

Synthesis and Characterization of Fatty Hydroxamic Acids from Triacylglycerides

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Abstract: In this study, fatty hydroxamic acids (FHAs), which have biological activities as antibiotics and antifungal, have been synthesized via refluxing of triacylglycerides, palm olein, palm stearin or corn oil with hydroxylamine hydrochloride. The products were characterized using the complex formation test of hydroxamic acid group with zinc(I), copper(II) and iron(III), various technique methods including nuclear magnetic resonance (^1H NMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy and elemental analysis. Parameters that may affect the conversion of oils to FHAs including the effect of reaction time, effect of organic solvent and effect of hydro/oil molar ratio were also investigated in this study. Results of characterization indicate that FHAs were successfully produced from triacylglycerides. The conversion percentages of palm stearin, palm olein and corn oil into their fatty hydroxamic acids are 82, 81 and 78, respectively. Results also showed that hexane is the best organic solvent to produce the FHAs from the three oils used in this study. The optimum reaction time to achieve the maximum conversion percentage of the oils to FHAs was found to be 10 hours for all the three oils, while the optimum molar ratio of hydro/oil was found to be 7:1 for all the different three oils.

Key words: hydroxamic acid, triacylglyceride, reflux, fatty hydroxamic acids, palm olein

1 INTRODUCTION

Hydroxamic acid (N-hydroxy carboxylic acids) which has general formula of RCONHOH may be regarded as derivatives of both hydroxylamine and carboxylic acids. A hydroxamic acid and their derivatives have attracted a lot of attention due to their biological activity as antifungal agents, food additives and chelators^{1,2} which chelate transition metals in particular. This property is mainly used in analytical chemistry, therapeutics and agronomy. Hydroxamic acids have been studied with respect to chelates with ferric ion for medical use and as siderophore models³. A hydroxamic acid that specifically inhibits urease; it retards alkalization of the urine caused by urease-producing bacteria and may inhibit bacterial growth. Used in the prevention and dissolution of uroliths, but in dogs causes a dose-related, reversible hemolytic anemia and blood dyscrasia^{4,5}. FHAs are also used as heavy metal conveyors in liquid-liquid extraction (aqueous phase/organic solvent) in the nuclear industry⁶. Other uses include, synthesized a series of alkyloxybenzoyl hydroxamic acids and fatty acyl hydrox-

amic acids and found that some of these chemicals possessed antifungal activity⁷⁻⁹.

Several studies were carried out for synthesis of hydroxamic acids from acids and esters with hydroxylamine¹⁰⁻¹³. Servat *et al.* (1990) reported that the synthesis of alkanohydroxamic acids (N-acyl hydroxylamines) by Rhizomucor miehei lipase-catalyzed hydroxylaminolysis of fatty acids and soybean methyl ester¹⁴. Dedy *et al.* (2005) synthesized fatty hydroxamic acids from palm oil using immobilized lipase as the catalyst¹⁵.

In this study, we have investigated the use of reflux method for preparation of fatty hydroxamic acids from different oils including palm olein, palm stearin and corn oil with hydroxylamine hydrochloride. Palm olein, palm stearin and corn oil are a mixture of triacylglycerides, just like any ordinary fat, which are esters of glycerol with different saturated and unsaturated fatty acids. Basiron *et al.* (2007) reported that Malaysia is currently the world's largest producer and exporter of palm oil and it is the major source of vegetable oil for industrial application¹⁶.

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Accepted September 4, 2009 (received for review May 1, 2009)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/>

No information was found in the accessible literature about production of fatty hydroxamic acids directly from triacylglycerides of oils without using enzymatic catalyst.

2 MATERIALS AND METHODS

2.1 Materials

The hydroxylamine hydrochloride used in this study was provided by Fluka, Switzerland. Both palm olein and palm stearin were obtained from Ngo Chew Hong oils and fats (M) Sdn.Bhd. Malaysia. The corn oil was purchased from local Malaysian market. Hexane, cyclohexane, chloroform and n-butane were provided by T. J. Baker, USA.

2.2 Synthesis of (FHAs)

Palm olein was dissolved in hexane with Hydroxylamine hydrochloride by reflux at boiling point of hexane for 10 h using a thermostated round bottom flask equipped with water-cooled condenser and mechanical stirrer. After the reaction had finished (product changed the colour to green with copper (II) due to its ability to form complex). The product was dissolved in hot hexane and separated from bottom layer by separating funnel. The hexane phase was cooled in an ice bath for 4 h to obtain FHAs and then filtered and washed by hexane for three times and dried in a vacuum desiccators over phosphorous pentoxide.

The same procedure was used to produce the FHAs from palm stearin and corn oil. Scheme 1 shows the equation of reaction.

2.3 Characterization

Qualitative analysis of hydroxamic acid group on FHAs were carried out by observing the colored complex formed after methanolic solution of the FHAs reacted with zinc (II), copper (II) and iron (III) in the dilute hydrochloric acid solution. The amount of the hydroxamic acid group was estimated based on nitrogen content of the dry FHAs determined by elemental analyzer model 932 (LECO, USA). The measure range of FTIR spectra was 4000- 280 cm^{-1} in a Parkin-Elmer 1650 infrared Fourier transform spectrometer, using the KBr pellet technique (about 1 mg of sample and 300 mg of KBr were used in the preparation of the pellets). The mixture was then pressed under 8 tons load for 1

min to produce the disc.

^1H Nuclear Magnetic Resonance (NMR) spectra were recorded using NMR spectrophotometer (Joel Ltd., Tokyo, Japan).

3 RESULTS AND DISCUSSION

3.1 Effect of Reaction Time

The effect of the reaction time on the conversion of FHAs from palm olein was recorded at an interval of one hour during the test. Figure 1 shows that the conversion percentage proportionally increased as the reaction time progressed. Where Y is the conversion percentage of FHAs and X is the time of reaction (hours).

After 10 h of the test, no significant increase in the conversion percentage was observed which leads to the conclusion that the reaction becomes in an equilibrium state or at low limit state of mass transfer. The figure indicates that the maximum conversion of FHAs (about 81%) was achieved after 10 h.

3.2 Effect of Organic Solvent

The type of organic solvent used in the synthesis of the FHAs may play a significant rule in the process as it is

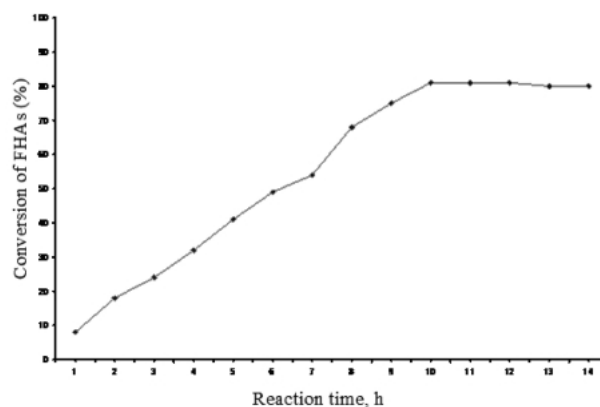
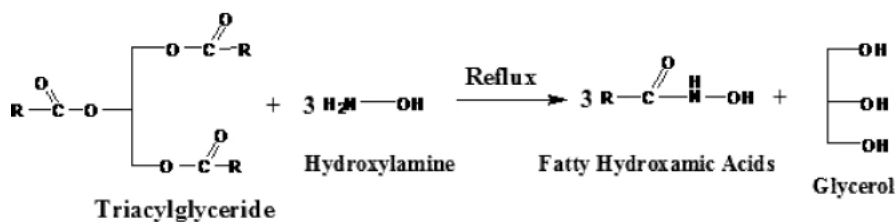


Fig.1 Effect of Time Reaction on Conversion Percentage of FHAs from Palm Olein.

Reaction condition hydroxylamine hydrochloride /palm olein molar ratio 7:1, hexane volume 17 mL and temperature 69 $^{\circ}\text{C}$.



Scheme 1 The Reaction Equation of Triacylglyceride with Hydroxylamine.

responsible for portioning of the triacylglycerides at different phases¹⁷. Four various organic solvents, namely, hexane, cyclohexane, chloroform, and n-butane were used individually in this study to examine the effect of the organic solvent on the conversion percentage of FHAs (Fig. 2).

The maximum conversion percentage of three oils into FHAs against the various organic solvents as shown in Table 1. It can be seen that hexane achieved the highest conversion percentage for all the three oils. The conversion percentage of the palm stearin, palm olein, and corn oil into FHAs using hexane as a solvent were 82%, 81%, and 78%, respectively. On the other hand, using of n-butane as an organic solvent relatively resulted in the lowest conversion percentage of the three different oils into FHAs due to higher solubility of the substrate and ability of separation product and substrate into 2 phases¹⁸. Additionally, the logP value of hexane is above 3.0. It can also be noticed that the conversion percentages of the palm stearin were the highest in all four organic solvents compared to those of the palm olein which is in turn were higher than those of

corn oil.

These results are in agreement with those published by Dedy *et al.* (2005) which show that hexane was the most efficient solvent for production of fatty hydrazine from oils¹⁵.

3.3 Effect of concentration of hydroxylamine

The molar ratio of hydroxylamine to oil can be one of the most important parameters that affect the conversion of the oil to FHAs. Stoichiometrically, each mole of palm olein required 3 mol of hydroxylaminolysis^{18,19}. In practice, the hydroxylaminolysis reaction generally requires a large excess of hydroxylamine to shift the equilibrium favourably. Various molar ratios of hydroxylamine to oil ranging from 1:1 to 10:1 were used with all three oils to determine the effect of Hydro/oil molar ratio on the conversion percentage of the oils to FHAs.

Figure 3 illustrates the conversion percentages of palm olein to FHAs against the molar ratio of hydro to palm olein. As can be noticed that the conversion percentage of the palm olein into FHAs directly increased as the molar

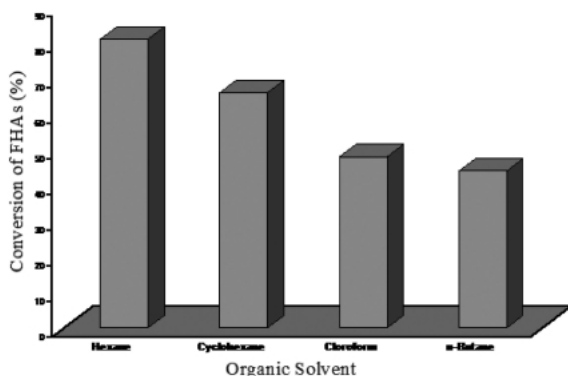


Fig.2 Conversion of Palm Olein as a Function of Organic Solvent.

Reaction conditions: hydroxylamine hydrochloride / palm olein molar ratio 7:1, hexane volume 17 mL, temperature 69 C° and reaction time 10 h.

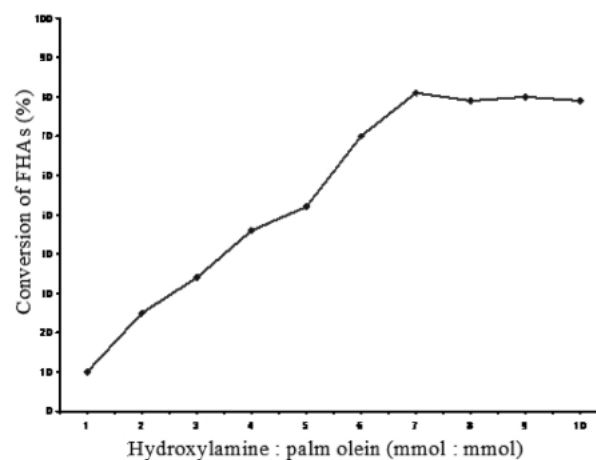


Fig.3 Effect of Molar Ratio of Hydroxylamine to Palm Olein on the Conversion Percentage of the Oil to FHAs (temperature of reaction is 69°C, time of reaction is 10 h, and organic solvent is hexane).

Table 1 Conversion Percentages of Palm Stearin, Palm Olein, and Corn Oil into FHAs Using Different Organic Solvents.

Solvent	Conversion (%)		
	Palm stearin	Palm olein	Corn oil
Hexane	82	81	78
Cyclohexane	71	70	66
Chloroform	54	52	49
n-Butane	51	49	44

ratio increased up to 7:1 beyond which no increase in the conversion percentage was observed. Tests conducted on palm stearin and corn oil also showed that 7:1 molar ratio of hydroxamic acid to oil was the optimum molar ratio that achieved the maximum conversion percentage of oil to FHAs. It should be noted that the 7:1 molar ratio was used in the tests performed to examine the effect of reaction time and organic solvent as discussed in Section 3.1 and 3.2.

3.4 Characterization of FHAs

3.4.1 Complex formation

The colour of complexes of FHAs with copper (II), iron (III) and zinc (II) are green, dark red and pale yellow, respectively. Haron *et al.* (1994) reported that observed colour after reaction between these metals with hydroxamic acids²⁰.

3.4.2 Fourier transform infrared spectroscopy (FTIR)

Results of FTIR spectra of palm oil and FHAs are graphically depicted in **Table 2**. FTIR spectrum conducted on palm oil reveals peaks at wavenumber of 2855 cm^{-1} and 2922 cm^{-1} which correspond to C-H stretching of alkyl chain, at 1742 cm^{-1} this peak corresponding to C=O stretching and at 1456 cm^{-1} which corresponds to C-H aliphatic bending, respectively. Peaks observed in FTIR spectrum of FHAs were at wavenumber of 3413 cm^{-1} and 3210 cm^{-1} which corresponds to N-H and O-H stretching, these absorption bands for amide¹⁶, at 2854 and 2917 cm^{-1} which corresponds to C-H stretch of alkyl chain, at 1641 cm^{-1} which correspond to C=O for secondary amide and at 1044 and 1113 cm^{-1} which corresponds to C-N stretch, respectively.

3.4.3 ^1H nuclear magnetic resonance (NMR)

The analysis of samples of FHAs using ^1H NMR technique shows the presence of the proton of N-H and O-H in

the FHAs structure in the region 8 and 2 ppm, respectively. FTIR and ^1H NMR spectrum of fatty hydroxamic acids from palm stearin and corn oil show similar characteristics.

3.4.4 Elemental analysis

Results of elemental analysis conducted on FHAs reveals the presence of nitrogen atoms in the product. The nitrogen content of fatty hydroxamic acids was found to be 4.91%.

4 CONCLUSIONS

This paper describes synthesis and characterization fatty hydroxamic acids from palm olein, palm stearin and corn oil with hydroxylamine using reflux method.

Based on test results, the following conclusions can be drawn:

- FTIR spectrum, ^1H NMR and elemental analysis tests conducted on FHAs reveal that FHAs were successfully produced from palm olein, palm stearin and corn oil
- The conversion percentage of oils to FHAs increases as the reaction time increases. The optimum reaction time for all the different three oils was found to be 10 h.
- The conversion percentage of oils to FHAs was found to be substantially affected by the organic solvent used in the production process. Hexane solvent results in the highest conversion percentage of the three different oils to FHAs.
- The conversion percentage of the oils to FHAs increases as the molar ratio of hydroxamine to oil increases. A molar ratio of 7:1 (hydroxamine to oil) results in the maximum conversion percent-

Table 2 Numerical Presentation of FTIR Spectrum Conducted on Palm Olein and FHAs.

Material	Wavenumber(cm^{-1})	Assignment
Palm olein	2922	C-H stretching
	2855	C-H stretching
	1742	C=O stretching
	1456	C-H bending
FHAs	3403	N-H stretching for amide
	3210	O-H stretching for amide
	2917	C-H stretching of alkyl
	2854	C-H stretching of alkyl
	1641	C=O stretching for amide
	1113	C-N stretching
	1044	C-N stretching

age of the three oils to FHAs.

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