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Effect of Type of Yeast and Yeast Concentration to The Level Ethanol in Bioethanol Production with Molasses Material

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ABSTRACT

Molasses is a byproduct of sugar manufacturing process and a sugar factory waste that can not be crystallized again with sucrose content of 48-56%. Molasses is one of the many ingredients that can be used as raw material for ethanol production. Ethanol is a volatile liquid, clear, colorless, odorless typical that can be obtained from fermentation with a substrate that contain lots of carbohydrates (sugar, starch or cellulose). The aims of this research was to investigate the effect of yeast type and yeast concentration between bread yeast and tape yeast to ethanol content obtained from the fermentation of molasses. The technique of data collected was conducted, namely fermentation which carried out during the 72 hours with bread yeast and tape yeast. It was followed by a distillation process. The result of the distillation was measured by gas chromatography hewlett paccard 5890 series II and mass spectroscopy Shimadzu QP-2010S. The data obtained were analyzed statistically by using t test and anova test with a level of 95%. The ethanol content with bread yeast and tape yeast with concentration 0,1 % (v/v); 0,2% (v/v); dan 0,5%(v/v respectively was adalah 0,40 %; 1,16 %; 2,56 % dan 0,85 %; 1,84 %; 3,66 %. Results of the test "t" showed no significant differences between bread yeast and tape yeast. In the anova test showed there are difference between the concentration of yeast 0,1%; 0,2%; and 0,5% which indicated the probability value 0,00 (<0,05).

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KEYWORDS: fermented molasses, ethanol content, bread yeast, tape yeast

INTRODUCTION

Indonesia is an agrarian country that annually produces abundant natural wealth in the form of agricultural products. These agricultural products are widely used for agricultural industry activities. Every year the agricultural industry produces a large amount of waste. In the sugar or cane sugar industry, in addition to producing granulated sugar, it also produces molasses. Molasses is a type of syrup that is a by-product produced during the bleaching process of sugars that can no longer be crystallized. Molasses contains a lot of organic acids and large amounts of sugars, both sucrose and reducing sugars. The total sugar content ranges from 48 - 56% with a pH of around 5.5 - 5.6, so it is an excellent raw material for the manufacture of bioethanol. .(Irvan, P. 2015: Trisakti B, 2015: Yuana, 2018)

Bioethanol is a liquid resulting from the fermentation process of sugar from carbohydrate sources (starch) using the help of microorganisms. The production of bioethanol from plants containing starch or carbohydrates is carried out through the process of converting carbohydrates into sugars (glucose) by several methods including acid hydrolysis and enzymatic. (Baharuddin, M, 2016; Banerjee, R. 2017; Ni'mah, L, 2015; Zabed, H, 2017)

In the ethanol fermentation process, the concentration of the level and type of yeast used can affect the ethanol levels produced. Therefore, it is necessary to conduct research to determine the influence of yeast concentration and the type of yeast used, in this case the comparison between baker's yeast and tape yeast on ethanol levels in the production of bioethanol and for efforts to increase the economic value of molasses. .(Andari, 2015; Malle, D, 2014; Ni'mah, L, 2015)

RESEARCH METHODS

1. Equipment and Materials

The equipment used in this study includes: glass tools in general (*pyrex*) such as a set of distillations, measuring cups, glass bags, measuring flasks and erlenmeyer. Picnometers, *waterbaths*, gas stoves, thermometers, digital pH meters, vials, analytical scales, gram scales, stirring rods, watch glasses, gram and milligram weights, static-clamps, hoses and a set of Hewlett Pacard 5890 series II gas chromatography tools. The material used is class 3 molasses (*blackstrap)* obtained from the Gondang Winangun Klaten sugar factory, Central Java.

2. Work Procedure

2.1 Preparation of ethanol from molasses

33.3 mL of molasses was taken, and diluted with 100 mL of aquadest in a glass freezer (133 mL of a mixture of molasses and water). Then stirred until evenly distributed, after which the pH was measured at 4.0 – 5.0. The temperature is increased through heating until the temperature is 28-32^{0}C above the *waterbath*. After that, it is transferred into a bottle, then mixed with yeast at a concentration of 0.1% (v/v), 0.2% (v/v) and 0.5% (v/v) in the form of a solution in aquadest of 50.0 mL, stirred until homogeneous. Tightly covered with plastic and fermented for 72 hours at room temperature. It is then distilled until ethanol is produced. (Baharuddin, M, 2016;Banerjee, R, 2015; Guerro, A, 2018;Irvan, P, 2015;Trisakti, B, 2015)

2.2 Analytical Methods

Preparation of Standard Solution 1 %

The concentrated n-butanol solution was taken with a measuring pipette of 1.24 mL to make a 10% n-butanol parent solution, then put into a 10mL measuring flask, added aquadest to the limit mark and shaken until homogeneous. A 10% n-butanol solution is taken as much as 1mL, then an aquadest is added until the mark is marked and cornered until homogeneous.

Calibration Curve Manufacturing / Standard Ethanol

Ethanol raw solution is made with a concentration of 0.5; 1; 2; 4 and 6% V/V are respectively inserted into a 10 mL measuring flask and 1 mL of 1 % n-butanol solution is added. The solution is then shaken until homogeneous.

Sample Solution Preparation

The distillate is put into a measuring flask of 10 mL until the mark is marked, then 1 mL of 1% n-butanol is added. The solution is cornered until homogeneous. The sample solution was then taken as much as 2 mL and placed in a GC sample vial to be tested for ethanol content.

Penentuan alkohol dalam sampel dengan metode GC,

The sample filtrate was maintained at 20±0.1 °C. Dissolve 2 mL of sample with 20 mL of internal standard n-butanol. Inject 0.5-1 µL of each standard ethanol solution into the GC. The same treatment was also carried out on the sample solution in n-butanol. The peak areas of the internal standard ethanol and sample were calculated. Calculation, plot the graph of the peak area of ethanol peak area of internal standard against the concentration of ethanol % V/V (after correcting for purity) (Aziz, Y.S. 2019; Coning, P.D, 2019; Dhony, 2020; Hasan, M.M, 2019; Honig,V, 2015; Muchtaridi,M.I, 2012; Paramita A, 2022; Widya, A.,2018;Yurika, 2022)

RESULTS AND DISCUSSION

1. Measurement of ethanol content using gas chromatography

Ethanol analysis on fermented samples was carried out in 2 stages, namely, the separation stage and the quantitative level of measurement with gas chromatography to measure ethanol levels

Ethanol as a food and beverage product is produced from the fermentation process, namely from the process of carbohydrate fermentation that is enzymatically lyated. One type of enzyme converts carbohydrates into glucose then into ethanol, the other type of enzyme produces vinegar (acetic acid), with ethanol as an intermediary. Fermentation is carried out with the help of certain yeast species such as Saccharomyces Cerevisiae. This yeast metabolizes sugar (glucose) in the absence of oxygen to produce ethanol and CO2,. (Aziz, Y.S. 2019; Coning, P.D, 2019; Dhony, 2020; Hasan, M.M, 2019; Honig,V, 2015; Muchtaridi,M.I, 2012; Paramita A, 2022; Widya, A.,2018;Yurika, 2022)

Determination of ethanol levels using the GC method can be done because ethanol is a volatile compound. The process is carried out by means of an ethanol solution being injected using a micro syringe into the GC separator column. At the injection site, the ethanol solution immediately evaporates and enters the column along with the inert carrier gas. In the GC column, the separation of gas components occurs according to the boiling point and polarity of each compound. The gas components that have been separated then exit the column and enter the detector, then the detected signal is recorded on the recorder or integrator. The ethanol content can be determined by calculating the peak area of the ethanol chromatogram recorded on the recorder**.** (Aziz, Y.S. 2019; Coning, P.D, 2019; Dhony, 2020; Hasan, M.M, 2019; Honig,V, 2015; Malle, D. 2014; Mc.Nair, H.M, 2011;Muchtaridi,M.I, 2012; Paramita A, 2022; Widya, A.,2018;Yurika, 2022)

Tabel 1. Results of ethanol content measurement by Gas Chromatography (GC)

Replication	Baker's yeast			Tapai Yeast		
	Baker's yeast concentration			Concentration of tape yeast		
	0,1 %	0,2 %	0,5 %	0,1 %	0,2 %	0.5 %
1	0,44 %	1,14 %	2,49 %	0,91 %	1,96 %	3,11 %
2	0,38 %	1,17 %	2,54 %	0,81 %	1.83%	3,26 %
3	0,37 %	1,16 %	2,66 %	0,82 %	2,02 %	4,60 %
4	0,40 %	1.15 %	2.53 %	0.83 %	1,55 %	3.55 %
5	0.41 %	1.17 %	2.58 %	0.87 %	1.85%	3.78 %
X	0,40 %	1,16 %	2,56 %	0,85 %	1,84 %	3,66 %

2. Ethanol content measurement by gas chromatography (GC) Hewlett Paccard 5890 series II

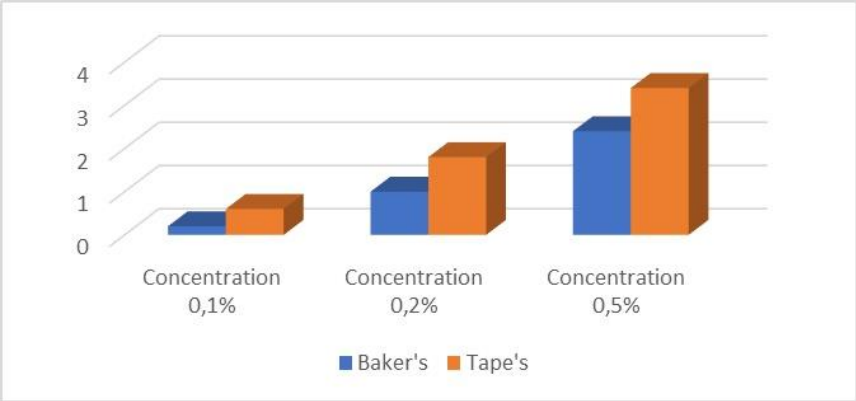


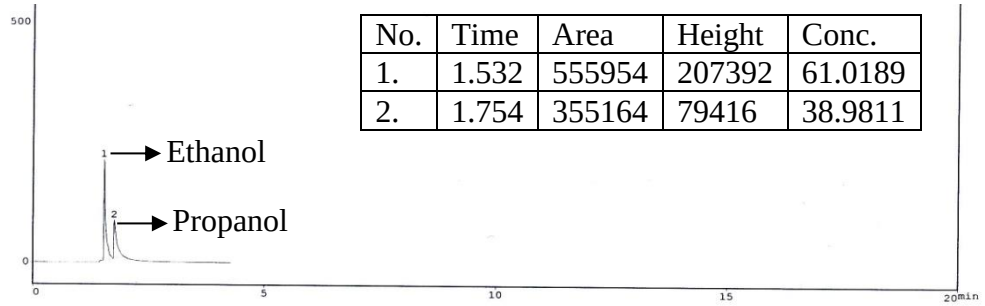
Figure 1. Graph of ethanol levels of baker's yeast and tape's yeast

The ethanol levels of baker's yeast and tape's yeast with concentrations of 0.1%; 0.2%; and 0.5% respectively were 0.40%; 1.16%; 2.56% and 0.85%; 1.84%; 3.66%. A higher number of initial cells will accelerate the adaptation phase. The amount of concentration of 0.5 % is higher when compared to the concentration of 0.1 % and 0.2 %. This larger amount of yeast makes the ability of yeast to produce enzymes and break down sucrose into glucose even greater so that the number of enzymes produced is also more. With more glucose formed, the ethanol levels produced are greater. So that in the fermentation time of 72 hours, the yeast concentration of 0.5% has the highest ethanol content when compared to the concentration of 0.1% and 0.2%.. (Dirayati, A.G, 2020)(Yuana, S.E.N, 2018)(Zabed, H.S, 2017)

In addition, yeast has different microbial content. The main microbe in baker's yeast is *Saccharomyces cerevisae*.. Yeast for tapai is a mixed population of genera where there are species of the genus *Aspergillus*, genus *Saccharomyces*, genus *Candida*, genus *Hansenula*. The genus lives together synergistically. *Aspergillus* can simplify amylum, while *Saccharomyces*, *Candida and Hansenula* can decompose sugars into ethanol. Due to the synergistic work of microbial cultures contained in tape yeast, the number of enzymes produced to convert glucose increases when compared to baker's yeast, causing the ethanol levels produced to be different when compared to baker's yeast. (Hasanah, H, 2012;Muchtaridi, M.I, 2012, Mohammed, A.D.H, 2018; Trisakti, 2015; Zabed, H, 2017)

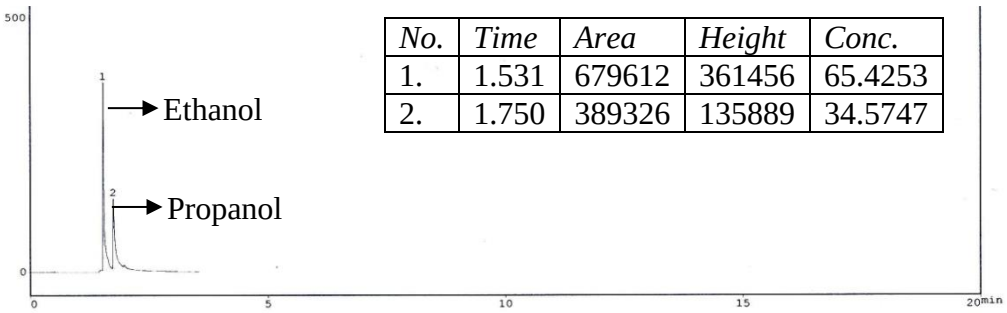
Polysaccharides will be broken down into simpler carbohydrates. The well-known process of glycolysis or breakdown of glucose is the Embden-Meyerhoff Parnas (EMP) pathway. This glucose breakdown pathway consists of several stages and in principle this process is the breakdown of glucose into pyruvic acid. This path will be continued at a later stage if the expected result is ethanol. The reaction of converting pyruvic acid to ethanol starts from the conversion of pyruvic acid to acetaldehyde through the process of decarboxylase. The decarboxylase process is the loss of one carboxyl group into acetaldehyde and then reduced to alcohol.(Baharuddin,M. 2016;Dirayati,A.G, 2017)

The chromatogram results based on ethanol measurements with gas chromatography can be seen in figure 2 (baker's yeast) and figure 3 (tape yeast).



No.	Time	Area	Height	Conc.
1.	1.532	555954	207392	61.0189
2.	1.754	355164	79416	38.9811

Figure 2. Chromatogram of gas chromatography measurements (Bread Yeast)



No.	*Time*	*Area*	*Height*	*Conc.*
1.	1.531	679612	361456	65.4253
2.	1.750	389326	135889	34.5747

Figure 3. Chromatogram of gas chromatography measurement (Yeast Tape)

The results of the gas chromatography measurements above show the *peak* and area counts of the ethanol and propanol samples. The peak is the result of a qualitative analysis of gas chromatography, this analysis provides an overview of what compounds are detected by gas chromatography. Meanwhile, area *counts* are a quantitative analysis indicated by measuring ethanol levels.

The retention time of the measurement results indicates the polarity of a compound. The polarity properties of ethanol and propanol are different, ethanol is more polar while propanol is non-polar. Propanol is an internal standard solution, that is, a standard solution added to an ethanol sample. Its function is to control injection errors in measurements by gas chromatography. The stationary phase used in measurements with non-polar gas chromatography is found in the column of the gas chromatography tool, the detector used is the FID (*Flame Ionization Detector*) type. The more polar the compound enters the column, the faster the compound will flow into the detector because the compound will not be retained in the column for long, this is based on the working system of a gas chromatography device that undergoes a reverse phase. So the first peak shows ethanol compounds while the second peak shows propanol compounds.

3. Determination of the molecular structure of organic compounds and the molecular weight of compounds.

The mass spectrum of baker's yeast ethanol is presented in figure 4 and for the mass spectrum of tapai yeast ethanol is presented in figure 5 and the mass spectrum of ethanol compounds is presented in figure 6.

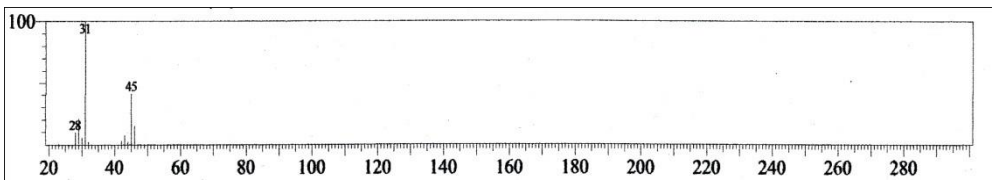


Figure 4. Mass spectrum of baker's yeast fermentation compounds

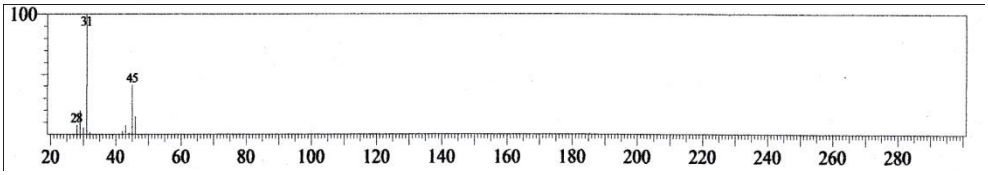


Figure 5. Mass spectrum of compounds from fermentation of tapai yeast

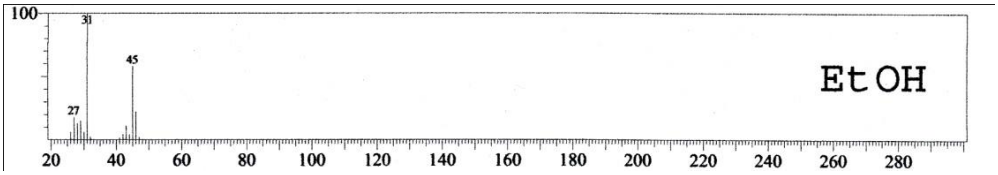


Figure 6. Mass spectrum of ethanol compounds

Above is a picture of three spectrums, the spectrum of compounds resulting from the fermentation of baker's yeast, tape yeast, and the spectrum of ethanol compounds. The spectrum of fermented compounds shows the same fragmentation results as the spectrum of ethanol compounds. The spectrum of compounds from the fermentation of baker's yeast and tape yeast above shows that the compounds are ethanol. The spectrum of fermentation results of tape yeast appeared at a retention time of 2.161 minutes and baker's yeast appeared at a retention time of 2.162 minutes. Ethanol compounds bombarded with high-energy electrons result in the release of one electron of the ethanol compound, by releasing one radical hydrogen atom resulting in fragments with m/z 45. Another fragment formed with a value of m/z 31 obtained from ethanol releases methyl radical compounds. Another fragmentation with a value of m/z 29, ethanol compounds release hydroxyl radicals. The ethanol fragmentation pattern is presented in figure 7.

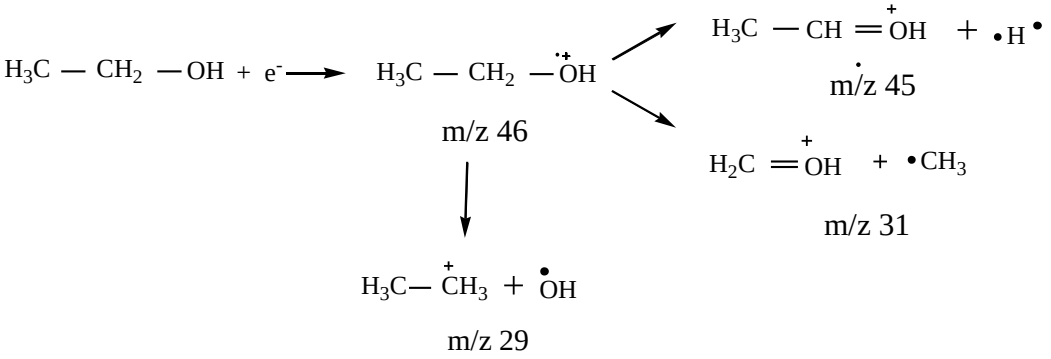


Figure 7. Ethanol fragmentation patterns

CONCLUSION

1. The type of yeast has no effect on ethanol levels
2. Yeast concentration affects ethanol levels from molasses fermentation
3. Molasses can be fermented into ethanol by baker's yeast and tape yeast. Ethanol content in baker's yeast concentration 0.1 %; 0,2 %; and 0.5% respectively is 0.40%; 1,16 %; and 2.56 percent. The ethanol content in fermented yeast concentration is 0.1 %; 0,2 %; and 0.5% respectively is 0.85%; 1,84 %; and 3.66 percent.

REFERENCES

1. Andari, Y., Mulyadi, A. H., & Puspawiningtyas, E. (2015). Pengaruh konsentrasi ragi dan waktu fermentasi pada proses pembuatan bioetanol dari air kelapa. In Seminar Nasional Teknologi 2015 (pp. 56–61). Purwokerto: Fakultas Teknik Universitas Muhammadiyah Purwokerto.
2. Anghelescu, G., N. Mirescu, A. Tănăsescu and F. Grama. 2017. Quantitative determination of ethyl alcohol in blood, by gas chromatography. In ECAI 2017-International Conference on Electronics, Computers and Artificial Intelligence (ECAI) – 9th Edition. Turkey. (pp. 1–5).
3. Aziz, YS, Hariyani, D, 2019, Penetapan Kadar Etanol Pada Arak Jowo Yang Beredar Di Wilayah Ponorogo Pada Bulan Januari–Maret 2019 Dengan Metode Kromatografi Gas, Jurnal MEDFARM: Farmasi dan Kesehatan, Vol. 8, No.1, hal 15-20
4. Baharuddin, M., Sappewali, S., Karisma, K., & Fitriyani, J. (2016). Produksi bioetanol dari jerami padi (Oryza sativa L) dan kulit pohon dao (Dracontamelon) melalui proses sakarifikasi dan fermentasi serentak (SFS). Chimica et Natura Acta, 4(1), 1. https://doi.org/10.24198/cna.v4.n1.10441
5. Bai, L., Smuts, J., Schenk, J., Cochran, J., & Schug, K. A. (2018). Comparison of GC-VUV, GCFID, and comprehensive two-dimensional GC-MS for the characterization of weathered and unweathered diesel fuels. FUEL, 214, 521-527.
6. Banerjee, R., Chintagunta, A. D., & Ray, S. (2017). A cleaner and eco-friendly bioprocess for enhancing reducing sugar production from pineapple leaf waste. Journal of Cleaner Production, 149, 387–395. https://doi.org/10.1016/j.jclepro.2017.02.088

7. Breme, K., & Guggenbühl, B. (2014). Aroma profile of a red-berries yoghurt drink by HS-SPMEGC-MS-O and influence of matrix texture on volatile aroma compound release of flavored dairy products. Flavour science. Academic Press.
8. Coning, P. D., & Swinley, J. (2019). A practical guide to gas analysis by gas chromatography. Elsevier Science Ltd.
9. Dhony H, Ayu, I.G.S.A, Hioar, R, Shofiyana, L.M, Ismilayli, N. 2020, Penentuan Kandungan Etanol dalam Makanan dan Minuman Fermentasi Tradisional Menggunakan Metode Kromatografi Gas, Chempublish Journal Vol. 5 No. 2, 105-115
10. Dirayati, A.G., dan Erlidawati, 2017, "Pengaruh Jenis Singkong Dan Ragi Terhadap Kadar Etanol Tape Singkong". Jurnal IPA dan Pembelajaran IPA (JIPI), 1(1): p. 26-33.
11. Guerrero, A. B., Ballesteros, I., & Ballesteros, M. (2018). The potential of agricultural banana waste for bioethanol production. Fuel, 213, 176–185. https://doi.org/10.1016/j.fuel.2017.10.105
12. Hasan, M. M., Mahmud, M. R. & Islam, M. G. (2019). GC-MS analysis of bio-active compounds in ethanol extract of Putranjiva roxburghii Wall. fruit peel. Pharmacogn Journal, 11(1), 146-149.
13. Hasanah, H., Jannah,Akyunul dan A. Ghanaim Fasya, 2012, "Pengaruh Lama Fermentasi Terhadap Kadar Alkohol Tape Sing (Manihot utilissima Pohl)". ALCHEMY, 2(1): p. 68-79
14. Hönig, V., J. Táborský, M. Orsák and R. Ilves. 2015. Using gas chromatography to determine the amount of alcohols in diesel fuels. Agronomy Research, 13(5):1234–1240.
15. Irvan, Prawati, P., & Trisakti, B. (2015). Pembuatan bioetanol dari tepung ampas tebu melalui proses hidrolisis termal dan fermentasi: Pengaruh pH, jenis ragi, dan waktu fermentasi. Jurnal Teknik Kimia USU, 4(2), 27–31.
16. Malle, D., Kapelle, I. B. D., & Lopulalan, F. (2014). Pembuatan bioetanol dari limbah air kelapa melalui proses fermentasi. Indonesian Journal of Chemical Research, 2(1), 155–159.
17. McNair, H. M., & Miller, J. M. (2011). Basic gas chromatography (2nd ed.). John Wiley & Sons.
18. Mohammed, A. H., A. K. Mohammed, F. H. Kamar, A. A. Abbas and G. Nechifor. 2018. Determination of ethanol in fermented broth by headspace gas chromatography using capillary column. Revista de Chimie-Bucharest, 69(11):2016–2019
19. Muchtaridi, M., I. Musfiroh, N. N. Hambali and W. Indrayati. 2012. determination of alcohol contents of fermentated black tape ketan based on different fermentation time using specific gravity , refractive index and GC-MS method. Journal of Microbiology, Biotechnology and Food Sciences, 2(3):933–946.
20. Ni'mah, L., Ardiyanto, A., & Zainuddin, M. (2015). Pembuatan bioetanol dari limbah serat kelapa sawit melalui proses pretreatment, hidrolisis asam dan fermentasi menggunakan ragi tape. INFO-TEKNIK, 16(2), 227–242.
21. Paramita A, Suryani M,Ym Safitrim R.A, 2022, Analisa Kandungan Non Halal (Kadar Ethanol) Pada Peuyeum Menggunakan Gas Chromatography YL6500 Quantum Terhadap Metode Validasi , Proseding Seminar Nasional Unimus, Vol. 5, 925-934
22. Trisakti, B., Silitonga, Y. B. R., & Irvan. (2015). Pembuatan bioetanol dari tepung ampas tebu melalui proses hidrolisis termal dan fermentasi serta recycle vinasse (pengaruh konsentrasi tepung ampas tebu, suhu dan waktu hidrolisis). Jurnal Teknik Kimia USU, 4(3), 17–22.
23. Widya, A. 2018. Validasi Metode Dalam Penentuan Kadar Etanol Pada Arak Menggunakan Kromatografi Gas Detektor Ionisasi Nyala. Journal Of Chemistry. Vol.12, No.2
24. Yuana Susmiati, 2018, Prospek Produksi Bioetanol dari Limbah Pertanian dan Sampah Organik, Industria: Jurnal Teknologi dan Manajemen Agroindustri, Volume 7 Nomor 2 : 67-80
25. Yurika, S, Evelyn N, Sherlin A.F, Oki. K, 2022, Comparison of gas chromatography detectors and its application in food analysis, Journal of Food and Agricultural Product, Vol. 2, issue, 1, p. 23-36
26. Zabed, H., Sahu, J. N., Suely, A., Boyce, A. N., & Faruq, G. (2017). Bioethanol production from renewable sources: Current perspectives and technological progress. Renewable and Sustainable Energy Reviews, 71, 475–501. https://doi.org/10.1016/j.rser.2016.12.076