

## Synthesis, Characterization and Optimum Reaction Conditions of Fatty Hydrazone from Triacylglycerides

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**Abstract:** Fatty Hydrazone (FH) has been synthesized via reaction of palm olein with hydrazine hydrate by reflux and characterized using spectroscopic methods and CHN analyzer. Optimum reaction conditions were studied in order to optimize the reaction time and hydrazine hydrate/palm olein ratio. When the hydrazinolysis reaction was carried out 7:1 molar ratio of hydrazine hydrate to palm olein, the conversion of palm olein was 82% and the maximum of conversion to FH was 84% when the reaction time was 12 h. Besides, hexane when used as an organic solvent could increase the conversion up to 81%. FH was also prepared from palm stearin and corn oil and then, the conversion percentages of these oils into their fatty hydrazides were investigated to compare with palm olein.

**Key words:** Synthesis, fatty hydrazone, triacylglycerides, palm stearin

### INTRODUCTION

Hydrazides are considered as significant intermediates in the synthesis polypeptid chains (Bodanszky *et al.*, 1976). The hydrazone functional group is used as a carboxyl-protecting group in peptide synthesis, a resin-linker for the solid-phase synthesis of peptide esters and amides, thioesters and lapidated (Wieland *et al.*, 1970; Millington *et al.*, 1998; Camarero *et al.*, 2004; Lumbierres *et al.*, 2005). Raddatz *et al.* (2002) reported that the reactions of hydrazides modified oligonucleotides like amines (Fig. 1) but they have lower pka (4-5) than pka of primary amine (10-11). Recently, the hydrazone has attracted scientist and researchers due to their biological and environmental activities. It can be used as tuberculostatic (Yadav *et al.*, 2005), antibacterial, anticancer agent (Malhotra *et al.*, 1992; Zhang *et al.*, 2004) and an organic reagent to determine trace of vanadium in aqueous solution based on the reaction of hydrazone with vanadium (Khalid *et al.*, 1985; Pradana *et al.*, 2004; Shewale *et al.*, 2008; Uzma Ashiq *et al.*, 2008).

Synthesis of hydrazone by reaction of ester with hydrazine hydrate has been prepared using different sources of heating (Yanqing and Song, 2001). Hydrazone compound is also synthesized by reaction between isonecotinate and hydrazine hydrate with different lipases. The effects of various parameters on the

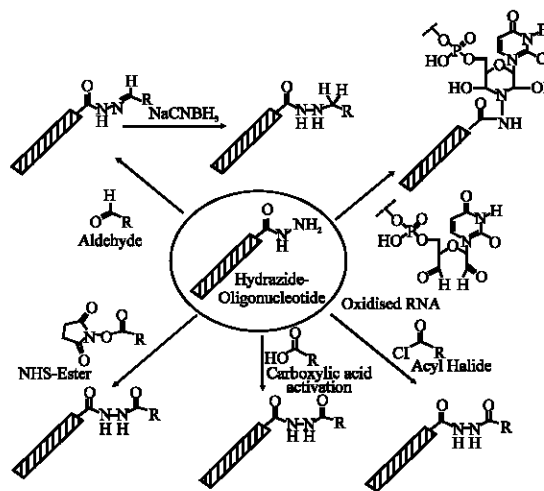


Fig. 1: Schematic diagram of the reactivity of hydrazone modified oligonucleotides (Stefan Raddatz *et al.*, 2002)

conversion and the rates of the reaction were studied in the presence of Novozym (Ganapati *et al.*, 2004). Nadia *et al.* (2005) had synthesized p-Amino salicylic hydrazone from ester with hydrazine hydrate and studied its properties. In addition, many studies have been carried out on the synthesis of hydrazone from amide and carboxylic acid (Yadav *et al.*, 2005; Kobayashi *et al.*, 1999). Enzymatic synthesis of fatty hydrazone from palm oil

products has been reported by Sharifah *et al.* (2008). Enzyme as catalyst has been used in most of previous studies to synthesis of hydrazide. Our search of the literature revealed that this study is the first for synthesis of fatty hydrazide directly from triacylglycerides without using enzyme.

In this research, fatty hidrazide has been synthesized from palm olein with hydrazine hydrate and characterized using spectroscopic methods, CHN analyzer then, studied optimum conditions of reaction. Palm olein is a mixture of triacylglycerides, just like any ordinary fat, which are esters of glycerol with different saturated and unsaturated fatty acids. Malaysia is currently the world's largest producer and exporter of palm oil and it is the major source of vegetable oil for industrial application (Basiron *et al.*, 2007). Palm stearin and corn oil were also used to know the best conversion to fatty hydrazide. So, inexpensive renewable material has been used to prepare these fatty hydrazides.

## MATERIALS AND METHODS

Hydrazine hydrate (Sigma-Aldrich), palm olein and palm stearin were obtained from Ngo Chew HONG oils and Fats (M) Sdn. Bhd. Malaysia. Corn oil was from local Malaysian market. Hexane, cyclohexane, chloroform and n-butane were from T.J. Beaker (USA).

**Synthesis of FH:** Palm olein in hexane with hydrazine hydrate were refluxed at boiling point of hexane for 12 h using thermostated round bottom flask equipped with a water-cooled condenser and a magnetic stirrer. After the reaction had finished (product change the color to purple with vanadium (III) due to its ability to form a complex), the product was dissolved in hot hexane and separated from bottom layer by separating funnel. The hexane phase was cooled in a refrigerator for 5 h to obtain fatty hydrazide and then filtered and washed by hexane for several times and dried in a vacuum desiccator over phosphorous pentoxide.

FH was also synthesized from palm stearin and corn oil similarly to above procedure.

**Characterizations:** FTIR spectra were measured in the range 4000-600  $\text{cm}^{-1}$  in a Perkin-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 at 8 tons for 1 min to produce the disc.

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

CHN analyzer (LECO CHNS-932) was used for quantities analysis of carbon, nitrogen and hydrogen contents. The determination was carried out under  $\text{O}_2$  and  $\text{N}_2$  atmospheric conditions using the sulfamethazine as standard.

## RESULTS AND DISCUSSION

**Characterization of FH:** CHN analysis, NMR and FTIR spectra were used to verify that FH is successfully produced from palm olein.

The FTIR spectra of palm olein are illuminated in Fig. 2a shown in transmittance ratio. The peaks caused by C-H stretching are around 2847 and 2915  $\text{cm}^{-1}$  for symmetric and asymmetric C-H stretching vibration, respectively (Rosario-Castro *et al.*, 2005). Moreover, the peak at 1740  $\text{cm}^{-1}$  corresponds to C = O stretching and at 1447  $\text{cm}^{-1}$  is related to the aliphatic C-H bending vibration (Guo *et al.*, 2006). The FTIR spectrum of unsaturated fatty hydrazide is shown in Fig. 2b. The spectrum exhibits peaks at 3426, 3300, 3199, 3040, 1630 and 1600  $\text{cm}^{-1}$ , which can be assigned to, N-H stretching primary amide (Vaysse *et al.*, 1997),  $\text{NH}_2$  group asymmetric stretching,  $\text{NH}_2$  group symmetric stretching (Albro *et al.*, 1985), C-H alkenes (C = C-H) stretch, C = O stretching amide and C = C alkenes, respectively. In addition, N-H bending is

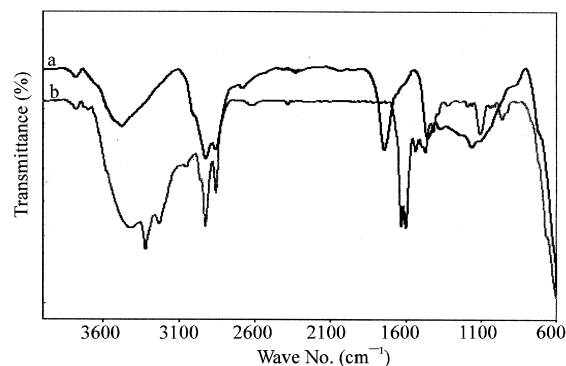


Fig. 2: FTIR spectra of samples (a) palm olein and (b) FH from palm olein

Table 1: Assignment of absorption of FTIR spectrum observed in both palm olein and FH

Material	Wave No. ( $\text{cm}^{-1}$ )	Assignment
Palm olein	2915	Asymmetric C-H stretching vibration
	2847	Symmetric C-H stretching vibration
	1740	C = O stretching
	1447	The aliphatic C-H bending vibration
FH	3426	N-H stretching primary amide
	3300	$\text{NH}_2$ group asymmetric stretching
	3199	$\text{NH}_2$ group symmetric stretching
	3040	C-H alkenes (C = C-H) stretch
	1630	C = O stretching and N-H bending
	1600	C = C stretching
	1038-1124	C-N stretching

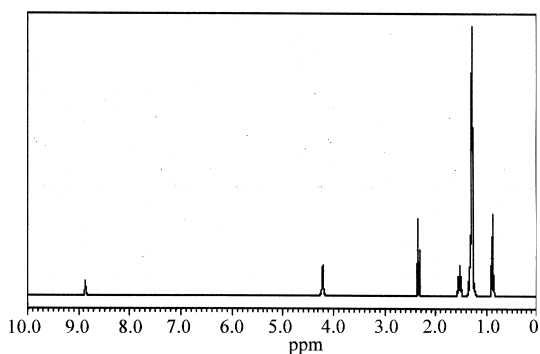


Fig. 3:  $^1\text{H}$  NMR spectrum of FH produced from palm olein

at  $1630\text{ cm}^{-1}$  and C-N stretching is at  $1038\text{--}1124\text{ cm}^{-1}$  (Shalaby *et al.*, 2000; Ramachandran *et al.*, 2007). The major absorption bands of both palm olein and FH are summarized in Table 1.

$^1\text{H}$  NMR technique was used for the analysis of FH sample. The proton of N-H and  $\text{NH}_2$  in the FH structure was observed in the region 8.85 and 4.2 ppm, respectively as shown in Fig. 3 indicating that hydrazine hydrate successfully reacts with triacylglycerides to form fatty hydrazide. FTIR and  $^1\text{H}$  NMR spectrum of fatty hydrazides from palm stearin and corn oil show similar characteristics.

CHN elemental analysis was also carried out to confirm the presence of nitrogen atoms in the products and consequently the sign of fatty hydrazide forming. The nitrogen content of palm olein, palm stearin and corn oil analysed by elemental analysis is found to be 9.94, 10.21 and 9.88%, respectively.

**Effect of hydrazine hydrate/palm olein ratio:** The molar ratio of hydrazine hydrate to palm olein is one of the important parameters that affect the conversion to fatty hydrazide. Stoichiometrically, 3 mol of hydrazine hydrate are required for each mole of palm olein; but in practice, the hydrazinolysis reaction generally requires a large excess of hydrazine hydrate to shift the equilibrium favourably. Figure 4 graphically illustrates the change of the conversion under these reaction conditions as a function hydrazine hydrate/palm olein ratio (mmol/mmol). As shown in this graph, when the hydrazine hydrate-feeding amount increased, the conversion was increased considerably. The maximum conversion of about 84% was obtained when the molar ratio was very close to 7:1. Beyond the molar ratio of 7:1, there was no substantial increase in the conversion. Therefore, a 7:1 molar ratio of hydrazine hydrate to palm oil was suitable for obtaining high conversion.

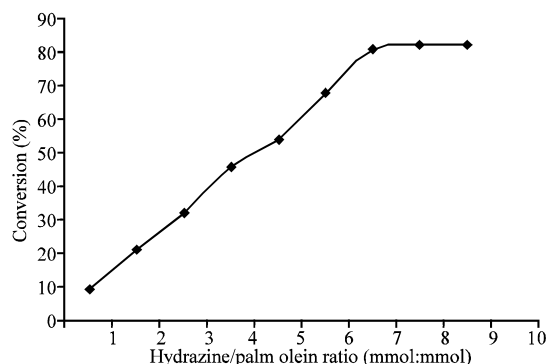


Fig. 4: Conversion to palm olein as a function of hydrazine hydrate/palm olein molar ratio. Reaction conditions: Hexane volume 15 mL, reaction time 12 h and reaction temperature 342 K

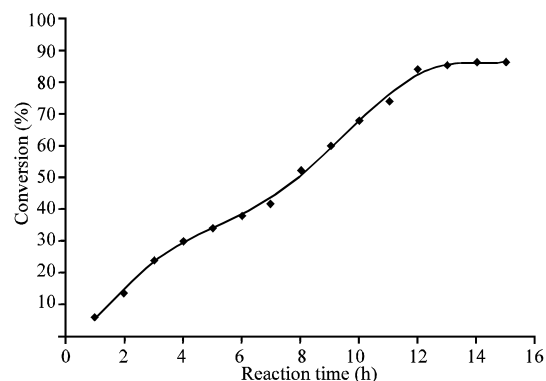


Fig. 5: Conversion of palm olein as a function of reaction time. Reaction conditions: Hydrazine hydrate/palm olein molar ratio 7:1, hexane volume 15 mL and reaction temperature 342 K

**Effect of reaction time:** The optimum reaction time for the production of fatty hydrazide was determined by performing reaction at varying reaction time in the range 1-15 h. The experimental results, reported in Fig. 5 that the conversion increased rapidly in the reaction time range between 1 and 12 h and thereafter the increase was just slightly for longer reaction times due to either the reaction become an equilibrium state or a lower limit of the mass transfer rate. Thus, the maximum conversion of palm oil was achieved at 12 h of reaction time.

**Effect of organic solvent:** Organic solvent in reaction system offer the potential for high substrate and product solubilities, facilitated recovery of product and shift of reaction equilibrium. In this study, the effects of organic solvent on FH of palm olein were investigated. Hexane, cyclohexane, chloroform and butanol were individually used

Table 2: The optimum experimental conditions for synthesis of FH from palm olein

Parameters	Optimum
Solvent	Hexane
Reaction period	12 h
Reaction temperature	342 K
Hydrazine hydrate/palm olein molar ratio	7:1

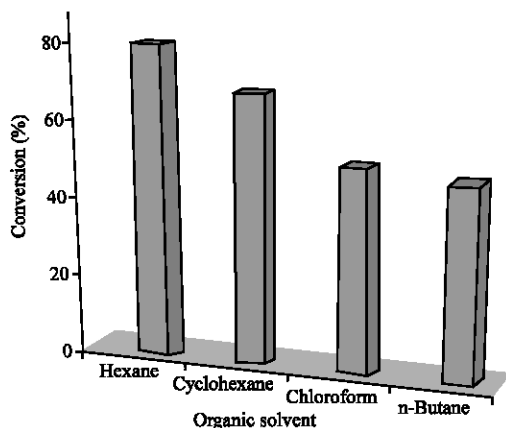


Fig. 6: Conversion of palm olein as a function of organic solvent. Reaction conditions: Hydrazine hydrate/palm olein molar ratio 7:1, reaction time 12 h, hexane volume 15 mL and reaction temperature 342 K

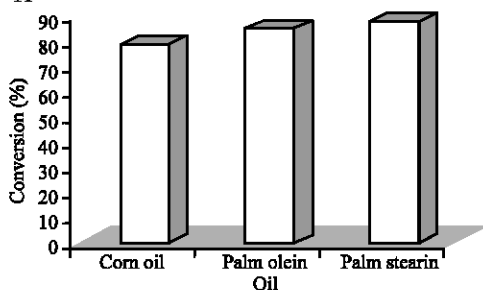


Fig. 7: Conversion into FH of different oils. Reaction conditions: Hydrazine hydrate/palm olein molar ratio 7:1, reaction time 12 h, hexane volume 15 mL and reaction temperature 342 K

as organic solvent. It was found that hexane is the most effective solvent for FH of palm oil as shown in Fig. 6 due to higher solubility of the substrate and ability of separation product and substrate into 2 phases.

From above discussion, we focus on the effect of parameters on conversion of palm olein for synthesis of FH to find the optimum conditions for each parameter. However, it is necessary to know the overall optimal synthesis conditions. These results are exhibit in Table 2.

Percentages of conversion into FH from palm stearin and corn oil were also investigated and compared to

palm olein at the optimum reaction conditions. Figure 7 displays percentages of conversion into FH. The percentages of conversion of corn oil, palm olein and palm stearin were 78, 84 and 87, respectively.

## CONCLUSION

This study shows that FH has been synthesized successfully from palm olein and hydrazine hydrate by reflux and characterized using FTIR spectroscopy, NMR spectrophotometer and CHN analyzer. Factors affecting the conversion of palm olein for FH synthesis were investigated to find the optimum conditions for each parameter. It was found that the amount of hydrazine hydrate/palm olein ratio (mmol:mmol), reaction time and reaction temperature are 7: 1, 12 h and 342 K, respectively. The effects of organic solvent on FH of palm oil were also studied. Hexane was the best solvent than the others because it gave the highest conversion. Palm stearin and corn oil were also used to prepare fatty hydrazide. The percentages of conversion of these oils into their fatty hydrazides were investigated to compare with palm olein. In the present research, fatty hydrazide was manufactured under simple conditions from inexpensive renewable resources that are considered environmentally friendly.

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