Synthesis of *Fatty Hydroxamic Acids (FHAs)* from Coconut Oil Using Lipase as a Catalyst

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Abstract

Fatty hydroxamic acids (FHAs) have been successfully synthesized from coconut oil by a one-step lipase catalyzed reaction. FHAs are the acids that are based on fatty acids. Their hydrophobicity can be use for some applications such as surfactant and metal ions extraction from aqueous media. This paper describes enzymatic synthesis of FHAs from coconut oil by reacting hydroxylamine with the substrate catalyzed by a lipase. The lipase used was Lipozyme, a commercially lipase of Mucor meihe fixed on a polymer anion exchange resin. The use of immobilized lipase as the catalyst for the preparation reaction provides an easy isolation of the enzyme from the products and other components in the reaction mixture. In addition, it also allows the reaction to be carried out under mild conditions, which reduces the reaction's side products. The optimum preparation conditions obtained were as follows; the reaction temperature was 30^{o}C, the reaction time was 30 h, the ratio of coconut oil: lipozyme (g:g) was 29.5, and the ratio of hydroxylamine: coconut oil (mmol:mmol) was 6. The solvent used was hexane. The purified products were characterized by qualitative test, such as FTIR spectroscopy and HPLC.

Keywords: *Coconut Oil*, *Enzymatic Synthesis*, *Fatty Hydroxamic Acids*, *Lipase*

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A. INTRODUCTION

Hydroxamic acids, hydroxamic acids, are chelating agents derived from hydroxylamines and carboxylic acids, therefore hydroxamic acids are also called N-hydroxy carboxylic amide with the general formula R-CO-NHOH. Recently, hydroxamic acid and its derivatives have received serious attention from many researchers due to their biological activities, such as growth promoters, antibiotics, antifungi (Kurzak et al., 1992), enzyme inhibitors (Anandan et al., 2007), anti-tumors (Holmes et al. 2001), prevention of iron corrosion (Deng et al. 2008) and anti-oxidants (Liu et al. 2008). The complex between hydroxamic acid and various metal ions is widely used for analytical chemical purposes, namely as a reagent for gravimetric and spectrometric metal determination (Pacco, 2008), as a chemical sensor in the determination of tiny metals (Isha et al., 2007), as a collector for rare earth elements (Agrawal & Kaur, 1999), and for extracting metal ions from solution (Suhendra et al., 2005a & 2005b).

The very diverse uses of hydroxamic acids and the need for chelating agents for research and teaching needs in the field of analytical chemistry are high, not accompanied by sufficient availability of material. In fact, the literature review conducted, shows that there have been many studies to synthesize hydroxamic acids

from various basic ingredients, and even many that are already available as commercial products. However, until now has not found a commercial product to acids, long chain hidroksamic.

For the purpose of teaching in analytical chemistry, especially in the separation or refining of metal ions, many chelating agents are needed. However, lately, the price of chemicals, especially chelating agents, is very expensive, so it needs to be thought to look for chelating agents that are cheap and can be prepared from the basic ingredients that are around. There are two ways of separating metals or extracting metals from solutions using a chelating agent, namely solvent extraction and immobilization of the chelating agent into a non-polar polymer support. For this purpose, a chelating agent is needed which has both hydrophobic and hydrophilic properties. When viewed from its functional group, hydroxamic acid is polar (hydrophilic), therefore it is needed medium to long chain alkyl groups to also be hydrophobic.

The main component of vegetable oil is triacylglycerol, which is an ester of fatty acids with glycerol. Synthesis of hydroxamic acids using vegetable oil as a precursor has been carried out using soybean oil (Servat et al., 1990), and palm olein oil (palm olein) (Suhendra et al., 2005a). The main component of soybean oil is linoleic acid (54%), while the main component of palm oil olein is palmitic acid which is 37% (Zamora 2005). Judging from the length of the fatty acid chain, the main components of the two oils are long chain fatty acids. Actually, for the purpose of extracting metals in solution, these two types of oil already meet the requirements as a basic ingredient in the synthesis of hydroxamic acids, but lately, the prices of these two commodities are quite expensive. Therefore, in this study coconut oil was used as the basis for the synthesis of chelating agent hydroxamic acid. With consideration; a relatively cheaper base material, abundant availability, and its main fatty acid content is lauric acid which is 47% (Zamora 2005), which is a medium chain fatty acid.

Synthesis of hydroxamic acids can be carried out by two methods, namely chemical and enzymatic. The first way is done in an alkaline atmosphere and a high enough temperature. For the synthesis of hydroxamic acids from vegetable oils, this method is not appropriate because there are fatty acids making up of oil that have double bonds. Under alkaline conditions and high temperatures, the double bonds can be oxidized. The second way is the way that feels most appropriate. This is due to the way enzymatics work in a neutral atmosphere and low temperatures, besides that, it is environmentally friendly and the enzymes used can be reused. This research is focused on the synthesis of Fatty hydroxamic acids (FHAs) from coconut oil which is catalyzed by the lipase enzyme.

B. METHOD

Hydroxylaminolis. The hydroxylaminolis procedure used refers to the procedure used by Suhendra et al. (2005a), with a slight modification. The amount of coconut oil dissolved in hexane is reacted with hydroxylamine hydrochloride which has been neutralized with NaOH 6 N and a number of lipase enzymes in a 100 ml lid erlenmeyer. The reaction mixture is then incubated into a batch water shaker with a shock speed of 100 rpm. The hydroxamic fatty acids (FHAs) formed at the water-hexane interface are then separated from the water and lipase by filtering. To obtain solid FHAs, the hexane fraction is cooled in the refrigerator (<-5^{o}C) for five hours and then filtered and rinsed with hexane several times to remove the remaining oil. The formed FHAs is dried in a vacuum desiccator over phosphorus pentoxide for 24 hours.

Product Characterization. Qualitative analysis of hydroxamic acid groups in FHAS was performed using FTIR spectroscopy (Perkin Elmer FTIR-Spectrum BX, USA). The composition of fatty acids in FHAs was determined using High-Performance Liquid Chromatography (HPLC) following a procedure developed by Gutnikov and Streng (1991), which was modified. HPLC used is a product from Waters HPLC-USA which is equipped with various devices, namely Waters Delta-600 pump, Waters 600-controller, Waters-2487 Dual l Absorbance Detector and Shimadzu CTO-6A column oven.

C. RESULT AND DISCUSSION

Effect of Reaction Time. Reaction time is an indicator of enzyme performance. The effect of this reaction time is used as a benchmark to obtain the shortest reaction time with the best results. The determination of the optimum reaction time also aims to minimize excess process expansion (Yee et al., 1997). Figure 1 shows that reaction time rises rapidly to the first 30 hours. Over 30 hours the reaction does not show a significant increase, this is likely due to the formation of solids in the reaction so that there is an inhibition of mass transfer (mass-transfer limitations). Another possibility is the achievement of an equilibrium reaction, ie the reaction in the direction of the formation of the product is the same as the reaction of the decomposition of the product so that the concentration of the product does not change.

Effect of Reaction Temperature. Changes in reaction temperature can affect the activity and stability of enzymes and of course also affect the rate of reaction (Mc Gilvery & Goldstein, 1983). Figure 2 shows the increase in yield on the temperature rise

from 30°C to 40°C. However, when the temperature is raised to 70°C, the decrease falls dramatically. This is probably caused by the degradation (denaturing) of lipase at temperatures above 40°C.

Although the optimum reaction temperature in this study was 40°C, but at scaling up, all reactions took place at room temperature 30-34°C. The choice of room temperature is due not only to avoiding the degradation of the double bonds of the product at high temperatures as well as the increase in yield between temperatures of 30 and 40°C is not large, so the implementation of the reaction at room temperature can save electrical energy.

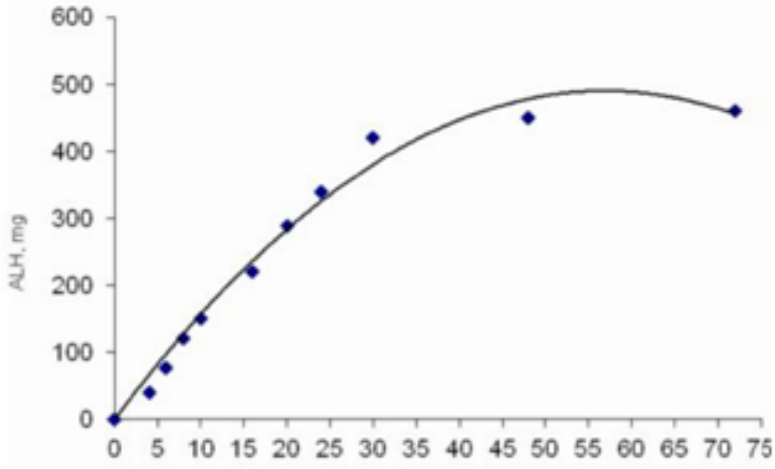


Figure 1 Effect of Reaction Time

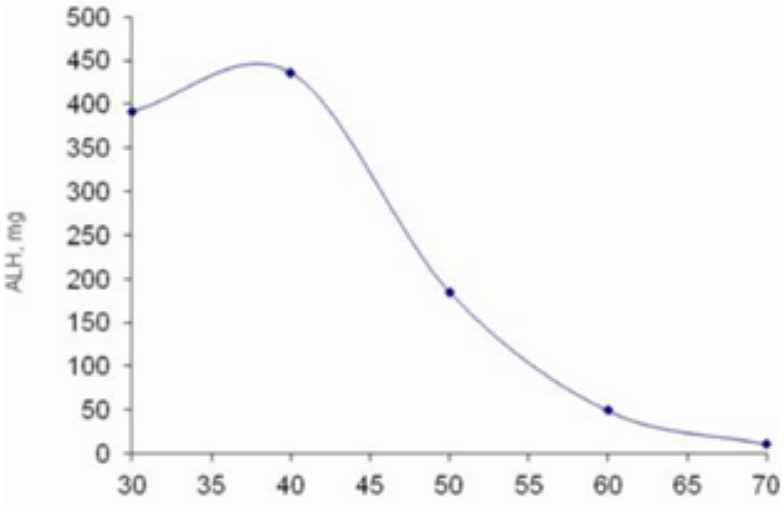
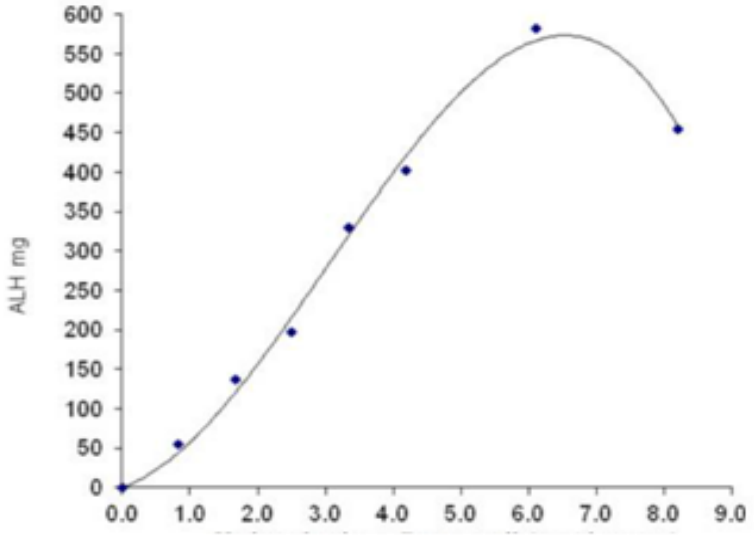


Figure 2 Effect of Reaction Temperature

Effect of Hydroxylamine Concentration. The optimum comparison of the substrate used is an important implementation in industry (Arcos et al., 1998). Figure 3 shows an increase in the FHAS ratio with an increase in hydroxylamine concentration.

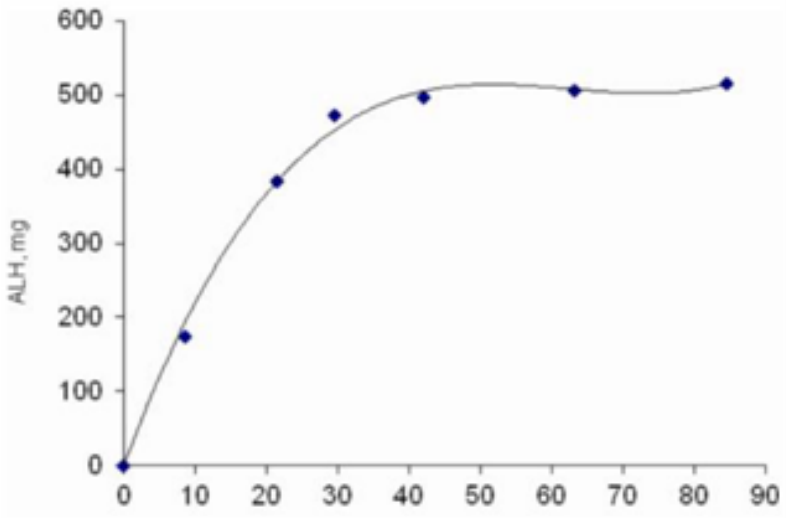
These results are consistent with the results obtained by Suhendra et al., (2005), on the synthesis of FHAs from palm oil. Figure 3 also shows that the higher the hydroxylamine concentration, the lower the yield. This phenomenon is consistent with the results shown by Vaysse et al., (1997), which shows the formation of inhibitors if the hydroxylamine concentration is high enough.

Effect of Enzyme Amount. For industrial purposes, the amount of enzymes used in the reaction is expected to be as small as possible to obtain as many results as possible. Therefore, the amount of enzymes is very crucial in suppressing production costs. The results of this study (Figure 4) show that high amounts of enzymes (ratio of enzymes: substrates above 30) did not contribute to the yield of FHAs.



Hydroxylamine: Coconut oil (mmol:mgram)

Figure 3 Effect of Hydroxylamine Concentration



[Lipozyme (mg): substrate (gram)] x 1000

Figure 4 Effect of Enzyme Amount

FHAs Characterization. FTIR Spectra. The FTIR spectrum of the substrate (coconut oil) and product (FHAs) is presented in Figure 5. In the spectrum of coconut oil the strong peaks at 2848 cm-1 and 2916 cm-1 show the presence of stretching C-H groups of stretching alkyl groups. This peak is supported by a peak at 1452 cm-1 which is the peak of the C-H group of bending vibrations (Skoog et al., 1998). Another peak is at 1740 cm-1 which is the typical peak of the C = O stretching group. The presence of OH and NH groups in the product indicates the formation of FHAS.

The FHAS spectrum looks similar to the substrate spectrum, but the difference is clearly seen, namely the presence of OH groups marked by peaks at 3426 cm-1 which are typical areas for OH stretching and the presence of NH groups marked with peaks at 3264 cm-1 which are peaks typical NH stretching. These peaks are supported by peaks at 1662 cm-1 which are typical peaks for C = O of secondary amides.

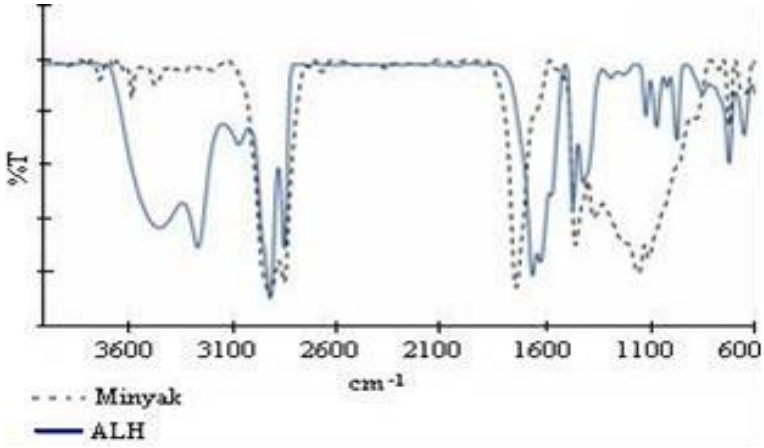


Figure 5 FTIR Spectrum of Coconut oil and FHAS

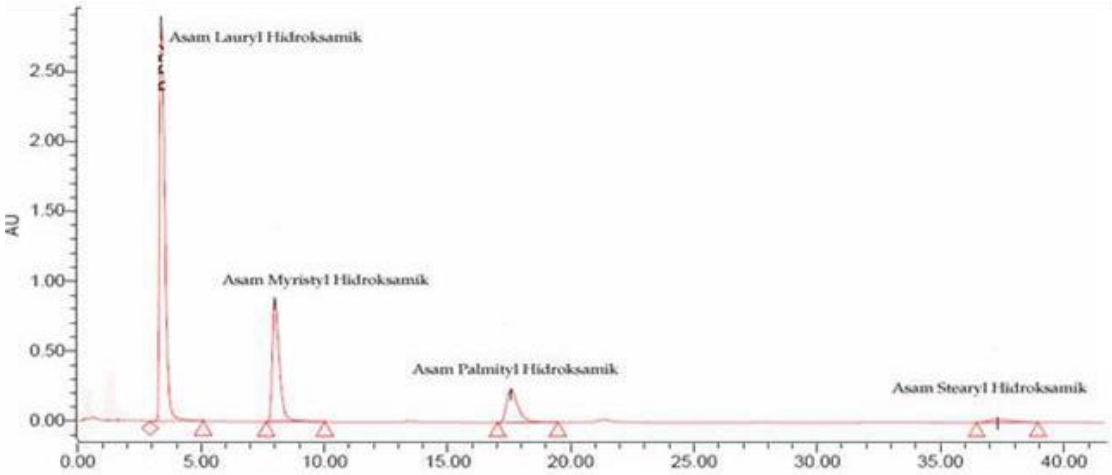


Figure 6 HPLC Chromatogram from FHAS Products

HPLC. HPLC is a separation method that is currently widely used. Extensive use due to this method can be used for qualitative and quantitative analysis at the same time. Each compound in the mixture, under the desired conditions, has its own time retention (qualitative analysis) and has a signal area related to the amount of the substance (quantitative analysis) (Meyer, 1994).

The retention time (tR) of a compound is typical for that compound (Snyder & Kirkland 1979). Therefore, if the tR value of an unknown compound is known in accordance with the tR value of a standard compound measured using the same conditions, then it is certain that the compound is the same. The composition of hydroxamic fatty acids from coconut oil can be seen in Figure 6. In the picture the composition of FHAS has been compared with standard chromatograms, namely hydroxamic laurylic acid (67.5%), hydroxyl myristic acid (20.9%), Palmityl hydroxamic acid (9.3%) and stearyl hydroxamic acid (2.3%).

D. CONCLUSION

This research has successfully synthesized FHAs from coconut oil with a commercial lipase catalyst (lipozyme). The optimum conditions for FHAs synthesis from coconut oil are the reaction time of 30 hours, reaction temperature = room temperature (30^{o}C), Lipozime ratio (mg): Substrate (g) = 29.5, and Hydroclaminate (mmol) ratio: Substrate (g) is 6.

REFERENCES

1. Agrawal, Y. K., & Kaur, H. (1999). Poly (β-styryl) hydroxamic acids: synthesis and ion exchange separation of rear earths. *React Funct Polym*, 39: 155-164.
2. Anandan, S. K., Ward, J. S., Brokx, R. D., Mark, T. D. Bray, R., Patel D. V., & Yi Xiao, X. (2007). Design and synthesis of thiazole-5 -hydroxamic acids as novel histone deacetylase inhibitors. *Bioorg Med Chem Lett*, 17: 5995-5999.
3. Arcos, J. A., Barnabe, M., & Otero, C. (1998). Quantitative enzymatic production of 6-O-Acylglucose esters. *Biotechnol Bioeng*, *57*: 505-509.
4. Deng, H., Nanjo, H., Qian, P., Xia, Z., Ishikawa, I., & Suzuki, T. M. (2008). Corrosion prevention of iron with novel organic inhibitor of hydroxamic acid and UV irradiation. *Electrochim Acta*, 53: 2972-2983.
5. Gutnikov, G., & Streng, J. R. (1991). Rapid high performance liquid chromatographic determination of fatty acid profiles of lipids by conversion to their hydroxamic acids. *J Chromatogr*, 587: 292-296.
6. Holmes, J., Mast, K., Marcotte, P., Elmore, I., Li, J., Pease, L., Glaser, K., Morgan, D., Michaelides, M., & Davidsen, S. (2001). Discovery of hydroxamic acid inhibitors of tumor necrosis factor-⍟ converting enzyme. *Bioorg Med Chem Lett, 11:* 2907–2910.
7. Isha, A,. Suhendra, D., Yusof, N. A., Ahmad, M., Wan Yunus, W. M. Z., & Zainal, Z. (2007). Optical fiber chemical sensor for trace vanadium (V) determination based on

newly synthesized palm based fatty hydroxamic acid immobilized in polyvinyl chloride membrane. *Spectrochim Acta A*, 67: 1398–1402.

8. Kurzak, B., Kozlowski, A & Farkas, F. (1992). Hydroxamic and amino hydroxamic acids and their complexes with metal ions. *Coordin Chem Rev,* 114: 169-200.
9. Liu, Y. H., Lin, S. Y., Lee, C., & Hou, W. C. (2008). Antioxidant and nitric oxide production inhibitory activities of galacturonyl hydroxamic acid. *Food Chem*, 109: 159–166.
10. McGilvery, R. W., & Goldstein, G. W. (1983). *Rates of Enzymatic Reaction in Biochemistry*: A Functional Approach. London: W.B. Sounders Co., p. 296-307.
11. Meyer, V. E. (1994). *Practical High-Performance Liquid Chromatography.* Chichester: John Wiley & Sons.
12. Pacco, A., Absillis, G., Binnemans, K & Parac-Vogt, T.N. 2008. Copper (II) 15 - metallacrown -5 lanthanide (III) complexes derived from 1-serine and l-threonine hydroxamic acids. *J Alloy Compd* 451: 38-41.
13. Servat, F., Montet, D., Pina, M., Gazly, P., Arnaud, A., Ledon, H., Marcau, L & Graillie, J. (1990). Synthesis of fatty hydroxamic acids catalyzed by the lipase of *mucor meihei*. *J Am Oil Chem Soc* 67(10): 646-649.
14. Skoog, D. A., Holler, F. J., & Neiman, T. A. (1998). *Principles of Instrumental Analysis, Fifth ed.,* Singapore: Thomson Learning Academic Resource Centre.
15. Snyder, L. R., & Kirkland, J. J. (1979). *Introduction to Modern Liquid Chromatography,* Toronto: John Wiley & Sons.
16. Suhendra, D., Haron, M. J., Silong, S., Basri, M., & Wan Yunus, W. M. Z. (2006). Separation and preconcentration of copper (II) ion by fatty hydroxamic acids immobilized onto amberlite XAD– 4 Resin. *Ind J Chem*, 6(2): 165-169.
17. Suhendra, D., Haron, M. J., Silong, S., Basri, M & Wan Yunus, W. M. Z. (2005a). Enzymatic synthesis of fatty hydroxamic acids from palm oil. *J Oleo Sci*, 54(1): 33-38.
18. Suhendra, D., Yeen, K. P., Haron, M. J., Silong, S., Basri, M., & Wan Yunus, W. M. Z. (2005b). Copper ion extraction by a mixture of fatty hydroxamic acids synthesized from commercial palm olein. *Solvent Extr Ion Exc J*, 23(5): 713-723.
19. Vaysse, L., Dubreueq, E., Pirat, J. L., & Galzy, P. (1997). Fatty hydroxamic acid biosynthesis in aqoeous medium in the presence of the lipase-acyltransferase from *candida parapsilosis. Journal of Biotechnology,* 53: 41-46.
20. Yee, L. N., Akoh, C. C., & Philips, R. S. (1997). Lipase PS-catalyzed Transesterification of Citronellyl Butyrate and Geranyl Caproate: Effect of Reaction Parameters. *J Am Oil Chem Soc*, 74: 255-259.
21. Zamora, A. (2005). *Fats, Oils, Fatty Acids, Triglycerides*. Retrieved from https://www.scientificpsychic.com/fitness/fattyacids1.html.