UNIVERSITI PUTRA MALAYSIA

ENZYME CATALYZED SYNTHESIS OF FATTY MONOETHANOLAMIDES FROM PALM KERNEL OIL FRACTIONS

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BY

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LIST OF ABBREVIATIONS

PKL	palm kernel olein
PKS	palm kernel stearin
PKO	palm kernel oil
FAME	fatty acid methyl ester
HLB	hydrophile-lipophile balance
O/W	oil in water
Log P	logarithm of the partition coefficient
Aw	water activity
TLC	thin layer chromatography
FT-IR	fourier transform infra red
GLC	gas liquid chromatography
GC	gas chromatography
HPLC	high performance liquid chromatography
NMR	Nuclear magnetic resonans
TNBS	trinitrobenzene sulfonate
PEG	monomethoxypolyethylene glycol
FFA	Free Fatty Acid

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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Faculty : Science and Environmental Studies

Fatty monoethanolamides were synthesized in organic solvent from palm kernel olein (PKL) and palm kernel stearin (PKS) using a lipase from *Candida rugosa* The transamidation reactions of PKL and PKS were enhanced in the presence of lipase Fatty monoethanolamides were characterized by melting point, spectroscopic (Infrared red and nuclear magnetic resonance) and gas chromatography

The optimal yield was achieved at reaction time 72 hours for both systems with PKL and PKS as the substrates For PKL as the substrates, the optimal reaction temperature was 40°C, whereas with PKS as the substrate no optimum temperature was found (in the range of temperature studied) where the relative yield increased with the increasing temperature It suggested that the temperature effect on the transamidation of PKS was more significant compared to the transamidation for PKL In both systems the precipitation of

high melting point fatty monoethanolamides hindered the progress of reactions. Lipase functioned better in hydrophobic solvents compared to hydrophilic solvents. The best solvent for the reactions was isooctane. The increasing amount of monoethanolamine used also resulted in the increase solubility of the reactants and products, hence, increase the yield. For PKL, increasing the mole ratio of PKL : monoethanolamine to 1:15 increased the relative yield to 4.45-fold than the relative yield of that PKL : monoethanolamine at mole ratio 1:1. However for PKS, its relative yield was only 2.5-fold more than the relative yield of that PKS : monoethanolamine at mole ratio 1:1. The optimal ratio of enzyme/PKL (or PKS) was 0.035. An excess of enzyme caused mass transfer limitation. In the water activity studies, the preequilibrium and the direct salt hydrate addition methods were used. Both observations were not in agreement and did not show the actual effects of water activity in transamidation reactions. The enzymes tend to aggregate and did not fully express its function. The addition of support hardly improved the conditions. Overall, PKL always showed the higher relative yield compared to PKS. At the optimum conditions, the yield of PKL monoethanolamide was 77.64% and the yield of PKS monoethanolamide was 39.32%. Kinetic studies also showed a clear preference to PKL which its K_m value 10-fold lower than that of PKS at room temperature.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia bagi memenuhi keperluan Ijazah Master Sains.

PENYEDIAAN MONOETANOLAMIDA BERLEMAK DARIPADA FRAKSI MINYAK ISIRONG KELAPA SAWIT DENGAN MENGGUNAKAN ENZIM SEBAGAI MANGKIN

oleh

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Monoetanolamida berlemak disediakan daripada fraksi olein (PKL) dan stearin (PKS) minyak isirong kelapa sawit dalam pelarut organik. Lipase *Candida rugosa* digunakan sebagai mangkin dalam tindak balas ini. Keputusan menunjukkan kehadiran lipase mempertingkatkan tindak balas transamidasi PKL and PKS. Monoetanolamida berlemak yang terbentuk dicirikan dengan cara penentuan takat lebur, cara spektroskopik (Infra merah dan resonans magnetik nuklear) dan analisis kromatografi gas.

Pembentukkan monoetanolamida berlemak mencapai optimum dalam masa 72 jam bila PKL atau PKS sebagai substrat. Bila PKL sebagai substrat, suhu tindak balas optimum adalah 40°C; tetapi bila PKS sebagai substrat, tiada suhu optimum ditemui dalam jarak suhu yang dikaji dan hasil relatifnya didapati meningkat dengan penambahan suhu. Ini mencadangkan kesan suhu ke atas tindakbalas transamidasi PKS adalah lebih ketara daripada tindakbalas

transamidasi ke atas PKL. Dalam kedua-dua sistem ini, hasil pepejal yang tinggi telah menghalang tindak balas untuk berterusan. Lipase berfungsi dengan lebih baik dalam pelarut hidrofobik berbanding dengan pelarut hidrofilik. Pelarut yang paling baik untuk kedua-dua sistem adalah isooctana. Penggunaan monoetanolamina yang banyak dalam tindak balas telah menambahkan kelarutan substrat dan hasil pepejal, dengan itu meningkatkan hasil. Dalam sistem PKL sebagai substrat, secara relatif hasil tertinggi dicapai pada nisbah mol PKL : monoetanolamina 1:15, ini merupakan 4.5 kali lebih tinggi daripada hasil pada nisbah mol PKL : monoetanolamina 1:1. Namun, dalam sistem PKS sebagai substrat pula, secara relatif hasil hanya 2.5 kali lebih tinggi daripada hasil pada nisbah mol PKS : monoetanolamina 1:1. Selain daripada itu, didapati nisbah optima bagi enzim/PKL (atau PKS) adalah 0.035. Enzim yang terlalu banyak boleh menghalang pemindahan jisim. Dalam kajian aktiviti air, cara keseimbangan dan cara penambahan terus garam terhidrat digunakan. Kedua-dua cara ini menunjukkan keputusan yang berlainan dan juga tidak dapat mencerminkan kesan sebenar aktiviti air ke atas tindakbalas transamidasi. Ini adalah kerana enzim berkecenderungan berkumpul bersama dan membentuk mendakan, Maka ia tidak dapat berfungsi dengan sepenuhnya. Malah, penambahan bahan sokongan ke dalam sistem juga tidak banyak membaiki keadaan. Secara keseluruhan, PKL menunjukkan relatif hasil yang lebih tinggi berbanding dengan PKS. Dalam keadaan optimum, PKL monoethanolamida yang terbentuk ialah 77.64% dan PKS monoethanolamida yang terbentuk ialah 39.32%. Kajian kinetik juga menunjukkan nilai K_m bagi PKL adalah 10 kali lebih rendah daripada nilai bagi PKS pada suhu bilik.

CHAPTER I

INTRODUCTION

Enzymes are catalysts which perform the function of inducing and governing reactors, as well as increasing the reaction rates. The conventional wisdom for the enzyme knowledge has been derived from studies of enzymes in aqueous solutions. However, two alternative approaches conducting enzymatic processes in nonaqueous media have been successfully developed. In the first approach (Luisi, 1985, Martinek *et al.*, 1986), enzymes are dissolved in micropools of water which are emulsified in water immiscible solvents; the microemulsion is stabilized by surfactants that form "reverse micelles". In the second approach (Klibanov, 1986), powdered enzymes are directly suspended in organic solvents.

In reverse micelles, the enzyme is confined to a water pool which, in turn, is insulated from the organic solvent by a monolayer of surfactant. Therefore, inherent catalytic properties of enzymes in reverse micelles are generally similar to those in aqueous solutions (Luisi, 1985; Martinek *et al.*, 1986). In contrast, solid enzymes dispersed in organic solvents are directly exposed to the solvents and hence exhibit some remarkable novel properties compared to those in water (Klibanov, 1986).

Enzymes in organic solvent greatly increased thermal stability (Zaks and Klibanov, 1984, Wheeler and Croteau, 1986, Ayala *et al*, 1986) and have strikingly different substrate specificity (Zaks and Klibanov, 1984, Zaks and Klibanov, 1986) Also, many transformations that are impossible in aqueous solutions due to kinetic or thermodynamic reasons, can be readily catalyzed by enzymes in organic solvents (Klibanov, 1986, Zaks and Klibanov 1985) Consequently, a number of interesting and useful enzymatic conversion in organic solvent have been accomplished such as esterification, interesterification, transesterification, amidation and peptide synthesis

The growing need for highly specific chemicals and biochemicals, which are only slight soluble or completely insoluble in water, and which sometimes cannot be synthesized in a purely chemical way, has been a challenge and a driving force for the fundamental study of the behaviour of enzymes in organic media and of the possible applications of these biocatalysts in technological processes The hydrolase, i e lipases and proteases is the major enzyme group that is being investigated However, most of the studies carried out involved in the synthesis of organic esters (Yamane *et al*, 1989, Basri *et al*, 1995, Habulin *et al*, 1996 and Linko and Wu, 1996) Not much work on the synthesis of fatty monoalkanolamide has been reported

Fatty alkanolamides are among the high volume and industrially important secondary fatty amides produced In 1989, 107 million kg of fatty alkanoamides were produced in USA (Fearheller *et al*, 1994), they comprise about 10% of all fatty nitrogen compounds produced Fatty monoalkanolamides have a broad spectrum of uses due to their diversity of physical properties, economy and ease of preparation

Fatty monoethanolamides are prepared commercially at temperature ranging 150-200°C Reaction times for these fatty monoethanolamides preparations can be as long as 10 hours and the process are energy intensive Some of them give rise to a variety of undesirable side reactions (Horvath, 1985)

Fearheller *et al*, (1994) showed that if the amidation was conducted with triglyceride and monoethanolamine at a fat monoethanoamine molar ratio of 1 10 at temperature 50-60°C for 8 hours, fatty monoethanolamide would be produced in high yield However, a high concentration of toxic monoethanolamine residue was left in the product mixture Therefore, the application of the product was restricted The separation of toxic monoethanolamine residue from the product mixture was not economic

The application of new biotechnological techniques such as the use of biocatalyst may offer an improvement to the manufacture of fatty monoalkanolamides over these conventional methods Enzyme catalyzes reactions at mild temperature and atmospheric pressure Thus, it may require less expensive equipment The enzyme and the organic solvent also can be recovered for reuse, this could further reduce the production cost In addition, enzyme works to modify specific chemical bonds at specific sites on a molecule, in contrast to ordinary chemical reactions which occur randomly in response to the law of thermodynamics This enables the enzyme to promote a highly selective transformation (Whitesides and Wong, 1985) Therefore, the relatively pure products can be produced in a very simple procedure with high efficiency The unwanted side products that normally appear in the waste stream also would be reduced The enzyme-based processes tend to have

lower waste treatment costs (Posorske, 1984) Finally, the enzymatic process is also non-toxic and non-corrosive, it is relatively environmental friendly compared with the conventional process which may involved toxic materials (Salleh, 1996)

Organic chemists using enzymes as catalysts tend to focus on the starting materials and the end products without paying much attention to the intermediate "biochemical machinery" necessary for catalysis No matter how useful this approach may be, it is necessary to pay attention to the reaction conditions (Halling *et al*, 1992) In the synthesis of fatty monoethanolamide, the reactants are carboxylic acid (or ester) and monoethanolamine This reaction is complicated by competing reactivity of the several functional groups present such as hydroxyl group and amide group Consequently, the composition of the products can vary considerably depending on the mole ratio and reaction conditions employed Enzyme properties and the rate of reaction also depend upon the reaction condition Therefore, transamidation in various reaction conditions was investigated in this study

To date there has been no published report on the enzymatic synthesis of fatty monoethanolamide Also, no previous papers showed the direct usage of palm kernel fractions as the substrate for transamidation Since Malaysia is a major producer of palm kernel oil (see pg 6 Table 1), the oil is prevalent and available at cheaper price, it is of our interest to widen their uses in oleochemical industries

Therefore, the objectives of study are

1. To synthesize fatty monoethanolamides from palm kernel olein and palm kernel stearin using lipase.

2. To prepare the individual standards of fatty monoethanolamides.

3. To investigate the optimum reaction conditions, including the reaction time, temperature, mole ratio of the substrates, concentration of enzyme, solvents and water activity.

CHAPTER II

LITERATURE REVIEW

Palm Kernel Oil

Palm kernel oil is an important co-product from the milling of the fruits of the palm, *Elaeis guineensis* Malaysia is a major producer of palm kernel oil Currently the oil is exported in the crude and refined forms Only a small percentage is used locally in the oleochemical industry (Table 1) Therefore, there is a need to expand the oleochemical consumption of it

Table 1 Production and Oleochemical Consumption of Palm Kernel Oil in Malaysia

		1988	1990	1995
PKO (x 1000 MT)	Production	580	810	930
	Consumption (oleochemicals)	38 7	120 5	674 2

(PORLA, 1996)

The outer portion of the oil palm fruit is a soft pulp which contains palm oil Whereas the kernel of the fruit is enclosed in a hard shell which makes it

simple to separate the pulp from the interior seed. Palm kernel oil comes from this seed (Weiss, 1983) and oil content of dried kernels is 44-53% (Swern, 1979). The two oils are quite different from each other in composition. The fatty acids of palm oil are essentially 45% palmitic acid and 55% mixed 18 carbon acids (Weiss, 1983). Palm kernel oil is a lauric fat, with a smaller quantities of the shortest-chain, namely, caproic and caprylic; hence its titer is only slightly different from that of coconut oil (Swern, 1979).

Palm Kernel Stearin and Palm Kernel Olein

Palm kernel stearin is the solid phase and palm kernel olein is the liquid phase from the fractionation of palm kernel oil. Table 2 present some of the common chemical and physical characteristics of them as compared with palm kernel oil. Table 3 shows the fatty acid composition compared with palm kernel oil and palm oil. Table 4 shows the triglyceride compositions of palm kernel stearin and palm kernel olein compared with palm kernel oil

The lower iodine value of palm kernel stearin compared to the palm kernel oil indicates the reduction in unsaturated acid content (Table 2). PKS shows a significant increase in medium chain fatty acid (lauric and myristic acids) and the corresponding decrease in short chain fatty acid (caprylic, capric) and unsaturated fatty acid (oleic and linoleic acids) comparison with palm kernel oil (Table 3). This is an agreement with the triglyceride composition where there is a significant increase in C36, C38 and C40, which consist of mainly lauric (C12) and myristic (C14) acids. There is a corresponding reduction in the smaller triglycerides (C28, C30, and C34) which are those containing the short

Table 2 Physical and Chemical Characteristics of Palm Kernel Olein and Palm Kernel Stearin

Parameter	Palm kernel oil	Palm kernel olein	Palm kernel stearin
Iodine value (Wijs)	18 0	23 0	7 0
Saponification Value (mg KOH/g)	249	239	248
Unsaponifiable matter (% by wt)	0 31	0 36	0 32
FFA (as C 12 0)	1 25	3 03	1 3
Refractive index (40°C	1 4514	1 4518	1 4500
Apparent Density (g/mL 40°C	0 9047	0 9045	0 9046
Slip melting point (°C)	28 0	23 3	32 1

(PORIM 1996)