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Medium chain and behenic acid incorporated structured lipids from sal, mango and kokum fats by lipase acidolysis

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ABSTRACT

Medium chain (MC) and behenic fatty acids were incorporated into kokum, sal and mango fats using 1,3-specific lipase catalysed acidolysis. The incorporation of fatty acids increased with increase in concentration of fatty acids and duration of reaction. The order of incorporation of fatty acids was C22:0 > C10:0 > C8:0, to the extent of 53%, 42.5%, 35.8%, respectively, after 16 h, using kokum as substrate. The same trend was observed with sal or mango fats as substrates though the percentages incorporated were different. The modified products with higher contents of MC were liquids with no solid fats, even at 0 °C, and which showed low cloud point due to an increase in triacylglycerols containing lower chain fatty acids. The modified products after incorporating both MC and C22:0 showed long melting ranges and were suitable for use in bakery, confectionery, etc. as vanaspati substitutes.

1. Introduction

Structurally defined lipids (SL) have been developed by changing the position and composition of fatty acids from the native oils or fats to enhance nutritional quality and to meet the demands of health conscious consumers. Lipids can be restructured by incorporating essential fatty acids or particular fatty acids into specific positions of the glycerol backbone of triacylglycerols (TGs) for nutritive or therapeutic purposes. SL may be synthesised by enzymatic methods using a 1,3-specific lipase (Fomuso & Akoh, 1997, 1998), the advantage of which is due to its selectivity, mild reaction conditions, little or no side reactions, ease of product recovery, easy control over the process and less wastage (Akoh, 1997). In recent years, several researchers have reported the preparation of various types of SL from a variety of oils/fats with specific fatty acids (Akoh & Yee, 1997; Lee, Akoh, & Lee, 2008; Mangos, Jones, & Foglia, 1999; Mu, Xu, & Hoy, 1998; Schmid, 1998; Senanayake & Shahidi, 2002; Tynek & Ledochowska, 2005; Xu, Fomuso, & Akoh, 2000). SL containing short chain, or medium chain, or both, with long chain fatty acids, preferably on the same glycerol molecule, exhibit maximum nutritional benefits (Akoh, 1995).

Medium chain triglycerides (MCT) consist of caprylic (C8:0) and capric (C10:0) acids, and minor amounts of caproic (C6:0) and lauric (C12:0) acids (Bach & Babayan, 1982). Compared to long chain TGs, MCTs are less likely to be deposited as body fat because they are not readily re-esterified into TGs (Geleibter, Torbay, Bracco, Hashim, & Van Itallie, 1983). They are metabolised as rapidly as glucose and have higher caloric densities than carbohydrates or proteins (Babayan, 1987).Hence, they may be utilised as rapid sources of energy. With increasing consumer awareness of the risks associated with high fat intake, there is an increasing demand for reduced calorie fats. Reduced calorie SL are designed by taking advantage of the limited absorption of long chain saturated fatty acids or the lower calorie content of short chain fatty acids (Haumann, 1997; Wardlaw et al., 1995). The synthesis of TG with long and short chain fatty acids is one of the methods used to obtain low calorie fats. Such examples include TGs containing behenic (C22:0), caproic and caprylic acids. The absorption of behenic acid in the intestine was reported to be about 28% in animal experiments (Yang, Jang, Jun Han, & Rhee, 2001). Incorporation of MC and behenic acids into palm olein and palm stearin and their suitability for use in various food products have previously been reported (Mounika & Yella Reddy, 2012a, 2012b).

The purpose of the present study was to incorporate both medium chain fatty acids, such as caprylic and capric acids, and low calorie behenic acid into non-traditional (NTOs) fats like kokum, mango and sal fats, to prepare value added and nutritionally superior speciality fats. Sal, mango and kokum fats are major non-traditional oilseeds in India and have good potential for use in various food applications including chocolate, bakery, etc. and processes to prepare such fats have already need reported

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(Maheshwari & Yella Reddy, 2005; Yella Reddy & Jeyarani, 2001; Yella Reddy & Prabhakar, 1990). Development of such value added and nutritionally superior fats/oils from these NTOs will boost the production of these underutilised sources of fats.

2. Materials and methods

2.1. Materials

Palm oil was procured from Palmtech India Ltd., Mysore, India. immobilised 1,3-specific lipase, Lipozyme RMIMLux00212, was procured from Novozymes, South Asia Ltd., Bangalore, India. All the chemicals and solvents used were of analar and HPLC grade.

2.2. Methods

2.2.1. Lipase acidolysis

The formulated blend was stirred at 90 °C under vacuum on a magnetic stirrer to dry the sample. The dried blend was mixed with 10% (w/w) of 1,3-specific lipase and 2% (w/v) of hexane and stirred continuously at 65–70 °C, till the end of the reaction. The reactions were conducted at intervals of 1, 2, 4, 6, 8, 16, and 24 h. After the end of the reaction, the enzyme was removed by vacuum filtration and washed with hexane. The solvent from the esterified sample was removed under vacuum and the sample was used for further analysis. Enzyme at 5% and water at 1% by wt of substrate were used to study their effect on the incorporation of fatty acids.

2.2.2. Removal of free fatty acids (FFA)

FFA from re-esterified products were removed as per the procedure described by Tynek and Ledochowska (2005). The samples after acidolysis were dissolved in hexane at a 1:1w/v ratio at 65 °C, and FFA were neutralised with 0.1 N KOH in 50% ethanol. The hexane layer was separated and washed with 50% ethanol to remove soap until it was neutral to phenolphthalein and it was then dried over anhydrous sodium sulfate. The solvent was removed under vacuum and purified samples were used for further analysis.

2.2.3. Fatty acid composition

The purified samples after lipase acidolysis were methylated using KOH/MeOH as per the AOCS (2002-03) method. Fatty acid methyl esters so obtained were analysed by gas liquid chromatograph (Varian GC, model 450; Varian BV, The Netherlands) with an FID detector. A fused silica capillary column SP-2340 (Supelco, Bellefonte, USA) 50 m × 0.25 mm; 0.25 μm was used; nitrogen flow, 1 mL/min; hydrogen flow, 1 mL/min; air flow, 1 mL/min; column temperature, 50–220 °C at 5 °C/min and at 220 °C maintained for 10 min; injector temperature, 220 °C, detector temperature, 230 °C. The fatty acids were identified using standard fatty acid methyl esters and were reported as the relative percentage of total fatty acids.

2.2.4. Differential scanning calorimetry (DSC)

A Mettler (Zurich, Switzerland) DSC-30 instrument was used to obtain the melting characteristics of the samples. The heat flow of the instrument was calibrated using indium. The PT-100 sensor was calibrated using indium, zinc and lead. To ensure homogeneity and to destroy all crystal nuclei, the samples were first heated to 60 °C. About 15 mg of the molten sample was accurately weighed into a standard aluminium crucible and crimped in place. An empty aluminium crucible with a pierced lid was used as a reference. For melting characteristics, the samples were stabilized according to the IUPAC method (1987), which includes maintaining the samples at 0 °C for 90 min, 26 °C for 40 h then 0 °C for 90 min prior to introduction into the DSC cell. Thermograms were recorded by heating at the rate of 2 °C/min from −5 to 60 °C. The peak temperatures, heats of fusion (ΔH), and % liquid at various temperatures were recorded directly using a TC-10A data processor and STARe program. The solid fat content (SFC) was calculated from the percentage of liquid and the melting profiles were drawn by plotting SFC against temperature.

2.2.5. Triacylglycerol composition

Triacylglycerol (TG) composition was determined by high performance liquid chromatography (HPLC) (Simadzu LC-10A, Simadzu Corp., Tokyo, Japan) with refractive index detector. The column used was a C-18 (ODS), length 25 cm × 9, 4.6 mm id, the mobile phase was 63.5% acetone and 36.5% acetonitrile and the flow rate 1 mL/min (Che Man, Haryati, Ghazali, & Buana, 1998). Peaks were identified by comparing the retention time with those of authentic standards and reported as relative percentages.

2.2.6. Slip point and cloud point

These were determined as per the AOCS (2002-03) procedure.

2.2.7. Statistical analysis

All experiments were performed in triplicate and data were reported as means ± standard deviation. Duncan Multiple Range Test (DMRT) at a level of $P < 0.05$ (Duncan, 1955) were carried out to assess the significance of differences among the mean values.

3. Results and discussion

3.1. Effect of concentrations of fatty acids and substrate on % incorporation of fatty acids into kokum or sal fats

The concentrations of fatty acids (FA) and substrate were found to have a considerable infuence on the incorporation of FA by lipase acidolysis. Incorporation of C8:0, C10:0 or C22:0 fatty acids increased with an increase in their concentrations from 40 to 60 wt.% and, at 70%, no effect was observed in kokum fat as substrate when FA was added individually (Table 1). Even when two FA were added in combination at a combined 60% level, incorporation was at a maximum, whereas at lower (40%) or higher (80%) levels, the level of incorporation was less. For instance, 22% of acids were incorporated when FAs were added at the 40% level,

Table 1
Effect of substrate and fatty acid (FA) proportions on fatty acid incorporation when added individually or in mixtures to kokum, sal or mango fats.

Substrate	Substrate/FA ratio (w/w/w)	Time (h)	C8:0	C10:0	C22:0
Kokum fat	6:4	2	4.0^{a}	19.3^{b}	16.1^{a}
	1:1	2	12.6^{b}	20.5^{b}	30.3^{b}
	4:6	2	15.1^{c}	27.1^{c}	39.5^{c}
	3:7	2	11.3^{b}	10.8^{a}	39.4^{c}
	4:3:3	2	9.0	9.6^{c}	–
	4:3:3	2	–	13.4^{e}	14.5^{c}
	6:2:2	2	5.3	7.1^{b}	–
	6:2:2	2	–	11.0^{d}	11.0^{b}
	2:4:4	2	3.2	4.0^{b}	–
	2:4:4	2	–	3.5^{a}	3.6^{a}
Sal fat	4:3:3	6	–	2.5^{b}	19.7^{a}
	5:2:3	6	–	8.6^{c}	26^{b}
	4:1:5	6	–	0.2^{a}	42.5^{d}
	4:2:4	6	–	13.6^{d}	28.4^{c}
Mango fat	4:3:3	6	–	3.5^{b}	21.1^{a}
	4:2:4	6	–	2.2^{a}	27.6^{b}
	4:1:5	6	–	2.6^{a}	45.0^{c}

The values in the same column within the group having different superscripts are significantly different ($p < 0.5$).