

Transformation of waste cooking oil into C-18 fatty acids using a novel lipase produced by *Penicillium chrysogenum* through solid state fermentation

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Abstract The prime aim of the current work was to illustrate the components existing in repeatedly used cooking oil and to develop an economical process for the production of fatty acids from low cost feedstock waste. The waste cooking oil was characterized by the occurrence of high molecular weight hydrocarbons and polymerized derivative of esters. Triacontanoic acid methyl ester, 2,3,5,8-Tetramethyldecane, 3,3 dimethyl heptane, and 2,2,3,3-teramethyl pentane were detected as thermal and oxidative contaminants that adversely affect the quality of cooking oil. Fundamentally, waste cooking oil comprises ester bonds of long chain fatty acids. The extracellular lipase produced from *P. chrysogenum* was explored for the hydrolysis of waste cooking oil. The incorporation of lipase to waste cooking oil in 1:1 proportion released 17 % oleic acid and 5 % stearic acid.

Keywords Waste cooking oil · Chromatography · Lipase · Fatty acid

Introduction

Vegetable oil and ghee are chemically characterized as triglyceride molecules and widely used for the cooking purpose. (Maher and Bressler 2007). Repetitive use of

cooking oil under elevated temperature causes deleterious changes in physical and chemical properties of oil by the process of hydrolysis, oxidation, and polymerization. The reactions occurring in repeated cooking of oil depend on original quality of oil, type of food materials, heating conditions and duration, and concentration of moisture and oxygen. High temperature and number of repeats decrease the flavor quality of oil and produce volatile or nonvolatile complexes. Most of volatile components evaporate and produce smoke that generates anoxigenic environment in working condition. The chemistry of nonvolatile compounds is complex and poorly understood process because of Diels–Alder and Amadori rearrangements (Martins et al. 2001). Therefore, it is not possible to predict the mechanism of formation of volatile or nonvolatile components due to isomerization, cyclization, thermal polymerization, hydrolysis and oxido-reduction process.

A huge amount of waste cooking oil generated from restaurant and food process industries is disposed without prior treatment. The Energy Information Administration in United States estimated that 100 million gallons of waste cooking oil is produced per day in USA (Radich 2006) while UK and EU countries generate approximately 200,000 tons and 700,000–1,000,000 tons of waste cooking in a year respectively. (Arjun et al. 2008). Theoretically, a single liter of oil can contaminate one million liters of water in spite of small volume ratio. These wastes have very high biological oxygen demand owing to highly reduced long chain fatty acids. Therefore, Environmental Protection Agency in 1994 released an ordinance under act of oil pollution in which animal fats and vegetable oils could not be exempted from regulations governing the cleanup of oil spills.

Rapidly advancing technologies and the use of custom-made biocatalysts have produced exciting opportunities for

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recycling of waste cooking oil. Transformation of waste cooking oil using enzymes makes process efficient in comparison to other physical and chemical treatments such as steam splitting and alkaline hydrolysis (Murty et al. 2002). Enzymes are recognized as highly selective and specific catalysts which accelerate the rate of reactions under mild conditions. Lipases (E.C. 3.1.1.3) are the members of the hydrolase family and exhibit positional specificity toward fatty acid and fatty acid selectivity in acylglycerol. Lipases hydrolyze the acylglycerol into fatty acids and glycerol. Lipases are unique biocatalysts that not only hydrolyze carboxylic ester bonds but also exploit to catalyze synthesis of fine chemicals through esterification, interesterification and transformations of racemic compounds. Lipase-mediated transformation of waste cooking oil could be a promising method for cost-effective production of FFA because 1.6×10^6 tons of fatty acids are produced every year from various plant and animal sources through lipases (Pronk et al. 1988). The current study opened a new avenue to generate C-18 fatty acids from waste cooking oil by lipase for various industrial applications.

Materials and methods

Enzyme production for hydrolysis of waste cooking oil

A locally isolated strain of *P. chrysogenum* SNP5 was explored for the production of extracellular lipase using grease waste, wheat bran and Czepek-dox media (1:1:2 w/w) as substrate. Enzyme was extracted after 7 days of solid state fermentation (SSF) and centrifuged at 8,000 rpm for 15 min. The clear supernatant was used as a crude extracellular lipase enzyme (Kumar et al. 2011). A 1:1 ratio of emulsified cooking oil waste and lipase was mixed to mediate lipolytic reaction of cooking oil (Kumar et al. 2012).

Gas chromatography

Fatty acids were methylated with $\text{BF}_3\text{-MeOH}$ as described by O'Fallon et al. (2007). The analytical conditions of GC for FAME detection were $75 \text{ m} \times 0.18 \text{ mm}$ SP-2560 capillary column. Nitrogen gas was used as mobile phase at rate of 20 ml/min. The injector temperature was set at $250 \text{ }^\circ\text{C}$ and detector temperature at $260 \text{ }^\circ\text{C}$ using flame ionization detector (FID). Oven temperature was programmed from 140 to $240 \text{ }^\circ\text{C}$ at $4 \text{ }^\circ\text{C}/\text{min}$.

Results and discussion

Characterization of waste cooking oil

The thermal and oxidative changes of vegetable oil under cooking temperature were studied by GC–MS to depict the chemical constituent of waste cooking oil. GC–MS analysis of waste cooking oil exhibited the presence of hydrocarbons and polymerized derivative of glyceride. It was observed that hydrocarbons such as n-hexadecane, 2,3,5,8-Tetramethyldecane, pentadecane, 2,6,10,14-tetra methylheptadecane, 3,3 dimethyl heptane, and 2,2,3,3,tera methyl pentane were detected in repeated cooking of oil. n-Tetradecane might be formed by removal of terminal oxirane group from 2-Tetradecyl oxirane (Fig. 1). 2-Tetradecyl oxirane is highly unstable compound owing to presence of oxirane group which further dissociates from parent chain yield derivative of multiple hydrocarbons. The entire mechanism for generation of cyclic and noncyclic hydrocarbon in vegetable oil during repeated cooking is not possible to predict owing to spectrum of reactions and formation of unstable intermediates. However, major transformations proceed via a free radical mechanism of chain reactions. The hydrogen with the weakest bond on the carbon of oil was removed first and forms free radical, because low energy is required to break weak C–H bond.

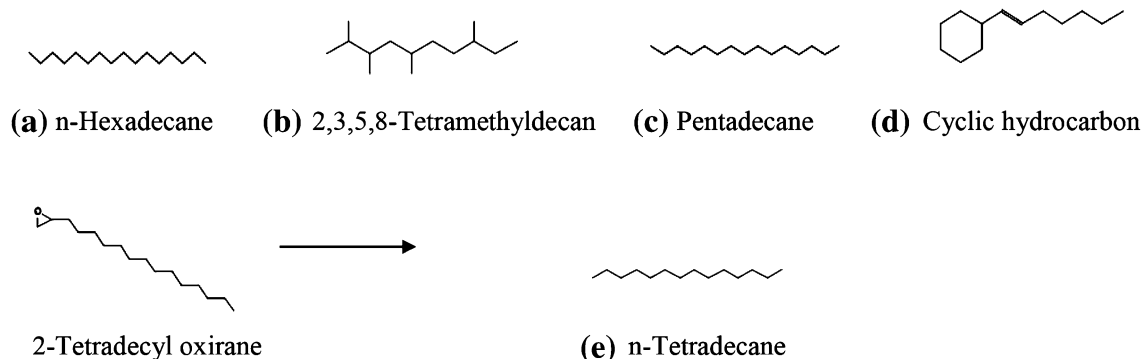


Fig. 1 Cyclic and noncyclic derivative of hydrocarbon in waste cooking oil

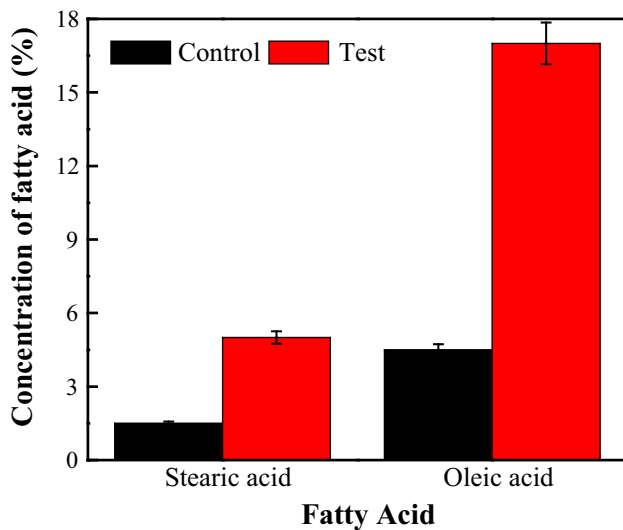


Fig. 4 Identification of fatty acids by gas chromatography

Table 1 C-18 fatty acids composition in uncooked and repeated cooking oil

S. no.	Fatty acids	Fatty acid (%)	
		Before use	After repeated cooking
2	Oleic acid	4–6	4–5
3	Stearic acid	3–5	1–2
4	Linoleic acid	55–60	28–32

indicated lipolytic efficiency of lipase. Initially, the concentration of linoleic acid in uncooked oil was 55–60 % and reduced to 28–32 % after repeated cooking of oil (Table 1). Approximately 30 % loss of linoleic acid indicates rapid oxidation to either oleic acid or stearic acid through proposed mechanism (Fig. 2). Thus, polyunsaturated linoleic acid containing double bond at carbon position 9 and 12 was to be reduced either to monounsaturated oleic acids or saturated stearic acid through oxidative free radical mechanism. However, these fatty acid were not detected during GC-MS analysis of waste cooking oil, which means multiple oxido-reduction process facilitated the formation of its conjugated derivatives. Therefore, percentage of fatty acid in repeated heating of oil was decreased but the percentage of fatty acid in lipase-treated waste cooking oil was increased compared to untreated waste cooking oil. This might be due to hydrolysis of polymerized derivatives of fatty acid by lipase. Kowalski (2007) reported concentration of total fatty acids was decreased from 86 to 75 % after 72 h and to 68 % after 120 h heating of olive oil at 90 °C, while sunflower oil consisting initially 80 % fatty acid reduced to 61 % after heating for 72 h and to 56 % after 120 h. The lower loss of

total fatty acids from olive oil compared to sunflower oil was because of its lower content of linoleic acid, which is readily oxidized. Edwinoliver et al. (2010) had used lipase to hydrolyze tallow and observed 36 % oleic acid, 19 % stearic acid and 27 % palmitic acid through gas chromatography.

Conclusions

Repeatedly used cooking oil consisted of various polymerized derivatives, hydrocarbons and glyceride molecules; its consumption is susceptible to health concern. It cannot be disposed due to environment issue. Therefore, lipase produced from *Penicillium chrysogenum* was exploited to hydrolyze waste cooking oil in order to procure value-added products as C-18 fatty acids. The 22 % C-18 fatty acid, which was obtained from waste cooking oil opened new avenues to explore lipase for various industrial applications such as for biofuels, soap and detergent industries. Current approach was very ecofriendly and profit-making under the principles of green chemistry.

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Conflict of interest The authors declare that they have no conflict of interest in the publication.

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