

Preparation of Fatty Hydroxamic Acid from Canola Oil

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Fatty hydroxamic acids were synthesized from canola oil by hydroxylaminolysis using lipozyme as catalyst. The products were converted to copper fatty hydroxamate and precipitated in acetone. The precipitate was treated with nitric acid solution to release fatty hydroxamic acids and extracted to chloroform. The fatty hydroxamic acids were obtained by solvent evaporation under reduced pressure. Qualitative identification of hydroxamic acids were carried out by observing colour of the complexes with vanadium(V), iron(III) and copper(II) which were purple, dark red and green, respectively. The FTIR spectrum of the product showed the existence of the characteristic amide peaks at 3270 and 1642 cm^{-1} . Among four oil samples studied, canola oil of Ladan brand gave the highest yield of fatty hydroxamic acids.

Key Words: Fatty hydroxamic acid, Copper fatty hydroxamate, Lipozyme, Canola oil.

INTRODUCTION

Hydroxamic acid (HA) research was first time appeared in 1869 as cited by Yale in 1943¹. Hydroxamic acids and their derivatives have attracted many researchers due to their ability to form stable complexes with a number of metal ions. They also constitute as components in some important compounds such as antifungal agents, growth factors, antibiotics, tumour inhibitors, cell division factors, enzyme inhibitors and food additives^{2,3}. Monohydroxamic acids such as acetohydroxamic acid acts as bidentate ligand forming octahedral complexes. These type of complexes have been characterized in previous studies with Fe(III), Cr(III), Ni(II), Co(II) and Zn(II) ions, which indicated the formation of octahedral complexes both in the solid state and in solution³. A number of applications of the metal complexes of hydroxamic acids in analytical chemistry were described by Agrawal and Patel⁴. Hydroxamic acids were used as collector for rare earth metals from minerals⁵ and were applied for metal ions extraction from aqueous media⁶. Metal complexation ability of hydroxamic acids increased the efficiency of surfactants in the detergent industry⁷.

Hydroxamic acids were generally synthesized by the chemical reaction of alkyl or aryl ester with hydroxylamine in high alkaline medium. However, due to its mild reaction condition, many researchers used enzyme as catalyst for preparation of hydroxamic acid. Hacking *et al.*⁸ successfully used the *Candida rugosa* lipase to catalyze acylation of hydroxylamine and hydrazine as well as their derivatives by octanoic acid.

Vaysse *et al.*⁹ applied *Candida parapsilopsis* for synthesis of fatty hydroxamic acids from oleic acid ethyl ester and linoleic acid ethyl ester. Biocatalyst was improved by the immobilization of enzyme on Duolite A-378 resin for the production of short-chain hydroxamic acids¹⁰.

In the present work we report the preparation of fatty hydroxamic acids (FHAs) by hydroxylaminolysis of canola oil using lipozyme as catalyst. The fatty hydroxamic acids product was converted to copper complex and precipitated in acetone. The precipitate was treated with nitric acid to release fatty hydroxamic acids and extracted to chloroform. The fatty hydroxamic acids was obtained by solvent evaporation under reduced pressure. Parameters investigated include effect of reactants mol ratio, pH, types of organic solvent, amount of enzyme and reaction time. The recovery of fatty hydroxamic acid through the precipitation of its copper complex in acetone followed by the extraction of fatty hydroxamic acid from nitric acid solution of the complex into chloroform is a new method for isolation of fatty hydroxamic acids from canola oil substrate.

EXPERIMENTAL

Canola oils used were of food grade and others chemicals used were of analytical grade. Hydroxylamine hydrochloride, copper(II) sulphate pentahydrate hexane, heptane, chloroform, toluene, xylene, acetone, petroleum ether and cyclohexane were supplied by System Co (UK). Sodium hydroxide was purchased from Aldrich (USA). The immobilized lipozyme

was obtained from Novo Nordisk (Denmark). Ladan, Moi, Krystal and Natural brands of canola oil were supplied by Sanaye Behshar Co. (Iran), Moi Food Sdn. Bhd. (Malaysia), FFM Co. (Malaysia) and Lam Soon Edible Oils Sdn Bhd. (Malaysia), respectively.

Synthesis and separation of fatty hydroxamic acids:

Hydroxylaminolysis of canola oil in hexane was catalyzed by lipozyme. Reaction was carried out using 3.0 mmol canola oil dissolved in 30.0 mL of hexane, 18.0 mmol hydroxylamine dissolved in 20.0 mL of distilled water and 100 mg lipozyme and sealed in 250 mL round bottom flask. The flask was shaken at 100 rpm at a controlled temperature in a water bath. After a predetermined time of reaction, the enzyme was removed by filtration. The fatty hydroxamic acids formed and the oil residue in the organic phase were separated from the aqueous phase by decantation. The organic phase was then mixed with 100.0 mL of 0.1 M copper sulphate solution and agitated at 500 rpm for 15 min for formation of green copper fatty hydroxamate (CuFHAs) complexes. The CuFHAs were precipitated by adding 50.0 mL acetone to the mixture. The precipitate was then filtered, washed several times with acetone and air dried. For obtaining fatty hydroxamic acids as final product, CuFHAs were treated with 50.0 mL of HNO₃ solution (15 %) and stirred at 500 rpm for about 15 min and then 50.0 mL of chloroform was added to the mixture and continue stirring until fatty hydroxamic acids was extracted to organic phase. The organic phase was separated by separation funnel and treated with 50.0 mL of nitric acid solution (15 %) again. The mixture was stirred at 500 rpm for about 10 min then organic phase was separated. Finally the solid fatty hydroxamic acids was recovered from chloroform by rotary evaporation and weight. The percentage of the yield was calculated as the percentage of the actual weight of the product to the theoretical weight of the product assuming canola oil is a triglyceride. Qualitative identification of hydroxamic acids were carried out by observing colour of their metal complexes. For this purpose solution of fatty hydroxamic acids in hexane were mixed by 0.1 M copper(II) iron(III) and vanadium(V) solutions separately and agitated for *ca.* 5 min. FTIR spectra of products were recorded by FTIR Spectrophotometer (Perkin-Elmer 1650 FTIR-Spectrum USA).

RESULTS AND DISCUSSION

Effect of organic solvent: The best solvent can dissolve the substrate and products, does not give negative influence on enzyme activity and stability and has the ability to distribute the substrate and products into different phases¹¹. We have investigated effect of different solvents on hydroxylaminolysis of canola oil. Fig. 1 shows the effect of different organic solvents on hydroxylaminolysis of canola oil. The results show that heptane gives the highest yield of the hydroxylaminolysis followed by hexane. However due to cheaper cost of hexane than heptane, it was chosen as solvent in the subsequent experiments. Furthermore the yield only differs by 1.2 % when using hexane compared to heptane.

Effect of initial pH of hydroxylamine solution: In this experiment we used aqueous solution of hydroxylamine at different pH. For this purpose NaOH (6M) was added to

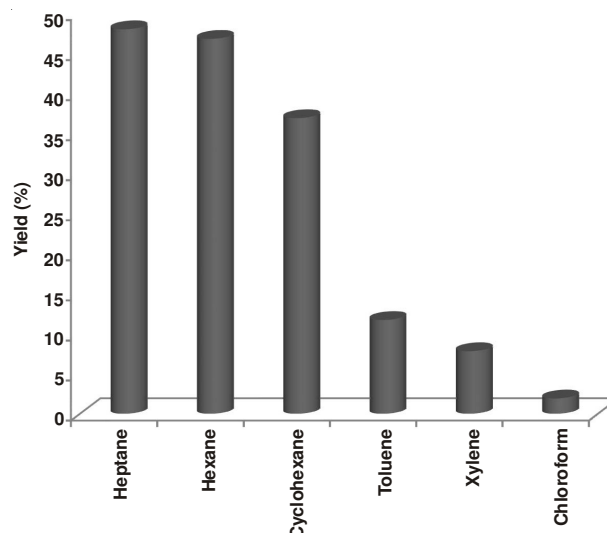


Fig. 1. Effects of organic solvents on the percentage yield of hydroxylaminolysis of canola oil (Krystal brand). Reaction conditions: canola oil 2.55 g, reaction time 42 h, NH₂OH·HCl 18.0 mmol, lipozyme 90 mg, organic solvent 30.0 mL, temperature 35 °C, shaking rate 100 rpm, initial pH 7.0, H₂O 18.0 mL, NaOH (6M) 3.0 mL

NH₂OH·HCl solution until attainment of the desired pH (pH 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0). Fig. 2 shows effect of initial pH of hydroxylamine solution on percentage yield of hydroxylaminolysis of canola oil. The result shows that pH 8.5 gives the highest yield of the reaction. However the pH value was higher compared to pH 6 obtained for hydroxylaminolysis of oleic acid by Vaysse *et al.*¹⁰.

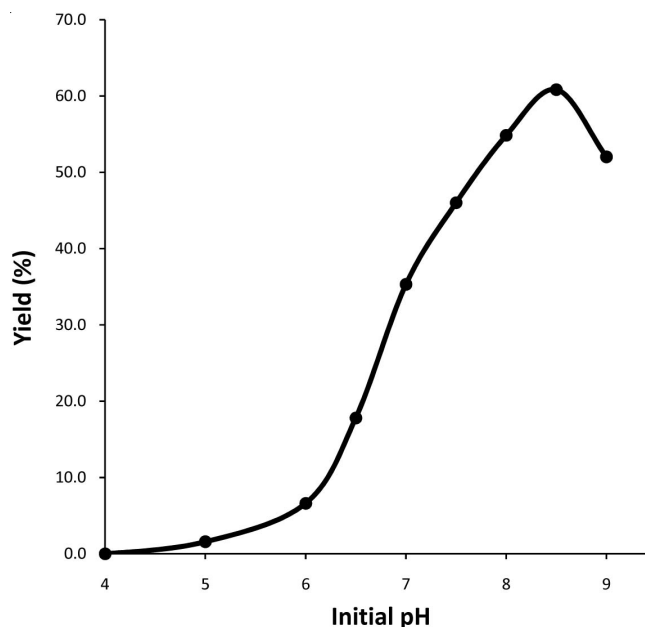


Fig. 2. Effects initial pH of hydroxylamine solution on the percentage yield of hydroxylaminolysis of canola oil (Krystal brand). Reaction conditions: canola oil 2.64 g, reaction time 42 h, NH₂OH·HCl 18.0 mmol, lipozyme 90 mg, hexane 30.0 mL, temperature 35 °C, shaking rate 100 rpm, H₂O 18.0 mL, NaOH (6M) variable

Effect of reaction time: Fig. 3 shows the effect of reaction time on yield of hydroxylaminolysis of canola oil. The result showed that the reaction progressed rapidly in the first 32 h and almost completed after 42 h.

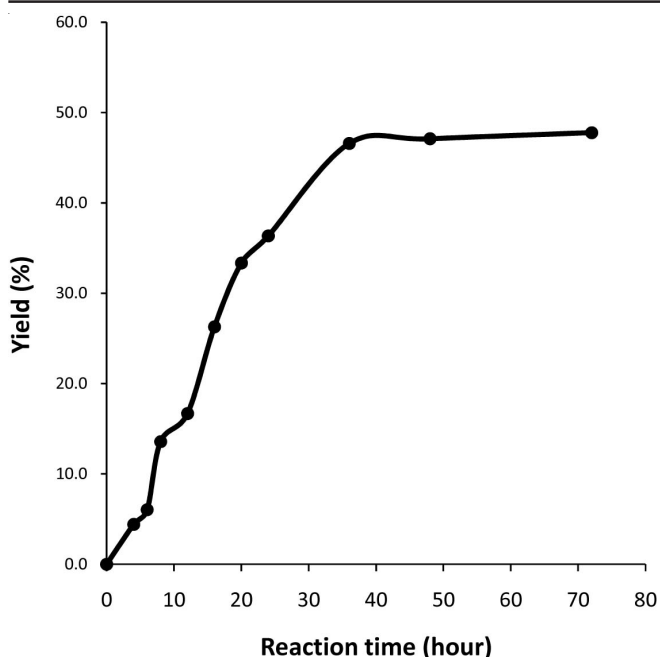


Fig. 3. Effects of reaction time on the percentage yield of hydroxylaminolysis of canola oil (Krystal brand). Reaction conditions: canola oil 2.55 g, $\text{NH}_2\text{OH}\cdot\text{HCl}$ 18.0 mmol, lipozyme 90 mg, hexane 30.0 mL, temperature 35 °C, shaking rate 100 rpm, initial pH 7.0, H_2O 18.0 mL, NaOH (6M) 3.0 mL

Effect of the amount of enzyme: In an enzymatic reaction, the reaction takes place on the active sites of the enzyme. Therefore the amount of product also depends on amount of the enzyme used. To determine the optimum amount of the enzyme needed the effect of the ratio of the enzyme and oil on the percentage of the conversion was studied. Fig. 4 shows the effect of the amount of lipozyme on hydroxylaminolysis of canola oil. The result shows that highest percentage of product was obtained when the ratio was 35.0 mg of the lipozyme to 1.0 mmol of oil.

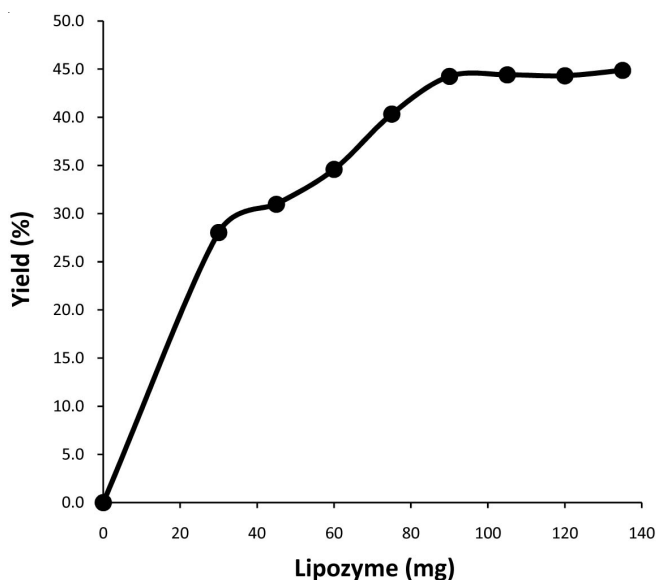


Fig. 4. Effects of lipozyme amount on yield of hydroxylaminolysis of canola oil (Krystal brand). Reaction conditions: canola oil 2.65 g (3 mmol), reaction time 32 h, $\text{NH}_2\text{OH}\cdot\text{HCl}$ 18.0 mmol, hexane 30.0 mL, temperature 35 °C, shaking rate 100 rpm, initial pH 7.0, H_2O 18.0 mL, NaOH (6M) 3.0 mL

Effect of amount of hydroxylamine: Maximum efficiency for chemical reaction is related to mole ratio of the reactants. This parameter is one of important factors in chemical industries¹². The best mole ratio of reactants for obtaining maximum efficiency depends on the nature of the reaction and it is related to equilibrium constant of reaction. Different mole ratio of reactants on hydroxylaminolysis of the canola oil was investigated. Fig. 5 shows the effect of amount of hydroxylamine on the yield of hydroxylaminolysis of canola oil. The results indicated that the optimum amount of hydroxylamine for the reaction was 18.0 mmol for 3.0 mmol of the canola oil.

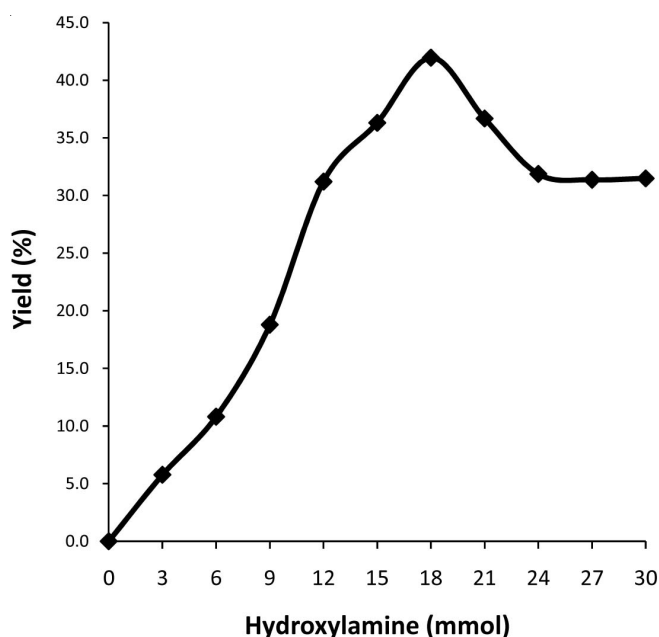


Fig. 5. Effects concentration of hydroxylamine on the percentage yield of hydroxylaminolysis of canola oil (Krystal brand). Reaction conditions: canola oil 2.65 g (3 mmol), reaction time 42 hours, Lipozyme 90 mg, hexane 30.0 mL, temperature 35°C, shaking rate 100 rpm, initial pH 7.0, H_2O 30.0 mL, NaOH (6M) variable.

Preparation of fatty hydroxamic acids from canola oils of different source: The optimum experimental conditions for the hydroxylaminolysis of canola oil (Table-1) were applied to four different commercial canola oils. Fig. 6 shows the canola oil from Ladan brand has the highest yield of fatty hydroxamic acids compared to other product.

TABLE-1 OPTIMUM CONDITIONS FOR HYDROXYLAMINOLYSIS OF CANOLA OILS	
Parameter	Condition
Solvent	Hexane
Initial pH	8.5
Ratio lipozyme (mg)/substrate (mmol)	35/1
Ratio hydroxylamine(mmol)/substrate(mmol)	6/1
Reaction time (h)	48

Characterization of fatty hydroxamic acids: The colour of the complexes of fatty hydroxamic acid with vanadium(V), iron(III) and copper(II) were purple, dark red and green, respectively. These are the common colour of the complexes observed when these metal ions were reacted with hydroxamic

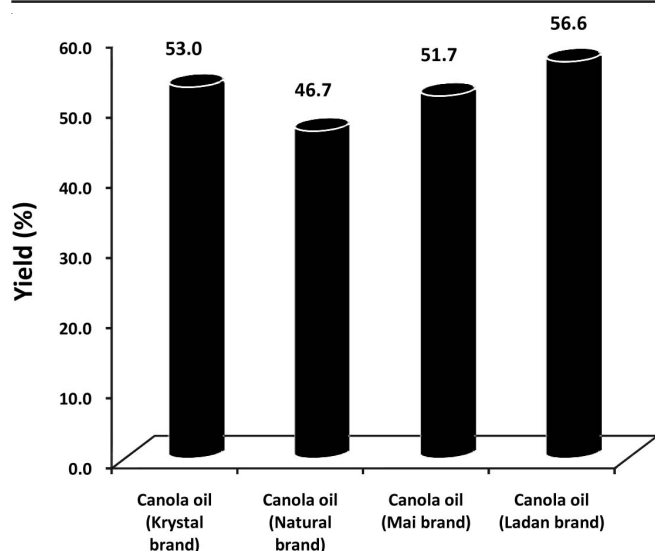


Fig. 6. Percentage yields of hydroxylaminolysis using different commercial brand of canola oil. Reaction conditions: canola oil 2.65 g (3 mmol), reaction time 48 h, $\text{NH}_2\text{OH}\cdot\text{HCl}$ 18.0 mmol, lipozyme 105 mg, hexane 30.0 mL, temperature 38 °C, shaking rate 100 rpm, initial pH 8.5, H_2O 25.0 mL, NaOH (6M) 3.0 mL

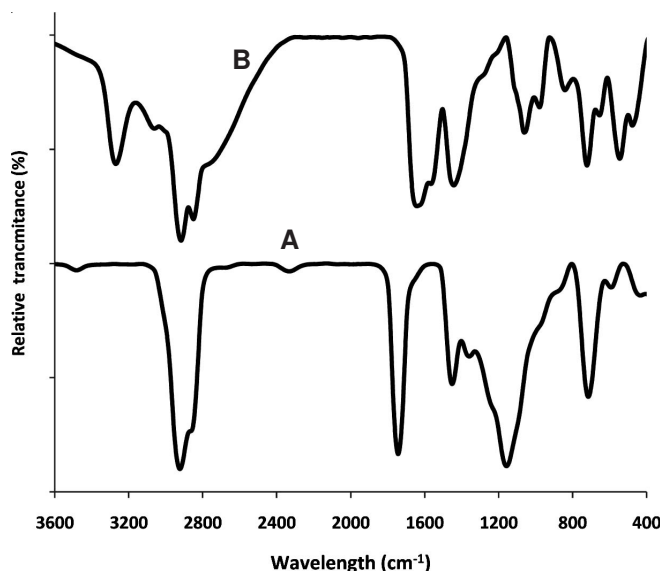


Fig. 7. FTIR spectra of canola oil (A) and fatty hydroxamic acids (B)

acids¹³. The FTIR spectrum of the canola oil in Fig. 7(A) shows the characteristic absorption bands at 2857 and 2923 cm^{-1} which correspond to C-H stretching of long alkyl chain. Additional characteristic absorption band of canola oil appear at 1743 cm^{-1} corresponding to C=O stretching. Absorption peak at 1452 cm^{-1} corresponds to alkenes (C=C) while peak at 1158 cm^{-1} corresponds to alkenes (C-O). The FTIR spectrum of the

fatty hydroxamic acids product in Fig. 7(B) shows the characteristic absorption bands of hydroxamic acid at 3270 cm^{-1} which corresponds to N-H and 3100 cm^{-1} (wide peak) corresponds to OH. Additional characteristic absorption bands of hydroxamic acid appear at 1642 cm^{-1} that correspond to C=O in secondary amide. Fig. 7(B) also shows the characteristic absorption bands at 2850 and 2917 cm^{-1} which correspond to C-H stretching of long alkyl chain. Peak at 1442 cm^{-1} corresponds to alkenes (C=C). Both qualitative tests show that the fatty hydroxamic acids are successfully produced from the commercial canola oil.

Conclusion

Fatty hydroxamic acids (FHAs) were successfully synthesized from canola oil by Lipozyme as catalyst. The fatty hydroxamic acids product was converted to a copper complex, precipitated in acetone and treated with in nitric acid and extracted into chloroform. The solid fatty hydroxamic acids were obtained by solvent evaporation under reduced pressure. The existence of the hydroxamic acid in the product was indicated by the colour of the complexes with vanadium(V), iron(III) and copper(II) which were purple, dark red and green, respectively. The FTIR spectrum of the product showed the existence of the characteristic amide peak at 1642 cm^{-1} . Optimum conditions for the fatty hydroxamic acids preparation were, canola oil 2.65 g, $\text{NH}_2\text{OH}\cdot\text{HCl}$ 18.0 mmol, lipozyme 105 mg, reaction period 42 h, hexane 30 mL, temperature 38 °C, H_2O 25 mL, shaking rate 100 rpm, NaOH (6M) 3.0 mL. Among four oil samples studied, canola oil of Ladan brand gave the highest yield of fatty hydroxamic acids.

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