from impurity compounds with the help of activated carbon. Liquid glucose is heated and stirred in a magnetic stirrer at 80 ℃ for 15 minutes then 2 grams of activated carbon is added. Liquid glucose is filtered to separate impurity compounds. The liquid glucose that has been filtered is then stirred in a magnetic stirrer and then the glucose isomerase enzyme is added with weight variations of 250, 300, 350, 400 and 450 mg. Next, the incubation process continued at a temperature of 60℃ for 40, 44, 48, 52, 56, 64, and 72 hours [15]. After the isomerization process was carried out, the fructose syrup produced was then analyzed for its fructose content using the luff scroll method at the Nutrition Laboratory, Airlangga University.

Result and Discussion

The isomerization process of glucose into fructose from cassava peel has been carried out in this research. The reaction is as follows in Equation 1. In Table 1, it can be seen that the percentage of fructose levels formed increases along with the amount of glucose isomerase enzyme used. Under the conditions of using 250 mg of the glucose isomerase enzyme with variations in isomerization time from 40 hours to 72 hours, fructose levels were obtained of 16.52% to 19.07% with a difference in the percentage interval of fructose levels of 2.55%. Meanwhile, under the conditions of using 450 mg of the glucose isomerase enzyme with variations in isomerization time from 40 hours to 72 hours, fructose levels were obtained of 18.34% to 26.05% with a difference in the percentage interval of fructose levels of 7.71%. Based on the results obtained, it is known that the use of the glucose isomerase enzyme weighing 450 mg can produce greater fructose than the use of the glucose isomerase enzyme weighing 250 mg. These results are similar to previous researchers who showed that the greater the enzyme added, the greater the fructose levels produced [15].

$$C_6H_{12}O_6 \text{ (glucose)} \rightarrow C_6H_{12}O_6 \text{ (fructose)} \ldots\ldots\ldots \quad (1)$$

The use of the glucose isomerase enzyme as a biocatalyst in this process. So that the use of more enzymes can effectively increase the levels of fructose produced. This is also because the enzymes still get enough nutrition to process glucose into fructose. The performance of the glucose isomerase enzyme is still optimal and has not yet reached the death or inactive phase [25].

Based on the results of the research that has been carried out, it is known that the longer the isomerization time takes, the higher the fructose content produced and it starts to remain constant at the isomerization time of 64 hours. In this study, the isomerization time parameters were varied in the range of 40 to 72 hours. In Table 1, it can be seen that the longer the isomerization time, such as when the glucose isomerase enzyme weight 450 mg, the lowest percentage is at an isomerization time of 40 hours, around 18.34%. The increase in the percentage of fructose content will continue to increase as the isomerization takes longer until it reaches 26.05% at an isomerization time of 72

hours. These results are similar to previous researchers who showed that the conversion of glucose to fructose increased with the length of isomerization time [17].

Table 1. Yield of Fructose

Time (hour)	% Yield of Fructose				
	Glucose Isomerase Enzyme Weight (mg)				
	250	300	350	400	450
40	16.52	17.09	17.62	18.19	18.34
44	17.27	18.89	20.73	21.08	21.47
48	18.06	19.91	22.04	22.50	22.67
52	18.42	20.06	22.84	23.47	23.76
56	18.70	21.18	23.09	24.15	24.36
64	19.00	22.15	24.56	25.72	25.94
72	19.07	22.21	24.71	25.84	26.05

From the research results, the best fructose level was 26.05%. Isomerization is a process to convert glucose from cassava peel sugar hydrolyzate into fructose syrup. This reaction is a reversible reaction so that the resulting yield may experience a decreasing trend depending on operational conditions such as temperature, time, enzyme use, and catalyst used[26]. Furthermore, it is necessary to vary the isomerization time and weight of the glucose isomerase enzyme over a longer range to determine the most optimal conditions for this process.

Based on the results of similar research, the use of enzymes as biocatalysts to convert glucose into fructose is also not very effective. The results of the review stated that the use of enzymes without a catalyst in the isomerization process only obtained 42% of the fructose content produced[27]. The performance of chemical catalysts is actually lower than enzymatic conversion, namely 30-40% on average[28].

Optimization was carried out to determine the optimal results of fructose formation using the response surface method in Minitab 19 software. This optimization was based on the basic theory of Yield Optimization. The equation obtained from optimization is as follows:

H_2O_2 has the property of very easily evaporating into oxygen and water.

$$\% \text{ Yield} = -13.86 + 1.096\ X_1 - 0.01396\ X_2 - 0.01065\ X_1^2 + 0.000708\ X_1X_2 \quad \text{.....(2)}$$

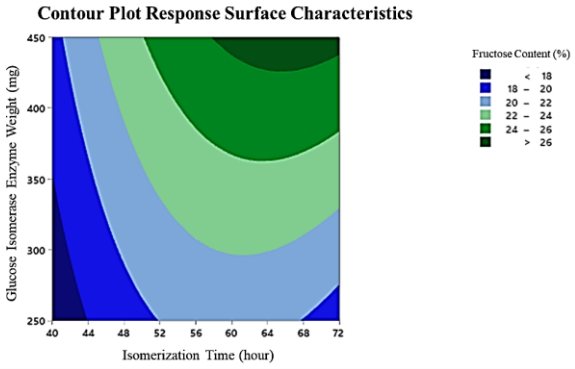


Figure 2. Contour Plot Response Surface Characteristics

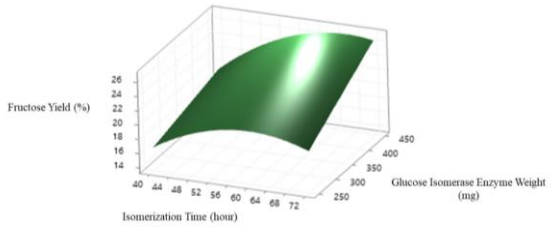


Figure 3. Optimization Result Graph

Where X1 is the isomerization time and X2 is the enzyme weight. The response plan in Minitab determines the fixed variable (Y) in the form of the percentage of fructose content formed, while the independent variable consists of isomerization time as factor 1 (X1), and the weight of the glucose isomerase enzyme as factor 2 (X2). Based on data analysis from Minitab 19 software, a regression equation was obtained which describes the effect of isomerization time and the weight of the glucose isomerase enzyme used on the percentage of fructose content produced. The optimization results from Minitab 19 show that the entire data is a second order polynomial equation or quadratic linear model. The optimization results will show the response equation function to changed conditions such as isomerization time and weight of the glucose isomerase enzyme. This research obtained a contour plot that illustrates the results of optimizing the percentage of fructose content resulting from the isomerization process. The contour plot graphic can be seen in Figure 2.

The contour plot image shows the differences in the percentage of fructose levels formed with different combinations of isomerization time and weight of the glucose isomerase enzyme. The variables of isomerization time and weight of the glucose isomerase enzyme obtained were used as independent variables in this study. The darker the blue colour on the contour plot graph, the lower the fructose levels formed, while the darker the green colour on the contour plot graph, the higher the fructose levels formed.

Response Optimization : Fructose Yield (%)

Parameters

Response	Goal	Lower	Target	Upper	Weight	Importance
Fructose Yield (%)	Maximum	16,52	26,47		1	1

Solution

Solution	Isomerization Time (hour)	Glucose Isomerase Weight (mg)	Fructose Yield (%) Fit	Composite Desirability
1	66,5051	450	26,8060	1

Multiple Response Prediction

Variable	Setting
Isomerization Time (hour)	66,5051
Glucose Isomerase Weight (mg)	450

Response	Fit	SE Fit	95% CI	95% PI
Fructose Yield (%)	26,81	0,00	(26,81; 26,81)	(26,81; 26,81)

Figure 4. Response Optimization

After applying RSM to the Minitab 19 program, treatment results can be optimized by analyzing contour plots and surface plots. This optimization process involves adjusting certain parameters to obtain the desired results. Optimization settings are configured to produce the percentage of fructose levels achieved under the most favorable conditions [29].

The parameters in the response results include the term lower for the lowest result and target for the highest result. The solution for optimization results using the Surface Response Method has optimum results at an isomerization time of 66.5 hours and a glucose isomerase enzyme weight of 450 mg with a resulting fructose content of 26.8% and a D (Desirability) value of 1. Value desirability which is close to the value of 1 is the most desired value which can be shown by the model because it shows the accuracy of the optimization, this shows the program's ability to produce the desired product which is close to perfect [23].

Based on Figure 4, the graph shows the optimization results. The graph shows the maximum optimum results. The highest point in the image above shows the highest point. Where if the red line is shifted to follow the shape of the curve it will show the results for each condition. From this figure it can be concluded that the optimum value for the percentage of extracted nickel occurs at an isomerization time of 66.5 hours using 450 mg of the glucose isomerase enzyme. These results also show that the resulting D (desirability) value is 1. The optimum conditions obtained can be used as a reference [30] for producing fructose sugar from cassava peel using the isomerization process

Conclusion

Production of fructose liquid sugar from cassava peel waste using the isomerization process produces the best fructose content under the isomerization time of 72 hours and the weight of the glucose isomerase enzyme is 450 mg with a yield of 26.05%. As for the optimization results using Response Surface Methodology (RSM), optimum results were obtained at an isomerization time of 66.5 hours and a glucose isomerase enzyme weight of 450 mg with a yield

of 26.8%. Variations in the use of isomerization time and weight of the glucose isomerase enzyme showed increasing results as these variables increased. Moreover, research needs to be developed with variations in longer isomerization times and the use of more glucose isomerase enzymes in order to get the highest peak point or best results that can be further improved.

Acknowledgement

The author expresses his gratitude to the University of Pembangunan Nasional "Veteran" Jawa Timur which has supported and facilitated this research.

Author Contributions

Concept, R.M.D; D.C.H; Methodology, R.M.D; D.C.H; Validation; F.N, I.U; Analysis, I.U; Review and Editing, R.M.D, I.U, D.C.M

Conflict of Interest

The authors declare no conflict of interest

References

1. M. F. Albar, I. Kusnodin, M. T. Lubis, and M. Jamhari, "Uji Organoleptik Yogurt Kulit Singkong (Manihot utilissima L .) sebagai Upaya Diversifikasi Pangan," in *Prosiding Seminar Nasional Biologi, Saintek, dan Pembelajarannya*, 2021, pp. 58–69.
2. Z. R. E. Ntelok, "Limbah Kulit Singkong (Manihot Esculenta L.): Alternatif Olahan Makanan Sehat," *J. Inov. Pendidik. Dasar*, vol. 01, no. 10, pp. 115–121, 2017.
3. A. S. Nurhaliza, D. Alfiah, T. F. Rahman, T. A. S. Putri, F. I. Maulana, and R. P. Sihombing, "Pengaruh Variasi Konsentrasi Kaolin Clay Terhadap Daya Serap Air Pada Bioplastik Dengan Penambahan Pvoh Bp-05," in *Prosiding SNAST*, 2022, no. November, pp. D114-125.
4. E. Z. Nasution and S. M. Ginting, "Production of Glucose Syrup Derived from Cassava Peel Starch Using Amylase Enzyme from Green Bean Seed Sprout Extract (Phaseolus radiatus L .)," vol. 6, no. 1, pp. 72–79, 2024.
5. Y. Jiang, X. Lyu, H. Chen, X. Wei, Z. Zhang, and X. Lu, "Catalytic Conversion of High Fructose Corn Syrup to Methyl Lactate with CoO@silicalite-1," *Catalysts*, vol. 12, no. 4. 2022.
6. H. Huang, X. G. Meng, W. W. Yu, L. Y. Chen, and Y. Y. Wu, "High Selective Isomerization of Glucose to Fructose Catalyzed by Amidoximed Polyacrylonitrile," *ACS Omega*, vol. 6, no. 30, pp. 19860–19866, 2021.
7. [7] S. Risnoyatiningsih, "Hydrolysis of starch saccharides from sweet potatoes using enzyme," *J. Tek. Kim.*, vol. 5, no. 2, pp. 417–424, 2011.
8. Z. Nst, E. B. Ginting, D. G. Romatua, and F. Fahdi, "Identifikasi Kadar Glukosa Dan Sukrosa Pada Madu Hutan," *J. Penelit. Farm. Herb.*, vol. 1, no. 2, pp. 5–10, 2019.
9. B. Merino, C. M. Fernandez-Diaz, I.-C. Cozar, and G. Perdomo, "Intestinal Fructose and Glucose Metabolism in Health and Disease," *Nutrients*, vol. 12, no. 1, p. 94, 2020.
10. E. Rohman, R. Tiyana, S. A. N. W. Al Falah, and M. N. Handayani, "Method of Sugar Production From Arrowroot Starch: A Review," 2021, vol. 520, no. Tvet 2020, pp. 143–147.
11. H. T. P., A. Z. A., and M. Nugroho, "Pengaruh Pre Gelatinisasi Terhadap Karakteristik Tepung Singkong," *Teknol. Pangan Media Inf. dan Komun. Ilm. Teknol. Pertan.*, vol. 1, no. 1, pp. 1–15, 2011.
12. A. Y. Rahmawati and A. Sutrisno, "Hidrolisis Tepung Ubi Jalar Ungu (Ipomea batatas L.) Secara Enzimatis Menjadi Sirup Glukosa Fungsional: Kajian Pustaka," *J. Pangan dan Agroindustri*, vol. 3, no. 3, pp. 1152–1159, 2015.
13. A. L. Salsabila and I. Fahruroji, "Hidrolisis Pada Sintesis Gula Berbasis Pati Jagung," *Edufortech*, vol. 6, no. 1, pp. 32–38, 2021.
14. T. H. Richardson *et al.*, "A Novel, High Performance Enzyme for Starch Liquefaction," *J. Biol. Chem.*, vol. 277, no. 29, pp. 26501–26507, 2002.
15. Mahreni and E. Sulistyowati, "Pembuatan 'High Fructose Syrup' Dari Tepung Maizena Secara Enzimatis (The Making Of High Fructose Syrup From Cornmeal Flour Through Enzymization)," in *Prosiding SNTPK*, 2004, pp. 7–9.
16. K. H. Nam, "Glucose Isomerase: Functions, Structures, and Applications," *Appl. Sci.*, vol. 12, no. 1, pp. 1–12, 2022.
17. D. Steinbach, A. Klier, A. Kruse, J. Sauer, S. Wild, and M. Zanker, "Isomerization of glucose to fructose in hydrolyzates from lignocellulosic biomass using hydrotalcite," *Processes*, vol. 7, pp. 1–15, 2020.
18. R. Johnson, G. Padmaja, and S. N. Moorthy, "Comparative Production of Glucose and High Fructose Syrup from Cassava and Sweet Potato Roots by Direct Conversion Techniques," *Innov. Food Sci. Emerg. Technol.*, vol. 10, no. 4, pp. 616–620, 2009.
19. F. Yulistiani, Saripudin, L. Maulani, W. S.

Ramdhayani, W. Wibisono, and A. R. Permanasari, "Fructose Syrup Production from Tapioca Solid Waste (Onggok) by Using Enzymatic Hydrolysis in Various pH and Isomerization Process," *J. Phys. Conf. Ser.*, vol. 1295, no. 1, pp. 1–8, 2019.
20. R. S. Budiarti, Harlis, and H. kapli, "Optimasi Pembentukan Gula Cair Dari Limbah Kulit Singkong (Manihot utilisima Pohl) Oleh Bacillus Licheniformis dalam Usaha Menumbuhkan Jiwa Kewirausahaan.," *Biospecies*, vol. 11, no. 2, pp. 108–114, 2018.
21. K. Keryanti, A. R. Permanasari, R. N. Hidayah, and R. Hasanah, "Penentuan pH dan Suhu Optimum Isomerisasi Pembuatan Sirup Fruktosa dari Hidrolisat Onggok Menggunakan Katalis Mg/Al," *CHEESA Chem. Eng. Res. Artic.*, vol. 5, no. 1, p. 1, 2022.
22. A. Becze, V. L. Babalau-Fuss, C. Varaticeanu, and C. Roman, "Optimization of High-Pressure Extraction Process of Antioxidant Compounds from Feteasca regala Leaves Using Response Surface Methodology," *Molecules*, vol. 25, no. 18, pp. 1–14, 2020.
23. D. C. Montgomery, *Design and Analysis of Experiments*. Arizona: John Wiley & Sons, Inc., 2013.
24. H. Manurung and I. Azhar, "Lignin utilization from black liquor as raw material lignin resorsinol formaldehyde adhesives (LRF)," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 1115, p. 12070, Dec. 2022.
25. Mahyati and O. S. Pasanda, "Produksi Fruktosa dari Tongkol Jagung sebagai Gula Rendah Kalori," *J. INTEK*, vol. 3, no. 2, pp. 109–111, 2016.
26. A. R. Permanasari, A. Fauzan, N. L. Rachmalia, R. Elfanti, and W. Wibisono, "Fructose syrup production from Onggok with isomerization process by Mg/Al hydrotalcite catalyst and glucose isomerase enzyme," *J. Phys. Conf. Ser.*, vol. 1450, no. 1, pp. 1–9, 2020.
27. X. Geng *et al.*, "Enhanced High-Fructose Corn Syrup Production: Immobilizing Serratia marcescens Glucose Isomerase on MOF (Co)-525 Reduces Co2+ Dependency in Glucose Isomerization to Fructose," *Foods*, vol. 13, no. 4, pp. 1–13, 2024.
28. S. Kumar, S. Sharma, S. K. Kansal, and S. Elumalai, "Efficient Conversion of Glucose into Fructose via Extraction-Assisted Isomerization Catalyzed by Endogenous Polyamine Spermine in the Aqueous Phase," *ACS Omega*, vol. 5, no. 5, pp. 2406–2418, 2020.
29. J. Manuel Pais-Chanfrau, J. Núñez-Pérez, R. del Carmen Espin-Valladares, M. Vinicio Lara-Fiallos, and L. Enrique Trujillo-Toledo, "Uses of the Response Surface Methodology for the Optimization of Agro-Industrial Processes," in *Response Surface Methodology in Engineering Science*, P. Kayaroganam, Ed. Rijeka: IntechOpen, 2021, p. Ch. 9.
30. A. H. Mulyadi and R. Adiningsih, "Optimasi Ekstraksi Minyak Depak Padi dengan Pelarut Etil Asetat Menggunakan Response Surface Methodology (RSM)," *TECHNO*, vol. 22, no. 1, pp. 69–76, 2021.