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RESEARCH NOTE

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Production of Lipase-catalyzed Structured Lipids from Mustard Oil with Capric acid

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Abstract To reduce the content of undesirable erucic acid in mustard oil (MO), it was enzymatically modified with capric acid using immobilized lipase TL IM to produce structured lipid (SL). After reaction, the content of erucic acid was reduced up to 21.7% under the performed reactions in this study. Meanwhile, unsaturated fatty acids existing at *sn*-2 position (oleic acid, linoleic acid, and linolenic acid) in MO were not much changed.

Keywords: mustard oil, capric acid, erucic acid, structured lipid

Introduction

Lipids (oils and fats) are known to play nutritional and functional roles in food products. Usually, consumers prefer cooking oils which are available in their region. Therefore, people of eastern and northern parts of India, Nepal, and Bangladesh commonly use mustard oil (MO) for cooking. MO has a strong pungent odor and hot taste. However, it has previously reported that MO contains high amount of erucic acid (1). The high level of erucic acid in human food is not desirable in European Union and other countries since erucic acid showed serious pathological changes in the heart and skeletal muscles in animals after feeding with rapeseed oil containing high amount of erucic acid (2). Hence, it is necessary to reduce erucic acid content from MO.

Lipase-catalyzed modification of triacylglycerol (TAG) provides a way to replace undesirable fatty acids with healthy fatty acids (3). This method shows several advantages over chemical-catalyzed process due to mild reaction, regiospecificity, and low by-products (4).

The objective of this research was to reduce erucic acid content from MO using lipase-catalyzed reaction with medium chain fatty acid (MCFA) such as capric acid. MCFA possesses characteristics of high stability against oxidation, low viscosity, and melting points. Moreover, MCFA can be easily hydrolyzed from TAG structure, and metabolized in liver as a quick energy source (5). Previous study revealed that MCFA increased energy expenditure, and less fat was deposited in the body as compared to long chain fatty acids (6). In this present study, the effects of reaction time and mole substrate ratio on the incorporation of capric acid (C10:0) into MO were studied.

Materials and Methods

Materials Mustard oil (MO) was supplied from Agricultural Marketing Co., Ltd. (Dhaka, Bangladesh). The immobilized lipase TL IM was obtained from Novozymes A/S (Bagsvaerd, Denmark). According to the manufacturer specification, the specific activity of lipozyme TL IM is 175 IUN/g catalytic activities, having 0.54 g/mL bulk density and 0.3-1.0 mm particle diameter. Hexane, 2-propanol, heptadecanoic acid, capric acid, and acetic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). The pancreatic lipase also from Sigma-Aldrich was purchased.

Fatty acid composition analysis The fatty acid composition of each sample was analyzed according to the method developed by our group Lee and Akoh (7).

Positional fatty acid composition Sample (7 mg) was taken in a test tube. Seven mL of Tris-HCl buffer (pH 7.6), 1.75 mL of 0.05%(w/v) bile salt in distilled water, 0.7 mL of 2.2%(w/v) $CaCl_2$ in distilled water, and 7 mg of pancreatic lipase were mixed together for hydrolysis. Other steps were followed as described by Lee and Akoh (7).

Effect of mole substrate ratio and reaction time MO and capric acid were mixed together in a screw-capped test tube at a mole substrate ratio of 1:1, 1:2, 1:3, 1:4, and 1:5. Three mL of hexane was added in all reaction mixtures. The reaction started after adding immobilized lipase TL IM (10% of the total weight of the substrate). After that, the combined blends were incubated in an orbital-shaking water bath at 180 rpm and 55°C for 24 hr. To know the effect of reaction time on the incorporation, substrate of 1:3 (MO:capric acid) was selected for 36 hr reaction. The reactant was passed through an anhydrous sodium sulfate column for separation of enzyme and removal of water. The solvent was evaporated with nitrogen gas in the heating module (60°C). The fatty acid compositions were analyzed by GC for the obtained reactant at the designated reaction time.

TAG profile analysis by high performance liquid chromatography (HPLC) The TAG composition of each sample was analyzed by HPLC according to Lee *et al.* (8).

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Statistics The statistical analysis system (SAS, Cary, NC, USA) was used to perform statistical analysis. Duncan's multiple-range tests was performed on the means of values.

Results and Discussion

Fatty acid composition The fatty acid compositions (mol%) of mustard oil (MO) are presented in Table 1. As observed, the most abundant fatty acid of MO was erucic acid, showing 42.9% of total fatty acids. Other compositional fatty acids were oleic acid (17.3%), linoleic acid (15.9%), and linolenic acids (18.1%). Our results showed good agreement with previous reports (9,10). It should be mentioned that unsaturated fatty acids consisted of predominantly in MO, accounting for 94.2% while only 5.8% of saturated fatty acids were found. However, high amount (42.9%) of erucic acid among the unsaturated fatty acids could not allow MO for healthy food application since erucic acid can cause some health problems (2). In order to confirm the positional presence of erucic acid in TAG structure, pancreatic hydrolysis was conducted. It found that unsaturated fatty acids mostly existed at *sn*-2 position while most of erucic acid (63.7%) was placed at *sn*-1,3 position (Table 1).

Table 1. Total and positional distribution of fatty acids content of mustard oil

Fatty acid	Composition (mol%)		
	sn-1,3	*sn*-2	Total
Palmitic acid C16:0	5.6	0.8	4.0
Stearic acid C18:0	2.5	0.4	1.8
Oleic acid C18:1	8.3	35.4	17.3
Linoleic acid C18:2	4.1	39.5	15.9
Linolenic acid C18:3	15.8	22.6	18.1
Erucic acid C22:1	63.7	1.3	42.9
Saturated fatty acids (ΣSFA)	8.1	1.2	5.8
Unsaturated fatty acids (ΣUFA)	91.9	98.8	94.2

Effect of reaction time on incorporation of capric acid One purpose of this study was to reduce erucic acid content in MO using lipase-catalyzed reaction with functional capric acid. Therefore, erucic acid in TAG molecules of MO was assumed to be replaced by capric acid through *sn*-1,3 specific lipase TL IM. The results of reaction time on the incorporation of capric acid into MO are given in Table 2. The incorporation of capric acid increased with increasing reaction time. Meanwhile, the decrease of erucic acid amount was simultaneously observed. The highest incorporation of capric acid (37.5%) was found at 36 hr. However, the lowest amount of erucic acid was found from 24 hr reaction, showing 24.3%. Previously, Turan *et al.* (11) observed that high incorporation of capric acid into soybean oil was obtained under the increased reaction time. However, a migration of capric acid, which was not expected, was also found after longer time reaction. Fomuso and Akoh (12) confirmed that the highest SL production was obtained from 24 hr reaction under their reaction condition, after which there was no significant increase in the production of SL. To consider the reaction efficiency and the prevention of acyl migration, the 24 hr reaction time was further considered at which the lowest content (24.3%) of erucic acid was obtained.

Effect of molar ratio on incorporation of capric acid The effect of substrate molar ratio on incorporation of capric acid into MO was also studied. Each reaction was conducted for 24 hr. As shown in Table 3, the incorporation of capric acid increased with increasing the molar ratio. On the contrary, the erucic acid content decreased with

Table 2. Effect of reaction time on the incorporation of capric acid into mustard oil

Reaction time (hr)	Fatty acid composition (mol%)						
	10:0	16:0	18:0	18:1	18:2	18:3	22:1
1	7.7$^{f1)}$	3.2^{a}	1.4^{a}	15.8^{a}	15.6^{a}	17.5^{a}	38.8^{a}
3	11.1^{e}	2.7^{b}	1.4^{a}	15.6^{b}	15.0^{b}	16.2^{b}	38.0^{b}
6	21.7^{d}	2.2^{c}	1.2^{b}	14.5^{c}	14.1^{c}	14.2^{c}	32.1^{c}
12	27.0^{c}	2.1^{d}	1.1^{c}	14.0^{e}	13.3^{e}	13.1^{d}	29.4^{e}
24	32.9^{b}	1.7^{f}	1.0^{d}	14.2^{d}	13.8^{d}	12.3^{e}	24.3^{f}
36	37.5^{a}	2.0^{e}	1.2^{b}	10.3^{f}	9.1^{f}	10.3^{f}	29.6^{d}

[1)]Different letters within column are significantly different ($p<0.05$).

Table 3. Effect of molar ratio on the incorporation of capric acid into mustard oil

Mole ratio	Fatty acid composition (mol%)						
	10:0	16:0	18:0	18:1	18:2	18:3	22:1
1:1	16.3$^{e1)}$	2.7^{a}	1.4^{a}	13.9^{b}	12.9^{b}	13.9^{a}	38.9^{a}
1:2	28.3^{d}	2.4^{b}	1.2^{b}	12.5^{c}	12.0^{c}	12.5^{b}	31.1^{b}
1:3	32.9^{c}	1.7^{d}	1.0^{d}	14.2^{a}	13.8^{a}	12.3^{c}	24.3^{d}
1:4	37.6^{b}	1.8^{c}	0.9^{e}	12.1^{d}	11.7^{d}	11.0^{d}	24.9^{c}
1:5	42.3^{a}	1.6^{e}	1.1^{c}	11.7^{e}	11.4^{e}	10.2^{e}	21.7^{e}

[1)]Different letters within column are significantly different ($p<0.05$).

Table 4. Total and positional fatty acid composition of reaction products with different molar ratio

Fatty acids	Molar ratio of mustard oil and capric acid														
	1:1			1:2			1:3			1:4			1:5		
	Total	*sn*-2	*sn*-1,3	Total	*sn*-2	*sn*-1,3	Total	*sn*-2	*sn*-1,3	Total	*sn*-2	*sn*-1,3	Total	*sn*-2	*sn*-1,3
C10:0	16.3	5.9	21.5	28.3	7.3	38.8	32.9	8.8	44.9	37.6	8.1	52.36	42.3	10.4	58.3
C16:0	2.7	3.1	2.5	2.4	2.8	2.2	1.7	2.5	1.3	1.8	4.8	0.3	1.6	3.2	0.8
C18:0	1.4	ND[1)]	2.1	1.2	ND	1.8	1.1	2.1	0.5	0.9	14.7	0.2	1.1	2.6	0.3
C18:1	13.9	34.5	3.6	12.5	30.8	3.4	14.2	30.5	6.1	12.1	28.4	3.9	11.7	32.8	1.2
C18:2	12.9	30.8	4.0	12.0	30.4	2.8	13.8	31.3	5.1	11.7	27.5	3.8	11.4	24.4	4.9
C18:3	13.9	24.5	8.6	12.5	23.3	7.1	12.3	21.5	7.7	11	13.2	9.9	10.2	15.4	7.6
C22:1	38.9	1.3	57.7	31.1	2.4	45.5	24.9	2.9	35.9	24.9	3.2	35.7	21.7	4.5	30.3

[1)]Not detected.

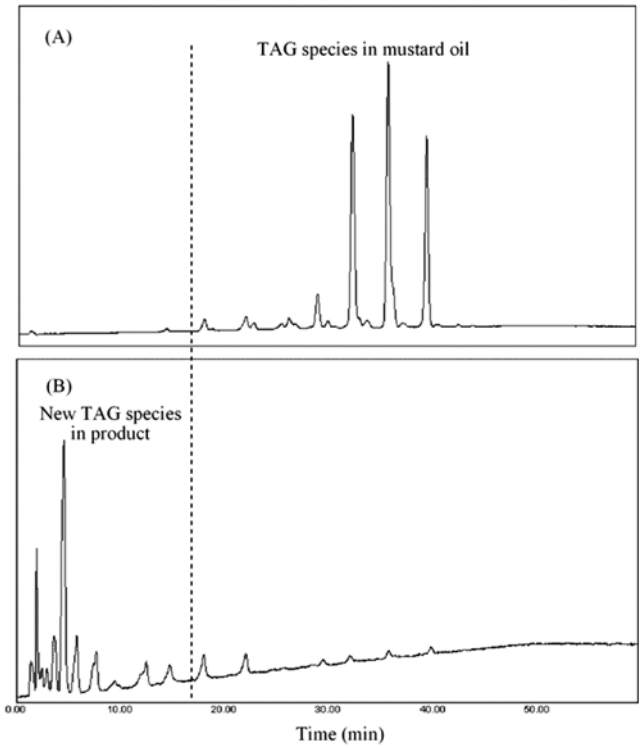

Fig. 1. Reverse-phase HPLC separation of TAG species in mustard oil (A) and reaction product of mustard oil with capric acid at molar ratio of 1:5 after 24 hr (B).

increasing the molar ratio. At 1:5 molar ratios, the highest incorporation of capric acid was found to be 42.3% with the lowest content of erucic acid (21.7%) was observed. Even though lipozyme TL IM is known as a *sn*-1,3 specific enzyme, capric acid was found at *sn*-2 position arranging from 5.9 to 10.4%, suggesting that acyl migration occurred during the reaction (Table 4). Meanwhile, unsaturated fatty acids existing at *sn*-2 position (oleic acid, linoleic acid, and linolenic acid) in MO were not much changed.

Separation of TAG molecules MO and selected reaction product (1:5) after 24 hr reaction were analyzed by reverse-phase HPLC. The chromatograms are presented in Fig. 1. In a reverse-phase HPLC, it was proved that the elution of TAG is according to their equivalent carbon number (ECN) (13). TAG with lower ECN value is eluted earlier. On the contrary, TAG with higher ECN value is eluted with longer retention time. As shown in Fig. 1 (A), TAG species from mustard oil with higher ECN values showed 3 major clusters at retention time 32.4, 35.8, and 39.5 min, accounting for 27.6, 34.1, and 21.0%, respectively. However, after reaction, the TAG species existing in MO disappeared while new TAG species with lower ECN values were produced and eluted earlier (Fig. 1B). The reason was contributed to the incorporation of capric acid into TAG species in MO and reduced the ECN values of the TAG species.

In conclusion, the purpose of this study was to obtain erucic acid-reduced mustard oil using lipase-catalyzed esterification with capric acid. The reaction condition was optimized at different time and different substrate molar ratio. The highest incorporation of capric acid (42.3%) was found at 24 hr and 1:5 substrate molar ratio in the reaction as well as the lowest amount of erucic acid (21.7%) was obtained.

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References

1. Samman S, Chow JWY, Foster MJ, Ahmad ZI, Phuyal JL, Petocz P. Fatty acid composition of edible oils derived from certified organic and conventional agricultural methods. Food Chem. 109: 670-674 (2008)
2. Food Standards Australia New Zealand. Erucic acid in food: A Toxicological Review and Risk Assessment. Tech. Report Series 21: 1448-3017 (2003)
3. Fomuso LB, Akoh CC. Stuctured lipids: Their food applications and physical property testing methods. Food Sci. Biotechnol. 10: 690-698 (2001)
4. Xu XB. Production of specific-structured triacylglycerols by lipase-catalyzed reactions: A review. Eur. J. Lipid Sci. Tech. 287-303 (2000)
5. Kim IH, Kim H, Lee KT, Chung SH, Ko SN. Lipase-catalyzed acidolysis of perilla oil with caprylic acid to produce structure lipids. J. Am. Oil. Chem. Soc. 79: 363-367 (2002)
6. Papamandjaris AA, MacDougall DE, Jones PJH. Medium chain fatty acid metabolism and energy expenditure: Obesity treatment implications. Life Sci. 62: 1203-1215 (1998)

7. Lee KT, Akoh CC. Immobilized lipase-catalyzed production of structured lipids with eicosapentaenoic acid a specific positions. J. Am. Oil. Chem. Soc. 73: 611-615 (1996)
8. Lee KT, Jone KC, Foglia TA. Separation of structured lipids by high performance liquid chromatography. Chromatographia 55: 197-201 (2001)
9. Pritchard JLR. Analysis and properties of oilseeds. pp. 80-95. In: Analysis of Oilseeds Fats and Fatty Foods. Rossell JB, Pritchard JLR (eds). Elsevier, London, UK (1991)
10. Fernandez-Escober J, Dominguez J, Marti A, Fernandez-Martinez JM. Genetics of erucic acid content in interspecific hybrids of Ethiopian mustard (*B. carinata A. Braun*) and rapeseed (*B. napus* L.). Plant Breeding 100: 310-315 (1988)
11. Turan S, Karabulut I, Vural H. Effects of reaction parameters on the incorporation of caprylic acid into soybean oil for production of structured lipids. J. Food Lipids 13: 306-317 (2006)
12. Fomuso LB, Akoh CC. Structured lipids: Lipase-catalyzed interesterification of tricaproin and trilinolein. J. Am. Oil. Chem. Soc. 75: 405-410 (1998)
13. Lai OM, Low CT, Akoh CC. Lipase-catalyzed acidolysis of palm olein and caprylic acid in a continuous bench-scale packed bed bioreactor. Food Chem. 92: 527-533 (2005)