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# Corn Cobs Hemicelluloses Isolation Method Comparison and its Characterization with Infra Red Spectrophotometry (FTIR) and High Performance Liquid Chromatography (HPLC)

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**Abstract:** Hemicellulose is a component of Non-Starch Polysaccarides (NSP) contained in agricultural byproduct and can be separated from the other components. Various studies about hemicelluloses isolation procedures from various agricultural byproduct materials have been conducted including corn cobs. Objective of this research is to modify the isolation method and compare with other procedures of isolation method and characterization of corn cobs hemicellulose.

To get the optimum of hemicelluloses yield, the isolating corn cobs hemicellulose using three procedures that had been done by other researchers and the modified procedure that had been conducted by the researcher himself. Characterization of corn cobs hemicellulose was done by infra red spectrophotometry (FTIR), high performance liquid chromatography (HPLC) with column Shim - pack VP - ODS (4.6 x 250 mm) using a UV - Vis detector at a wavelength of 280 nm with an isocratic elution system in aquabides solvent and a flow rate of 0.8 mL/min.

The modified procedure provided the greatest yield of corn cobs hemicellulose which is 12.04% with characteristic FTIR region of 1820-1600 $cm^{-1}$ and vibration widened near 3800-3000 $cm^{-1}$. This means that the corn cobs hemicellulose having functional groups within the structure, that is the carbonyl group, hydroxyl and carboxylic groups. The results showed that the yield of corn cobs hemicellulose with characteristic HPLC chromatograms from all procedures of isolation had a retention time of 1.8 minutes.

In conclusion that the four isolation methods had the same structure and the same characteristic with FTIR dan HPLC.The modified method showed the highest yield compared to other methods and produced environmentally friendly waste.

**Keywords:** isolation method. comparison. corn cobs. hemicelluloses. FTIR and HPLC.

## Introduction and Experiment

A corn cob is a major part of the corn's waste. It is estimated that 40-50% of corn is corn cob. The magnitude of corn cob is affected by corn's varieties. Corn cobs hemicellulose containing is the highest (12.4%) compared to the content of other agricultural-products [1].

However, corn cobs have not been optimally utilized primarily for the purposes of the pharmaceutical industry. Content of a corn cob is 35.5% fiber, 2.5% protein, 0.12% calcium and 0.04% phosphorus. The corn cob's fiber contains of 30-40% cellulose, 20-36% hemicellulose, 16% lignin and 8% other materials. This high hemicellulose content of a corn cob potentially can be developed into industrial raw materials such as xylose and xylitol. Xylose is used in food industry and also can be synthesized into xylitol, ethanol and organic acids [1].

To increase of the economic value of hemicellulose, efforts should be made by isolating or separating it from other components. The hemicellulose can be utilized as a new alternative polymer for various applications especially in pharmaceutical preparations. Several methods of isolations have been developed in order to isolate hemicellulose based on solubility [1,2,3].

Several researchs had been performed for the separation of hemicellulose from different plant byproduct with different methods of isolation. Lignification process conducted among others by NaOH in 70% ethanol, chlorine, sodium hypochlorite and 30% $H_2O_2$. Isolation of hemicellulose used alkaline compounds such as NaOH or KOH with various concentrations, whereas hemicellulose is used for purification of HCl and 90% ethanol [1,2,3].

Isolation of hemicellulose process must conformity with the principles of green chemistry. The latter sets guidelines for the chemical industry. In order to secure sustainable development while increasing process economy. Briefly, green chemistry, green engineering and the related call for an increase of in, and/ or upgrading of:

- Process economy, by preventing waste generation. This represents a much superior approach to waste treatment;
- Atom economy, by incorporating all reagents employed in the final product. This also contributes to reduction and / or elimination of waste;
- Process safety, e.g. by using non-toxic, non-inflammable solvents and reagents;
- Process efficiency, e.g. by: material recycling into the process; where possible, the use of catalytic pathways; use of catalysts that can be regenerated/recycled; rational use of energy, and reduction of the number of intermediate steps [4].

Green chemistry also calls for design for biodegradable end products, principally, by employing chemicals from renewable sources, dictates the use of real-time and on-line analysis for better process control [4].

The aim of this research are to compare the 4 (four) isolation methods of corn cobs hemicelluloses and to be able to identify the method that must be assured to be environmentally safe and does not cause a negative impact on human health.

## Materials

Corn Cobs were obtained from local corns in Medan. Indonesia, sodium hydroxide (E Merck). 50% sodium hydro chlorite (E Merck), 37% hydrochloride acid (E.Merck), 35% hydrogen peroxide (E.Merck), 96% ethanol (E.Merck), acetic acid 98% (E.Merck), and all other chemicals used were of analytical grade.

### Preparation of the Corn Cobs

Preparation begins with drying the corn cobs, then cut them small with the size of 1 cm per side, crushed and sieved. This fine powder is used to identify the corn cobs, determination the Kappa Numbers for delignification and isolation of corn cobs hemicelluloses [4,5,6] .

### Isolation of Hemicelluloses from Corn Cobs

Isolation of corn cobs hemicelluloses is conducted by 4 (four) methods of isolation.

### Method I:

Fifty grams of corn cobs powder was added to 500 ml of 0.05 M HCl and then heated at the temperature of 70° C for 2 hours. The formed suspension was left to reach the room temperature, then added 6 ml of $NH_4OH$ (p) to pH of 9.2. The suspension was stirred 12 hours at room temperature and filtered through whatman filter paper in a vacuum to extract cellulose and starch. The residue was added with 500 ml of 0.025M NaOH in 70% ethanol and heated at 75°C and stirred for 2 hours to dissolve the lignin. The suspension was allowed to cool until reach the room temperature and filtered through whatman filter paper. The residue was added with 500 ml of 0.1 M NaOH. and stirred for 16 hours at room temperature to dissolve the hemicellulose. Then filtered with whatman GF filter paper in a vacuum. Filtrate was heated to 65°C, added with 35 ml of 30% $H_2O_2$ in stages into the filtrate and stirred constantly. When the filtrate had turned white, left cool to reach the room temperature. Then added 32 ml of concentrated HCl to pH 5.3, then add 95% ethanol for 3 times of the filtrate's volume. The mixture was left fot 12 hours to precipitate hemicellulose. The filtrate was carefully separated using a suction pump, and the precipitate formed is hemicellulose and dry with a vacuum dryer and weighed and called as the yield K [7].

**Method II:**

Fifty grams of corn cobs powder was suspended into a mixture of 0.1 M NaOH and 0.05 M Ca $(OH)_2$ solution and heated for 1 hour. Then the obtained residue was centrifuged and suspended in distilled water and heated for 5 minutes and centrifuged again. The supernatant was added with $H_2O_2$ (0.1 g/g corn cobs), the pH was adjusted with 0.1 N NaOH to 11.5. allowed to stand at room temperature for 2 hours, then the pH adjusted to 4.0-4.5 with 0.1 M HCl. and the the precipitate obtained was hemicellulose A. The supernatant was added with the ethanol 2:1. precipitate Hemicellulose B then both collected and dried in a vacuum oven at 50$^{\circ}$ C. The results yield of hemicellulose called the yield of Y [3].

**Method III:**

Fifty grams of corn cobs powder put in a glass beaker and soaked with a solution of 575 ml of 0.5% Sodium hydrochlorite, for 5 hours, filtered and washed with distilled water to eliminate lignin, then dried at room temperature for 48 hours. The obtained precipitate added with 400 ml of 10% NaOH solution and left for 24 hours at room temperature while in stir. After that, it was filtered. The filtrate was accommodated and the residue was washed with distilled water, the washing results combined and the residue discarded. The filtrate was neutralized with 63.07 ml of 6 N HCl to pH 7. and centrifuged at a rate of 4000 rpm for 30 minutes. The supernatant was taken and added to 661.2 ml of 95% ethanol and the precipitate formed then it was separated and dried with a vacuum dryer. The results obtained were weighed. The results yield of hemicellulose called the yield of R[1].

**Method IV:**

Fifty grams of corn cobs powder put in a glass beaker and added 500 ml of 0.03M NaOH in 70% ethanol and heated at 60 ° C, stirred for 2 hours to dissolve the lignin. The suspension was allowed to cool to room temperature, filtered through whatman filter paper. The residue was added 500 mL of 0.2 M NaOH and stirred for 8 hours at room temperature to dissolve hemicellulose. The filtrate was heated at 65°C, add 35 ml of 3% $H_2O_2$ in stages the filtrate and stirred constantly and filtered. Added 10% acetic acid solution in 95% ethanol at a ratio of 1:4 (v / v) and stored for 1 hour. The filtrate was centrifuged at a rate of 3.000 rpm for 15 minutes and the filtrate was discarded. The precipitate was washed with 96% ethanol and dried with a vacuum dryer. The results yield a heavy weighed hemicellulose called The yield M [8,9].

The yield of K, Y, R and M are identified with Infra-red (FTIR) and HPLC.

**Identification with Infrared Spectrophotometry**

The yields of hemicellulose, sonification and respectively weighed 1 mg and 200 mg of Potassium Bromide. Put in the mortar, crushed to a homogeneous, and then analyzed over a range of wave numbers 4000 - 500 $cm^{-1}$ and infrared spectra recorded [10,11]. Compared to the fingerprint spectrum and the resulting functional group of corn cobs hemicelluloses.

**Identification with High Performance Liquid Chromatography**

The yield of K, Y, R and M each of which weighed 25 mg, then put in a 50 mL volumetric flask with distilled water and added to the line mark, shaken and filtered (the first few ml of the filtrate discarded). The solution was filtered through a membrane filter Cellulose Nitrate 0.2 mm and sonification for ± 20 minutes. The solution was injected into the HPLC system via a loop injector with a 20 mL using an isocratic elution system with a aquabidest are mobile phase with a flow rate of 0.8 ml/min. Detection using a UV detector at a wavelength of 280 nm [12,13,14]. The chromatograms were recorded and qualitative analysis is done by comparing the number and height of the peak yield of K, Y, R, and M and the retention time on the chromatogram.

## Results and Discussion

### The Yield of Corm cobs Hemicellulose

The four methods of isolation to produce corn cobs hemicellulose yield can be seen in the figure 1 below.

The four method of isolation in figure 1 shows the yield of each method produces different weight because of the differences in the delignification process, alkaline pH, type of chemicals, temperature, and time of isolation. The results showed that the method IV showed the highest yield compared to other methods.

F-test results in Table 1 above, with the level of 95% is seen that 10.817 with a significance of 0.000 [16]. This means that there is a marked difference between the yield of the four isolation procedures.

From Tukey's test[16] in Table 2 above appears that the results of the four hemicellulose yields has the following results. Yield K of corn cobs hemicellulose give significantly different results from yield R and yield M of corn cobs hemicellulose with an asterisk (*), and not significantly different from the yield Y of corn cobs hemicellulose. The The yield Y of corn cobs hemicellulose give results significantly different from yield M of corn cobs hemicellulose with an asterisk (*), but not significantly different from the yield K and yield Rof corn cobs hemicellulose.

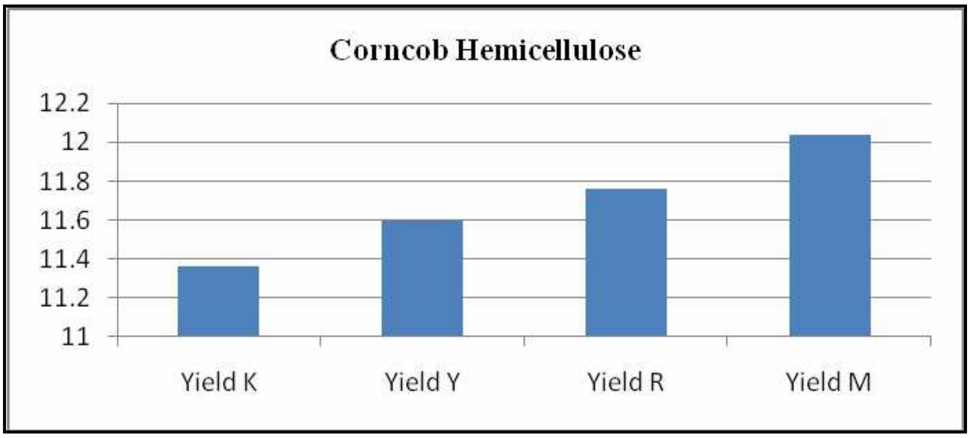


**Figure 1.** Yield percentage of isolation corn cob hemicellulose

**Table 1.** Analysis of Varians[16] Yield of Corn cobs Hemicellulose.

| Source | Type III Sum of Squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Between Yield | 2.627 | 3 | 0.876 | 10.817 | 0.00 |
| In Yield | 1.619 | 20 | 0.081 | | |
| Total | 4.247 | 23 | | | |

Based on observed means,
* The mean difference is significant at the, 05 level,

**Tabel 2.** Tukey's test for Corn Cobs Hemicellulose Yield

| Yield | Yield K | Yield Y | Yield R | Yield M |
|---|---|---|---|---|
| Yield K | | 0.1050 | -0.5550(*) | -0.8133(*) |
| Yield Y | -0.1050 | | -0.4500 | -0.7083(*) |
| Yield R | -0.5550(*) | -0.4500 | | -0.2583 |
| Yield M | -0.8133(*) | -0.7083(*) | -0.2583 | |

Based on observed means,
* The mean difference is significant at the .05 level

The yield R of corn cobs hemicellulose give significantly different results with yield K of corn cobs hemicellulose with an asterisk (*), and not significantly different with yield Y and and yield M hemicellulose of corn cobs. While the yield M of corn cobs hemicellulose give significantly different results with K and Y yield of corn cobs hemicellulose with an asterisk (*), and not significantly different from the yield of R.

This difference is partly due to the isolation process that had been performed to obtain the yield K and Y Those isolation processes of yield K and Y influenced the properties of the raw materials that were isolated. While the isolation process that produces yield R by Richana, et al., 2007[1] is the process of isolation of hemicellulose from the same properties of the raw materials, so that the results are not significantly different with the yield M, although there are differences in temperature, pH, amount and type of chemicals for delignification and isolation process.

All procedures using NaOH with different concentrations and it did not affect the process of hemicellulose isolation because these compounds are soluble in alkaline solvents [1,2,3,16].

Isolation of the four isolation process carried out at alkaline pH with NaOH at soaking time of each procedure ranged from 8 hours to 16 hours. Yet, immersion does not affect the yield of hemicellulose because the hemicellulose does not break down into their constituent monomers in alkaline conditions [17]. In the isolation process, the addition of acids used to precipitate hemicellulose, whereas the addition of ethanol is to increase the amount of hemicellulose which precipitates or clumping, making it easy to separate from the solution because hemicellulose is not soluble in acid and ethanol [1,2,3,8,16].

An important factor influencing the hemicellulose content of each procedure is delignification process, because some isolation procedures for delignification using different materials such as chlorine or $H_2O_2$ in alkaline atmosphere [1,5,6]. There is a big possibility that a few hemicellulose will dissolved in an alkaline atmosphere. The delignification process in the modified procedure was done twice: first using 0.05% NaOH in 70% ethanol at a temperature of 60°C. Even though hemicellulose is soluble in NaOH as 70% ethanol was used as a 0.05 % NaOH solvent, and then the hemicellulose did not participate in dissolved in 0.05 % NaOH. This is due to the hemicellulose is not soluble in ethanol, while lignin dissolved in ethanol[17]. Second, the delignification is conducted again after the isolation process. The second delignification process is done with 3% $H_2O_2$. The use of 3% $H_2O_2$ in the modified isolation method conducted by the researcher aimed to produce more white hemicellulose, in addition to the waste generated does not produce dioxins so there is no damage the health and pollute the environment [5,6,].

Yield of the four isolation procedures provide quantitative levels that did not differ, but the analysis of variance and Tukey's test gives a real difference. Based on the chemicals used in the isolation procedures, it can been seen that the three previuos procedures using a concentrated HCl to precipitate hemicellulose acid, but the modified isolation method conducted by the researcher using 10% acetic acid in 96% ethanol. This is done with the intention that the hemicellulose obtained will contain more pentosan and more soluble in water. It is based on researchs conducted by Doner and Hicks, 1977., Woolard, et.al., 1977 in Muralkrishna Rao and Subra 2007[17], which states that the deposition of alkaline extract with 50% acetic acid at pH 4.5 -5.0 produces

hemicellulose A which is a mixture of (1-3), (1-4) - β - D- glucans and arabinoxylans that are not soluble in water and hemicellulose B which are rich in pentosan. It also based on researchs conducted by Blake et.al 1971; Salimath and Thanranathan., 1982, in Muralkrishna and Subra Rao., 2007[17], which states it will easily dissolve in water with addition of three parts of volume into hemicellulose solution[18]. The procedure which is used by Richana, et al, 2007[1] while used HCl as acid, also used chlorine for delignification. As it is known that this waste produces dioxins [6,7], especially if it is used as a pharmaceutical raw material that will be produced in bulk, so that it will produce waste that will detrimental to environment's health.

The modification procedure is not carried out using HCl or chlorine, so it has a lower possibility of disturbing environmental waste and ethanol that are used can be recycled. Based on the above, the modified isolation procedure in the terms of procedures and waste generated, this procedure is better than the other procedures.

**Solubility of corn cobs hemicelluloses yield**

The solubility of hemicellulose resulting from the four methods can be seen in the table 3 below.

**Table 3.** Results of solubility yield of corn cobs hemicellulose

| Hemicellulose | Solubility | | | |
|---|---|---|---|---|
| | Distilled water | Hot distilled water | HCl 1% | 1% NaOH |
| The yield K | Low | Soluble | Insoluble | Soluble |
| The yield Y | Low | Soluble | Insoluble | Soluble |
| The yield R | Low | Soluble | Insoluble | Soluble |
| The yield M | Low | Soluble | Insoluble | Soluble |

From the results of the solubility tests, the four samples gave the same results and is consistent with the literature that is written that hemicellulose has a low solubility in distilled water, soluble in hot distilled water and form a solution that is transparent, insoluble in acid and easily soluble in NaOH 1%[16].

**Identification with Infrared spectrophotometry**

Identification of the infrared on the four samples isolated corn cobs hemicellulose provides the following figure 2 below.

The overlay results of the four yields in figure 2 above shows that all four yieldhave absorptions at 1820-1600 cm$^{-1}$ region, shows the existance of the C = O group and uptake widened near 3400-2400 cm $^{-1}$ indicates the presence of the same OH group and is located in the area of functional groups [10,11].

While in the finger print area, the four samples showed the same picture. This indicates that the four methods of isolation gives the shape and area of the same vibrational.

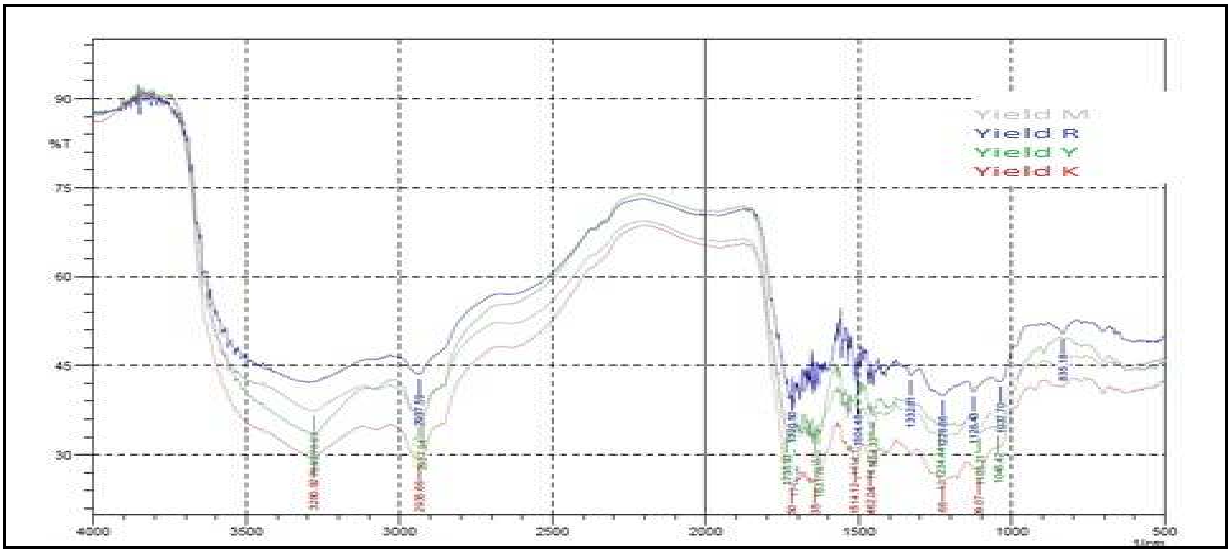


**Figure 2.** Characterization infra red vibration overlay test results yield K, Y, R and M of corn cobs hemicellulose with Infra red.

**Identification with High Performance Liquid Chromatography**

Identify chromatogram of corn cobs hemicellulose with HPLC by using a C18 column with solution of distilled water with flow rate 0.8 mL/min and the detector Ultra Violet rays at a wavelength of 280 nm[13,14,15].

Characterization testing with HPLC retention times associated in Table 4 above, can be seen that the four procedures of hemicellulose yield produce a retention time of 1.8 minutes is adjacent, with the same chromatogram shape, but the peak height, area and symmetry are different, it means that the four hemicellulose isolation procedures have the same yield with different levels[15].

HPLC chromatograms with aquabidest used as the mobile phase, C18 column, and the flow rate of 0.8 ml min with UV light detector at a wavelength of 280 nm. Mechanisms for identifying the supporting components monomer of the hemicellulose using an ODS column based on the presence of hydroxy functional groups in the sample, and the solubility in solubility test with NaOH exhibits a polarity of hemicellulose. Seen from the formed chromatogram. the four samples each have four peaks with the same retention time with the longest retention time is 1.8 minutes with the vast differences in the yield's respective areas. This means that identifying the HPLC indicates four methods of isolation gives the shape and area of the same peak.

**Table 4.** HPLC Chromatograms of the Yield Corn cobs Hemicellulose

| No. | Hemicellulose | Retention time | High Peak | Wide area | Symetris |
|---|---|---|---|---|---|
| 1. | K | 1.813 | 0.22605 | 1.18358 | 0.79 |
| 2. | Y | 1.813 | 0.67077 | 3.39732 | 0.81 |
| 3. | R | 1.804 | 0.75840 | 3.66488 | 0.92 |
| 4. | M | 1.802 | 1.06418 | 5.11558 | 0.82 |

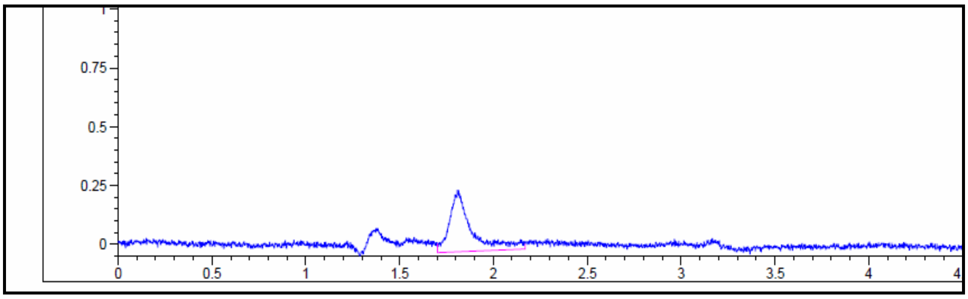


**Figure 3.a.** Characterization yield K of corn cobs hemicellulose by high performance liquid chromatography (HPLC)

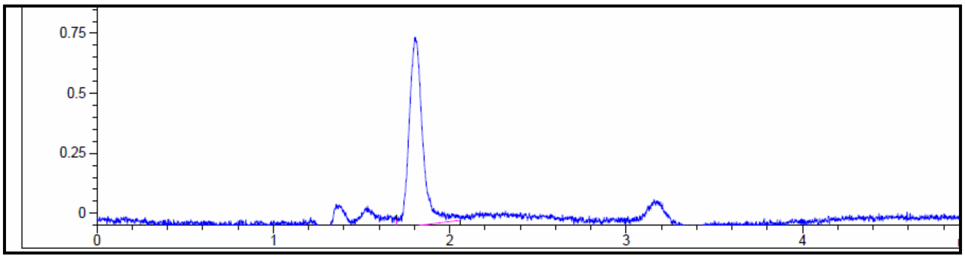


**Figure 3.b.** Characterization yield Y of corn cobs hemicellulose by high performance liquid chromatography (HPLC)

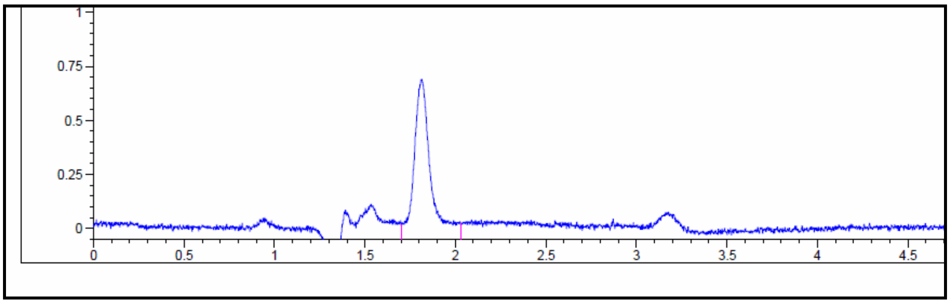


**Figure 3.c.** Characterization yield R of corn cobs hemicellulose byhigh performance liquid chromatography (HPLC).

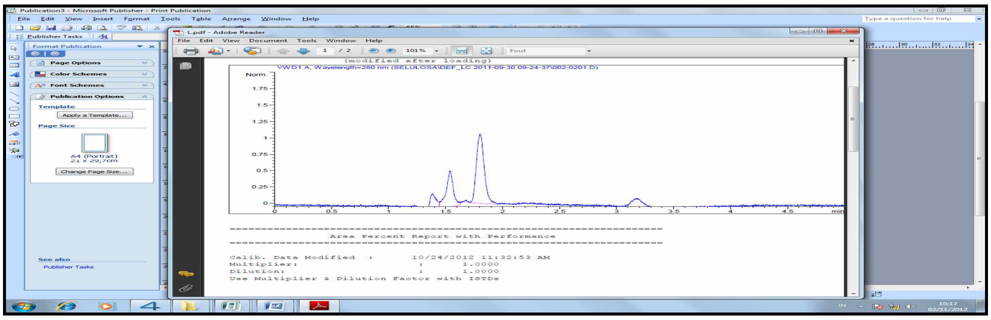

**Figure 3.d.** Characterization yield M of corn cobs hemicellulose by high performance liquid chromatography (HPLC).

## Conclusion

1. The highest result is the yield M corn cobs hemicelluloses at percentage of 12.04%.
2. The isolation method of hemicellulose effects on the improvement corn cobs hemicellulose yield.
3. HPLC analysis proved that the yield M corn cobs hemicellulose has the highest peak and area.
4. The modification isolation method of hemicellulose are produced environmentally friendly waste
5. Corn cobs have prospects for industrial raw materials of hemicellulose and can be developed in the pharmaceutical field as a dietary supplement and excipient.

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## References

1. Richana, N., Irawadi, T. T. and Nur, A. M.(2007). "The Extraction of Hemicellulose from Corn Cobs," *Postharvest Journal*, Vol. 4, No. 1, pp. (38-43)
2. Saha, C.B. (2003). "Hemicellulose Bioconversion," *Journal of Microbiologie Biotechnologie*, Vol. 30 No. 16, 2003, pp.(279-291).
3. Yadav, M.P., Johnston and Hicks, K.B.,(2007). "Structural Characterization of Corn Fiber Gums from Coarse and Fine Fiber and a Study of Their Emulsifying Properties," *Journal of Agricultural and Food Chemistry*, Vol. 55, No. 15, 2007, pp. 6366-6371. doi:10.1021/jf070024q.
4. El Seoud, O.A and Heinze, T (2005). "Organic Esters of Cellulose: New Perspectives for Old Polymers" *Adv Polym Sci* 186: pp. (103–149) Springer-Verlag Berlin Heidelberg DOI 10.1007/ b136818 Published online: 31 August 2005.
5. The National Agency for Standardization (2008). *ISO 0494:2008 Test Method Numbers Kappa pulp* Industry Research and Development Board Ministry of Industry Jakarta.
6. Batubara, R. (2006). Sustainable Bleaching Technology. Medan: University of North Sumatra. pp. 48-55.
7. Hadiernata, M., Budiyanto, A., Wiraatmadja, S., and Sugiarto, A., (2005). Kraft Pulp Bleaching Process Efficiency RDH (Rapid Displacement Heating) With Method ECF (elementally chlorine Free). Proceedings of the National Seminar on Innovative Technologies for Postharvest Development of Agriculture-Based Industries. Bogor: Center for Agricultural Postharvest Research and Development.pp 772-784.
8. Karaaslan. A. M., Tshabalala. MA., Buschle-DG (2010). Wood Hemisellulose /Chitosan-Basedsemi-Interpenetrating Networks hydrogels: Mechanical. Swelling And Control Drug Release *Properties. Journal Bioresources 5 (2).* Alabama. USA. p 1036-1054.
9. Muchlisyam, Harahap, U., Silalahi, J., and Alfian, Z.[a] (2013). "Isolation And Utilization Of Corn cobs hemisellulose As Chelating Agent For Lead Ions" *The Malaysian Journal of Analytical Sciences*, Vol 17, No. 1, pp (71 – 74).

10. Muchlisyam, Harahap, U., Silalahi, J. and Alfian, Z. (2013)."Binding Ability of Corn cobs hemicellulose toward Cadmium" *American Journal of Analytical Chemistry*, 4,pp.(86-93) doi:10.4236 /ajac.2013. 47A012. Published Online July 2013.
11. Giwangkara S. EG. (2007). "Infrared Spectroscopy" http://www.chem-is-try.org/
12. Britthin. HG (2006). *Spectroscopy of Pharmaceutical Solids.* Center for Pharmaceutical Physis Taylor & Francis Group. New York.
13. Kazakevich, Y. dan. LoBrutto, L. (2007). *HPLC for Pharmaceutical Scientists*. New Jersey: John Wiley & Sons, Inc. Hal. 8-15.
14. Meyer, V.R. (2004). Practical High-Performance Liquid Chromatography. Chichester: John Wiley and Sons Inc. Hal. 4.
15. Snyder. RL. Kirkland. J.. Joseph. DW (2010). *Introduction to Modern Liquid Chromatography.* A John Wiley & Sons. Inc. Publication Third Edition USA.
16. R. G. D. Steel and J. H. Torrie, "Principles and Procedures Statistics A Biometric Approach," No.2 PT Gramedia Pustaka Husada, Jakarta, 1991.
17. Dumitriu. S. (2005). Polysaccharides: Structural Diversity and Functional Versatility. Marcel Dekker. New York. USA.
18. Muralikrishna, G and Rao, S. M.V.S.S.T., (2007), Cereal Non-Fellulosic Polysaccharides: Structure anf Function Relationship- An Overview. *Critical Reviews in Foods Sceince and Nutrition* 47(6): 599-608.

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