

The separation of alkyldiethanolamide based on kernel oil of calophyllum inophyllum fruit using high-performance liquid chromatography

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DOI: 10.29303/aca.v5i1.78

Article info:

Received 17/10/2021

Revised 06/04/2022

Accepted 10/04/2021

Available online 17/04/2022

Abstract: This study aimed to separate the synthesized alkyldiethanolamide from kernel oil Calophyllum inophyllum (Local name: Nyamplung) using High-Performance Liquid Chromatography (HPLC). HPLC column utilized was SGE ODS-2 reverse phase and UV detector 213 nm. The variables to obtain the optimum conditions for separating alkyldiethanolamide were mobile phase and flow rate. The mobile phase composition and the optimum separation flow rate obtained acetonitrile: water (90:10) and 1.5 mL/minute, respectively. The percentage compositions of amide fatty acids that had been successfully synthesized based on HPLC were linoleoyl diethanolamide (46-49%), oleoyl diethanolamide (27-29%), palmitoyl diethanolamide (11-14%), and stearoyl diethanolamide (9-11%).

Keywords: alkyldiethanolamide, high performance liquid chromatography, mobile phase, flow rate

Citation: Iqbal, M., Suhendra, D., & Ryantin Gunawan, E. (2022). The separation of alkyldiethanolamide based on kernel oil of calophyllum inophyllum fruit using high-performance liquid chromatography. *Acta Chimica Asiana*, 5(1). 153-157. <https://doi.org/10.29303/aca.v5i1.78>

INTRODUCTION

The acid amide group has many uses. One of the essential functions of this compound is a surfactant because it has two active groups, namely polar and nonpolar. The surfactant property of this compound makes it widely applied in various industries such as lubricants, cosmetics, detergents, antimicrobials, shampoos, corrosion inhibitors, and various other types of industry [1-2].

This study used the kernel oil of Calophyllum inophyllum fruit as the raw material. The kernel oil of this fruit has the potential to be developed as a raw material for the synthesis of alkyldiethanolamide because it is a non-edible oil and easy to obtain. Calophyllum inophyllum seeds contain a very high oil content, namely 66-70% [3-4]. The composition of fatty acids in Calophyllum inophyllum oil consists of oleic acid (56-48%), linoleic acid (21-25%), palmitic acid (15-16%), and stearic acid (12-13%) [4-5].

The synthesis of alkyldiethanolamide can be carried out industrially by reacting fatty

acids, fatty acid methyl esters, or oils with alkanolamides using a chemical catalyst and a lipase enzyme catalyst [6-7].

The synthesis of alkyldiethanolamide with lipase as a biocatalyst has been developed. This method is very environmentally friendly because it requires lower temperatures and pressures than chemical catalysts [8-10].

Alkyldiethanolamide, having been successfully synthesized, is still composed of amide fatty acids components that have not been separated, so it is necessary to separate them. The separation of alkyldiethanolamide from its constituent amide fatty acids can be undertaken by using the High-Performance Liquid Chromatography [11]. The natures of alkyldiethanolamide compounds, which are low in volatility, non-reactive and stable, allow them to be very well separated by the High-Performance Liquid Chromatography [12-13]. This study aimed to separate the alkyldiethanolamide using the optimum variable conditions for the High-Performance Liquid Chromatography separation.

MATERIALS AND METHODS

The equipment in this research were: glassware in the laboratory, shocks, reflux, magnetic stirrer, column chromatography, filter paper, Whatmann filter paper, hot plate, pH meter, desiccator, Buchner, vacuum, rotary evaporator, chamber, horizontal shaker water bath, FT-IR spectrophotometer and a set of the High-Performance Liquid Chromatography (Shimadzu LC-10 ATVP).

The materials were the kernel of *Calophyllum inophyllum* fruit, anhydrous Na₂SO₄, chloroform (CHCl₃), diethanolamide, Lipozymes TL IM (Novozymes LA330045), n-hexane, methanol, DCM (CH₂Cl₂), silica gel, Na₂S₂O₃, KOH in ethanol 0.5N, ethanol 95%, TLC plate, diethyl ether, standard fatty acids, phenolphthalein indicators, acetonitrile (C₂H₃N) and distilled water.

Alkyl diethanolamide from the kernel oil of *Calophyllum inophyllum* fruit was synthesized based on the previous research method conducted by Suhendra et al. [6]. It was synthesized by reacting 10 g of triglycerides from *Cinophyllum* fruit core oil, 100 mL n-hexane, 50 mmol of diethanolamide, and then adding 7.5% commercial lipase enzymes (lipozymes). The mixture was incubated in a horizontal water bath shaker at a speed of 150 rpm at 40° C for 2 hours. Alkyl diethanolamide formed was separated from the water and enzyme layer using a separating funnel. The n-hexane (alkyl diethanolamide) phase was then cooled in the freezer (<-5) for 24 hours and filtered. The amount of alkyl diethanolamide formed was calculated by the gravimetric method.

The characterization of alkyl diethanolamide was done by several methods. Preliminary analysis was performed using Thin Layer Chromatography (TLC). The eluent was a mixture of methanol: chloroform (90:10 v/v) solvent [14-15]. The characterization of alkyl diethanolamide functional groups used an FT-IR spectrophotometer from Perkin Elmer Model Frontier (USA).

Separation of Fatty Amide Acids

The separation of amide fatty acids was performed under optimum conditions of the High-Performance Liquid Chromatography (HPLC). The High-Performance Liquid Chromatography column was SGE ODS-2 reversed-phase C-18 column and 213 nm UV detector. The sample concentration was made 5000 ppm, and the injection volume was 20 µL. The variables to obtain the optimum conditions for separating alkyl diethanolamide

were mobile phase and flow rate. The mobile phase was a mixture of acetonitrile: water solvent with a variation of the ratio of 85:15 v/v, 90:10 v/v, and 95: 5 v/v, while the variation of the flow rate was 0.5 ml/minute, 1.0 mL/minute, and 1.5 mL/minute. Fatty acids in alkyl diethanolamide successfully synthesized were identified by comparing the standard retention time to the retention time of the sample. The percentage of amide fatty acids was calculated based on the area of each peak on the chromatogram.

RESULTS AND DISCUSSION

Alkyl diethanolamide was synthesized by reacting *Calophyllum inophyllum* fruit core oil (triglycerides) and diethanolamide enzymatically. According to the optimum conditions of the previous research by Suhendra et al. [6]. Alkyl diethanolamide, which had been successfully synthesized, came in two forms: the solid form of 1.36 g and the liquid form of 2.66 g. if converted into % yield, the resulting alkyl diethanolamide was 40.20%. This result was not much different from the results obtained by Suhendra et al. [6] of 44%.

Characterization of Alkyl diethanolamide

A preliminary qualitative analysis of alkyl diethanolamide was performed by TLC with chloroform: methanol (90:10 v/v) as eluent. The analysis results showed that there were three spots of compounds resulting from the synthesis of alkyl diethanolamide with R_f values of 0.857; 0.750; and 0.550, respectively.

FTIR analysis results (Figure 1) were a spectrum of *Calophyllum inophyllum* oil (a) and a spectrum of alkyl diethanolamide (b). The most fundamental difference between the two spectra could be seen in the vibration absorption of C=O at wavenumbers 1747.89 cm⁻¹ and 1637.66 cm⁻¹, respectively. The success of the synthesis of alkyl diethanolamide was indicated by the O-H strain vibration absorption at wavenumbers 3434.21 cm⁻¹ indicating the presence of the O-H functional group. Alkyl diethanolamide had N-tertiary uptaken, so no peaks appeared on the spectrum. The absorption of amide compounds formed was supported by the appearance of C-N vibration absorption shown at the wavenumbers 1379.33 cm⁻¹ [6] and [16,17].

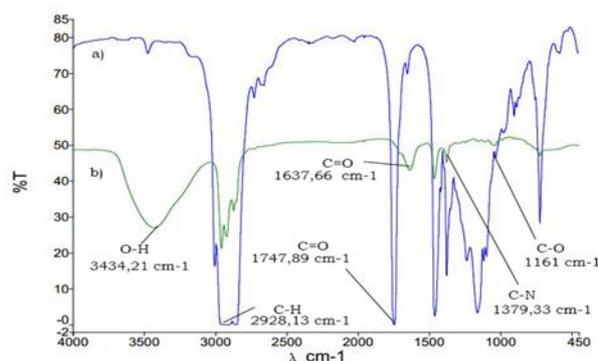


Figure 1. FTIR spectrum of nyamplung oil and alkyldiethanolamide

Table 1. Optimum conditions for High-Performance Liquid Chromatography

stationary phase	SGE C-18 ODS 2 (250 mm, ID 4 mm, Frit 4/m μ m)
Mobile phase	Acetonitrile mixture: water (90:10 v/v)
Flow rate	1.5 mL/minute
Injection Volume	20 μ L (0.05 mg in 10 mL Asetonitril)
Detector	UV 213 nm

The High-Performance Liquid Chromatography separated Alkyldiethanolamide samples based

on the Optimum conditions in the Table 1. The optimum conditions for separation for the variable mobile phase and flow rate were determined quantitatively based on the resolution value (R_s). Resolution value ≥ 1.5 for separation for the variable mobile phase and flow rate were determined quantitatively based on the resolution value (R_s). Resolution value ≥ 1.5 showed that the two chromatograms were well separated. Based on the resolution value obtained, the optimum conditions for separating alkyldiethanolamide were in the mobile phase composition of acetonitrile: water 90:10 v/v and a flow rate of 1.5 ml/minute (Figure 2). Identification of the components of alkyldiethanolamide was carried out by comparing the retention time of the sample to the standard alkyldiethanolamide synthesized from pure fatty acids.

From the chromatograms that had been compared to each standard, the composition of alkyldiethanolamide from 1 to 4 peaks was obtained, namely linoleoyl diethanolamide (46-49%), oleoyl diethanolamide (27-29%), palmitoyl diethanolamide (11-14%), and stearoyl diethanolamide (9-11%). The order of compounds was eluted based on the level of polarity.

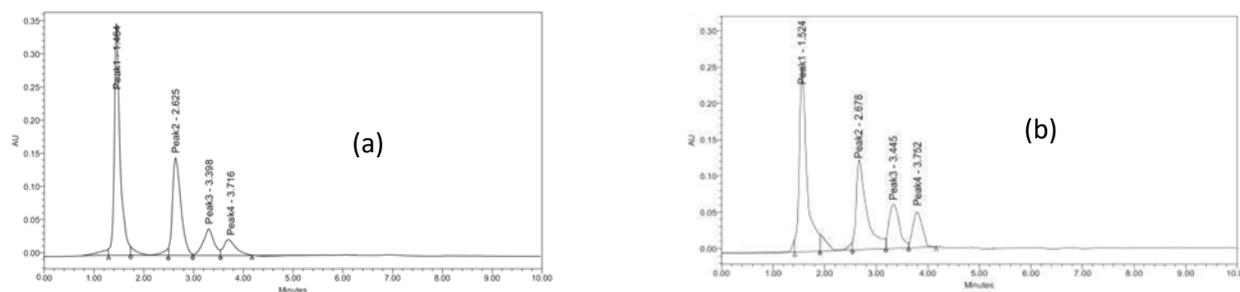


Figure 2. Chromatograms optimum conditions (a) and standard (b) of the separation of alkyldiethanolamide (acetonitrile: water 90:10 v/v and a flow rate of 1.5 ml/min)

Table 2. Optimum conditions for High-Performance Liquid Chromatography

Peak	RT Sample	RT Standard	Remarks	%
1	1.464	1.524	Linoleoyldiethanolamide	47.88
2	2.625	2.678	Oleoyldiethanolamide	28.65
3	3.398	3.445	Palmitoyldiethanolamide	12.83
4	3.716	3.752	Stearoyldiethanolamide	10.63

The nonpolar octadecylsilane (ODS or C18) stationary phase would bind more strongly to the nonpolar amide fatty acids. After synthesis, the composition order of linoleic fatty acids differed from those of oleic fatty acids. It was because linoleic acid was classified as a cis unsaturated fatty acid which was less stable, so it experienced more breakdown by enzymes.

Acknowledgement

This research was funded by the Ministry of Research and Technology, HIKOM Scheme, 2020

CONCLUSION

The optimum conditions for separating alkyldiethanolamide based on the kernel oil of Calophyllum inophyllum fruit were in the acetonitrile: water 90:10 v / v mobile phase with a flow rate of 1.5 ml/minute. The percentage of amides' composition successfully synthesized was calculated based on the results of the High-Performance Liquid Chromatography analysis, namely linoleoyl diethanolamide (46-49%), oleoyl diethanolamide (27-29%), palmitoyl diethanolamide (11-14%), and stearoyl diethanolamide (9- 11%).

REFERENCES

- Galilee, U. S., Modesto, T. C., & Soma, C. (2009). Biocatalytic synthesis of diethanolamide surfactants under mild reaction condition. *Philipp. J. Sci*, 138, 49-54.
- Silaen, D. S., Herawan, T., Masyithah, Z., & Ginting, H. A. (2017). Optimasi sintesis biosurfaktan lauril amida dari asam laurat dan dietanolamina menggunakan pelarut hexane dan enzim lipase terimobilisasi. *Jurnal Teknik Kimia USU*, 6(2), 19-23.
- Suhendra, D., Solehah, A., Asnawati, D., & Gunawan, E. R. (2019). Sintesis Poliuretan dari Asam Lemak Teroksidasi Minyak Inti Buah Nyamplung Melalui Proses Polimerisasi Menggunakan Toluene Diisosiyanat. *Chemistry Progress*, 6(2).
- Atabani, A. E., & da Silva César, A. (2014). Calophyllum inophyllum L.–A prospective non-edible biodiesel feedstock. Study of biodiesel production, properties, fatty acid composition, blending and engine performance. *Renewable and Sustainable Energy Reviews*, 37, 644-655.
- Gunawan, E. R., Suhendra, D., Hidayat, I., & Kurniawati, L. (2018). Optimization of Alkyldiethanolamides Synthesis from Terminalia catappa L. Kernel Oil through Enzymatic Reaction. *Journal of oleo science*, 67(8), 949-955.
- Suhendra, D., Gunawan, E. R., & Kusumawati, L. (2019). Synthesis of fatty diethanolamide from calophyllum inophyllum l. Kernel oil via enzymatic reaction. *Rasāyan Journal of Chemistry*, 12(2), 765-772.
- Manurung, R., Sinaga, R. A., & SimaTupang, R. T. (2013). Kinetics of Amidation for the Synthesis of Diethanolamide from Methyl Ester and Diethanolamina by Using Sulphuric Acid Catalyst. *International Journal of Innovative Research in Science, Engineering and Technology*, 2(9), 4205-4210.
- Wang, X., Chen, Y., Jin, Q., Huang, J., & Wang, X. (2013). Synthesis of linoleoyl ethanolamide. *Journal of oleo science*, 62(6), 427-433.
- Lokhande, A. R., Patil, V. S., & Wani, K. S. (2016). Kinetic Study on Synthesis of Alkanolamides of Fatty Acids of Mangokernel Oil. *International Journal of Engineering Development and Research*, 4.
- Gunawan, E. R., Suhendra, D. E. D. Y., Nurita, A. D., & Komalasari, D. E. S. Y. (2017). Four factor surface response optimization of ethyl palmitate from Terminalia cattapa L. kernel oil. *Asian J. Chem*, 29, 2107-2112.
- Masyithah, Z., Sembiring, S. B., Alfian, Z., & Herawan, T. (2011). The optimization of enzymatic synthesis for lauroyl-n-methyl glucamide surfactants. *Indonesian Journal of Chemistry*, 11(3), 223-228.
- Tinnis, F., Lundberg, H., & Adolffson, H. (2012). Direct Catalytic Formation of Primary and Tertiary Amides from Non-Activated Carboxylic Acids, Employing Carbamates as Amine Source.

Advanced Synthesis & Catalysis, 354(13), 2531-2536.

13. Masyithah, Z., & Herawan, T. (2017). Optimization of enzymatic synthesis of oleoyl-diethanolamide in solvent-free system. *J. Pure. App. Microbiol*, 11, 1327-1336.
14. Salleh, A. B., Basri, M., Tan, S. W., Abdurrahman, M. B., Dzulkefly, K., Rahman, R. N. Z., & Razak, C. N. A. (2001). Synthesis of fatty alkanolamides by using immobilized lipases. *Malaysian J. Anal. Sci*, 7, 281-285.
15. Rahman, M. A., Yap, C. L., Dzulkefly, K., Rahman, R. A., Salleh, A. B., & Basri, M. (2003). Synthesis of palm kernel oil alkanolamide using lipase. *Journal of Oleo Science*, 52(2), 65-72.
16. Liu, K. J., Nag, A., & Shaw, J. F. (2001). Lipase-catalyzed synthesis of fatty acid diethanolamides. *Journal of agricultural and food chemistry*, 49(12), 5761-5764.
17. Gunawan, E. R., Suhendra, D., Rohana, R., & Komalasari, D. (2020). Methyl Linoleate Synthesis From Cotton Seeds Oil: Optimization Study. *Acta Chimica Asiana*, 3(2), 163-169.