ENZYMATIC SYNTHESIS AND APPLICATION OF PALM OIL-BASED FATTY HYDROXAMIC ACIDS

By

DEDY SUHENDRA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

August 2004

DEDICATION

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

قَالُواْ سُبۡحَٰنَكَ لَا عِلۡمَ لَنَآ إِلَّا مَا عَلَّمۡتَنَآۖ إِنَّكَ أَنتَ ٱلۡعَلِيمُ ٱلۡحَكِيمُ ﴿٣٢﴾

"Mereka Menjawab: Maha Suci Engkau, tidak ada yang kami ketahui selain apa yang telah Engkau ajarkan kepada kami; sesungguhnya Engkaulah Yang Maha Mengetahui lagi Maha Bijaksana" (Al-Baqarah 32)

While this dissertation was in preparation, my beloved mother, Siti Rodiah binti Sugiono, passed away after a lengthy illness. I dedicate this thesis special to Her. I also dedicate this dissertation to all my family and Ibu Pertiwi.........Indonesia

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of requirement for the degree of Doctor of Philosophy

ENZYMATIC SYNTHESIS AND APPLICATION OF PALM OIL-BASED FATTY HYDROXAMIC ACIDS

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Chairman: Professor Wan Md Zin Wan Yunus, Ph. D

Faculty: Science and Environmental Studies

Fatty hydroxamic acids (FHA) have been successfully synthesized from palm oils by a one-step lipase catalyzed reaction. Conversion of palm oils into the FHA was carried out by treating the substrate with hydroxylamine at 30^0C and neutral pH for 30 h. The method employed offers technical simplicity and easy isolation of the enzyme from the products and other components in the reaction mixture. Moreover, it also allows the reaction to be carried out under mild conditions, which reduces unwanted products.

The percentages of conversion of commercial palm olein, RBD palm olein, RBD palm oil, RBD palm stearin and RBD palm kernel olein into their fatty hydroxamic acids, under the optimum conditions were 89, 79, 77, 90 and 98, respectively. The presence of the hydroxamic acid groups in the purified products

was confirmed by the qualitative test, FTIR analysis, CHN elemental analysis and HPLC.

Based on the ability of hydroxamic acid as a metal chelator, the synthesized FHA was used as a reagent for metal ion extraction and spectrophotometric determination. Spectrophotometry of complexes of vanadium (V) – and iron (III) – FHA shows that the molar extinction coefficient (ε) of vanadium (V) – FHA and iron (III) – FHA complexes were 6500 L mol^{-1} cm^{-1} and 9600 L mol^{-1} cm^{-1}, respectively. In addition, the detection limit of vanadium (V) – FHA and iron (III) – FHA complexes were 0.001 x 10^{-4} and 0.008 x 10^{-4} M, respectively. The mole ratio for the vanadium (V) – FHA complex was 3:1 while for the iron (III) – FHA was 2:1.

The FHA was also used as an extractant for recovery of copper (II) and iron (III) ions from aqueous media. Separations of copper (II) from other metal ions such as Co (II), Ni (II), Cd (II) and Zn (II) are conveniently achieved in the pH range of 4 to 6. A single extraction and stripping gave a good separation and preconcentration of copper (II) and iron (III). The separation of copper (II) can be accomplished quantitatively from other metal ions. However copper (II) cannot be quantitatively separated from iron (III) in a mixture. A preconcentration process has been proposed for the determination of copper (II) in water samples, which contains trace concentrations of copper (II), which cannot be measured directly by FAAS. It has been shown that the extraction of aqueous phase containing copper (II) with organic phase containing FHA and then stripping the organic phase with

10 % HNO_3 gave a solution of copper (II) 10 fold in concentrations. Meanwhile, extraction of iron (III) from aqueous solution shows a selective extraction, in which only iron could be extracted in pH 2.

The FHA synthesized from commercial palm olein was successfully immobilized onto Amberlite XAD-4 and Amberlite XAD-7 resins. The FHA loaded Amberlite XAD-4 (FHA-Amb) has been successfully used for the separation and preconcentration of copper (II) and iron (III) ions from aqueous solutions. The effect of factors such as pH, sample volume, flow rate and concentration of eluant on the preconcentration efficiency were investigated. It was found that quantitative recovery of copper (II) ion from FHA loaded Amberlite XAD-4 resin was obtained using HNO_3 (10%) as eluant with a preconcentration factor up to 60. The methods for the separation of copper (II) from Zn (II) and Cd (II) have been proposed. From the separation studies of iron (III) in a solution containing Cu (II), Zn (II), Cd (II) and Ni (II) ions by FHA-Amb, it was found that only iron (III) was extracted by the resin at pH 2. This indicates that FHA is a selective chelating agent for the separation of iron (III) ion from Cu (II), Zn (II), Ni (II) and Cd (II) ions at pH 2. While, the preconcentration studies of iron (III) ion from aqueous media showed that the percentage recoveries for all preconcentration factors are 100%. This indicates that the FHA-Amb can be applied for the preconcentration of iron (III) ion in aqueous media.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Doktor Falsafah

SINTESIS BERENZIM DAN APPLIKASI BAGI ASID LEMAK HIDROKSAMIK DARIPADA MINYAK SAWIT

Oleh

DEDY SUHENDRA

Ogos 2004

Pengerusi: Professor Wan Md. Zin Wan Yunus, Ph. D

Fakulti : Sains dan Pengajian Alam Sekitar

Asid-asid lemak hidroksamik (FHA) telah berjaya disintesiskan daripada minyak kelapa sawit dengan tindakbalas satu peringkat menggunakan enzim lipase sebagai mangkin. Penukaran daripada minyak kelapa sawit kepada FHA telah dilakukan dengan mencampurkan substrat dan hidroksilamina pada suhu 30^{o}C dan pH neutral selama 30 jam. Kaedah sintesis berenzim FHA yang dijelaskan di dalam laporan ini adalah ringkas, mudah dari segi teknikal dan pemisahan yang mudah bagi enzim daripada produk serta unsur-unsur lain di dalam campuran tindak balas. Selain itu ia juga disediakan dalam keadaan yang sesuai dimana ianya mengurangkan penghasilan bahan yang tidak dikehendaki melalui tindakbalas sampingan.

Peratus penukaran bagi Minyak Sawit Olein Komersial, Minyak Sawit Olein RBD, Minyak Sawit RBD, Stearin Minyak Sawit RBD dan olein isirong minyak sawit RBD kepada Asid lemak hidroksamik masing-masing adalah 89, 79, 77, 90 and 98. Analisis kualitatif menggunakan analisis FTIR, analisis unsur CHN dan kromatografi cecair berprestasi tinggi (HPLC) telah dijalankan untuk membuktikan kehadiran kumpulan asid hidroksamik di dalam produk yang telah ditulenkan.

Kajian aplikasi bagi produk sebagai reagen bagi kimia analisis telah dijalankan berdasarkan kebolehan FHA sebagai agen pengkelat. Penentuan spektrofotometrik bagi kompleks vanadium (V)-FHA serta besi (III)-FHA menunjukkan tahap pengesanan yang rendah ditunjukkan dengan keamatan warna kompleks yang tinggi. FHA yang disintesis daripada sawit olein komersial juga digunakan sebagi pengestrak bagi mendapatkan kuprum (II) dan besi (III) daripada media akueus. Pemisahan kuprum (II) daripada ion logam lain seperti Co (II), Ni (II), Cd (II) dan Zn (II) lebih mudah dicapai pada pH antara 4 ke 6. Pengestrakkan dan penanggalan tunggal memberikan pemisahan dan pra-pemekatan yang baik bagi kuprum (II) dan besi (III). Pemisahan bagi kuprum (II) boleh disempurnakan secara kuantitatif daripada ion logam lain. Walau bagaimanapun kuprum (II) tidak boleh dipisahkan daripada campuran besi (III). Proses pra-pemekatan dicadangkan bagi penentuan kandungan kuprum (II) di dalam sampel air, yang mengandungi paras kepekatan kuprum (II) yang tidak dapat diukur secara langsung oleh AAS. Ini telah ditunjukkan dengan pengestrak fasa akueus yang mengandungi kuprum (II) dengan fasa organik yang mengandungi FHA dan penanggalan daripada fasa organik dengan larutan 10% HNO_3 memberikan pemekatan larutan kuprum (II) sebanyak 10 kali ganda. Sementara itu pengekstrakan bagi besi (III) daripada larutan akueus menunjukkan pengekstrakkan yang selektif di mana hanya ion besi dapat diekstrak pada pH 2.

FHA yang disintesis daripada olein minyak sawit berjaya dipegunkan kedalam resin Amberlite XAD-4 dan Amberlite XAD-7. Amberlite XAD-4 yang pegunkan dengan FHA (FHA-Amb) telah berjaya digunakan bagi pemisahan dan pra-pemekatan bagi ion kuprum (II) dan besi (III) daripada larutan akueus. Kesan faktor pH, isipadu sampel, kadar aliran serta kepekatan bagi larutan pengelusi terhadap keberkesanan pra-kepekatan juga telah dikaji. Keputusan menunjukkan perolehan semula adalah kuantitatif bagi ion kuprum (II) daripada resin Amberlite XAD-4 dimasukkan FHA dapat diperoleh menggunakan HNO_3 (10%) sebagai larutan pengelusi dengan faktor pra-kepekatan sehingga 60. Kaedah pemisahan bagi kuprum (II) daripada Zn (II) dan Cd (II) telah dicadangkan. Di dalam kajian pemisahan bagi ion besi (III) daripada ion kuprum (II), Zn (II), Ni (II) dan Cd (II) pada pH 2. Kajian pra-pemekatan bagi ion besi (III) daripada media akuas menunjukkan peratusan perolehan semula bagi semua faktor pra-pemekatan adalah 100%. Ini menunjukkan bahawa FHA-Amb boleh diaplikasikan bagi pra-pemekatan untuk ion besi (III) di dalam media akuas.

ACKNOWLEDGEMENTS

In The Name of ALLAH, The Most Merciful and Most Beneficent

All praises do to Allah, Lord of the universe. Only by His grace and mercy this thesis can be completed.

This work was carried out with a hope to contribute towards the expansion of our currently limited knowledge on Analytical Chemistry. The completion of this thesis would have been impossible if not for the assistance and direct involvement of so many kindhearted individuals. Thus, I am very much indebted to my previous mentors and I have no way of repaying such a debt except to express my sincerest gratitude.

First and foremost, I am very grateful to my adviser Professor Wan Md. Zin Wan Yunus, Ph. D. for his strong support, guidance and patience for the very enriching and thought provoking discussions and lectures which helped to shape the thesis. He was always there to provide everything I needed in the laboratory. I would also like to thank him besides Assoc. Prof. Dr. Md. Jelas Haron for providing financial support during the period of study through the IRPA research fund.

I am also grateful to Prof. Dr. Mahiran Basri, Assoc. Prof. Dr. Md. Jelas Haron and Assoc. Prof. Dr. Sidik Silong in their capacities as members of the Supervisory Committee. Thank you for the comments and suggestions, which contributed a lot towards the improvement of the final manuscript. I am also indebted to the staff of the Department of Chemistry, Universiti Putra Malaysia for their help and cooperation.

Special thanks are extended to other 407 lab members (Aidil, Shahanna, Wee Chang, Su Fang, Kwa Pei, Wei Chee, Abby and Yahya al Iraqi) who helped me in every way possible and providing a congenial and enthusiastic atmosphere in the laboratory. Special thanks are also extended to Mr. Ekramul Mahmud and Umar al Faruq as editors of the first draft of this thesis. Acknowledgement is also extended to Indonesian Student Association that joined us in sweet friendship and made life easier during my stay in Malaysia.

I wish to express my deepest gratitude to my parents, brothers and sisters for their prayers, continuous moral support and unending encouragement. Last but not least, I wish especially to acknowledge my beloved wife, Erin Ryantin Gunawan, and my dearest children Erdy Izzatuffikri and Erdanisa Aghnia Ilmani for their love, support, patience and understanding.

I certify that an Examination Committee met on 19 August 2004 to conduct the final examination of Dedy Suhendra on his Doctor of Philosophy thesis entitled "Enzymatic Synthesis and Application of Palm Oil-based Fatty Hydroxamic Acids" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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DECLARATION

I HEREBY DECLARE THAT THE THESIS IS BASED ON MY ORIGINAL WORK EXCEPT FOR QUOTATIONS AND CITATIONS, WHICH HAVE BEEN DULY ACKNOWLEDGED. I ALSO DECLARE THAT IT HAS NOT BEEN PREVIOUSLY OR CONCURRENTLY SUBMITTED FOR ANY OTHER DEGREE AT UPM OR OTHER INSTITUTIONS.

DEDY SUHENDRA

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