Distinction Between Enzymically and Chemically Catalyzed Interesterification

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The 1,3-specific lipase-catalyzed interesterified fats were distinguished from chemically catalyzed products by the fatty acids in the 2-position. The fatty acid contents in the 2-position of the 1,3-lipase-catalyzed and the original triglycerides were similar but different from that of chemically interesterified fat. Also, the saturated-to-unsaturated fatty acid ratio in the 2-monoglycerides was lower for the 1,3-specific lipase-catalyzed interesterified fats than for the corresponding chemical products.

KEY WORDS: Interesterification (transesterification), lipase from *Mucor miehei* (Lipozyme), lipolysis, 2-monoglycerides.

Lipozyme catalyzes transesterification reactions with 1,3-positional specificity, and fatty acid distributions of the resulting triglycerides are significantly different from that produced by chemical nonenzymatic means. Random lipase-catalyzed reactions are similar to chemical transesterifications in terms of positional specificity of fatty acids (1). The specific lipase-catalyzed interesterification reaction can be distinguished from chemical or random lipase-catalyzed reactions on the basis of distribution of saturated and unsaturated fatty acids in the 1,3- and the 2-positions of the triglycerides.

Positional analysis of triglycerides by the technique of porcine pancreatic lipase hydrolysis and by stereospecific analysis according to Brockerhoff (2) is well established. In the present study, the porcine pancreatic lipase technique has been examined for the purpose of distinguishing 1,3-specific lipase-catalyzed interesterified products from those produced by chemical means.

MATERIALS AND METHODS

Source of lipases. Mucor miehei lipase (Lipozyme IM) was supplied by Novo Nordisk (Bagsvaard, Denmark). Steapsin, a pork pancreatic lipase, was obtained from Sigma Chemical Co. (St. Louis, MO).

Fat samples. All fat samples were refined, bleached and deodorized in the laboratory according to the standard method (3). Blends were made by simple mixing.

Chemical interesterification. Chemical interesterification was carried out with 0.2% sodium methoxide catalyst (30% w/w solution in dry methanol) at 90°C in nitrogen atmosphere for 0.5 h, followed by isolation of the product free of methyl esters (4).

Lipozyme-catalyzed interesterification. Lipozyme-catalyzed interesterification with *M. miehei* enzyme was carried out essentially by following published methods (5,6). Fifty grams of oil and 5 g *M. miehei* lipase preparation were stirred at 60°C with withdrawal of samples at 1-h intervals for checking both slip melting point and free fatty acid formation. These two remained almost constant from a 1-h to 4-h reaction period. The product with 0.5% free fatty acid was treated with hexane and isolated by filtering of the Lipozyme. Finally, the product was obtained by desolventizing and drying under vacuum.

Pancreatic lipase hydrolysis technique. The pancreatic lipase hydrolysis method of Luddy *et al.* (7) was adopted. About 50 mg of triglyceride was taken into a small, stoppered conical flask along with about 50 mg pancreatic lipase. Then, 1.0 mL of 1 M *tris*(hydroxymethyl) methylamine (pH 8.0), 0.1 mL of 22% $CaCl_2$ and 0.25 mL of 1% bile salt were added. After 1 min at 40°C, the mixture was extracted with diethyl ether. The ether was washed with water, dried over anhydrous sodium sulfate, filtered and evaporated. The individual products were quickly isolated by preparative thin-layer chromatography (8) on Silica Gel G with hexane and ether (60:40). The 2-monoglycerides were extracted with chloroform and then converted to methyl esters according to Metcalfe and Schmitz (9) and were examined by gas–liquid chromatography.

RESULTS AND DISCUSSION

Fatty acid compositions of triglycerides and 2-monoglycerides of vegetable oils and several blends were examined before and after interesterification by chemical means and by 1,3-specific *M. miehei* lipase (Lipozyme), as shown in Tables 1 and 2.

The distribution pattern of the fatty acids of the oils agreed with findings of previous workers. The positional distribution of fatty acids in palm oil triglycerides compared well with the pattern reported by Barrett *et al.* (10). The distribution in cottonseed oil also confirmed the finding of Mattson and Lutton (11) that cottonseed oil has nearly 90% of the unsaturated fatty acids in the 2-position. Linseed oil triglycerides contained more than 90% unsaturated fatty acids in the 2-position, with linolenic acid as the major component acid, in agreement with Brockerhoff and Yurkowski (12). Mustard oil had erucic acid almost exclusively in the 1,3-position, in confirmation of Mattson and Volphenin (13). The distribution pattern of palmstearin triglycerides showed that palmitic acid occupied the 2-position in much greater proportion in comparison with that of palm oil. The C_{18}-unsaturated acids were located predominantly in the 2-position of triglycerides.

The distribution of fatty acids in the 2-monoglycerides of chemically interesterified products were different from those of Lipozyme-catalyzed products. The chemically interesterified products had fatty acids in random distribution, in agreement with List *et al.* (14).

In the Lipozyme-interesterified fats, the 2-monoglycerides had much lower ratios of saturated to unsaturated acids and were similar to the original fat blends, in agreement with previous work (15).

The observations point out that Lipozyme (1,3-specific lipase)-catalyzed interesterified products were distinguished from the chemically catalyzed products by the composition of fatty acids in the whole triglycerides and in the 2-monoglycerides. The saturated/unsaturated ratio in

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TABLE 1

Fatty Acid Composition of the Whole Triglycerides and of the 2-Monoglycerides of Mustard Oil and Palmstearin Before Blending

	Fatty acids (% w/w)								
	C14:0	C16:0	C18:0	C20:0	C18:1	C18:2	C18:3	C20:1	C22:1
Mustard oil									
Whole triglycerides	—	2.3	1.0	0.4	11.8	14.2	19.9	0.7	48.8
2-Monoglycerides	—	—	—	—	35.9	29.2	28.3	—	6.6
Palmstearin									
Whole triglycerides	1.2	53.8	4.4	0.3	32.5	7.7	—	—	
2-Monoglycerides		43.7	4.2	—	45.1	9.4			

TABLE 2

Fatty Acid Composition of Whole Triglycerides and of the 2-Monoglycerides of Palmstearin and Mustard (70 + 30) Blend and Its Corresponding Interesterified Products

	Fatty acids (% w/w)									
	C14:0	C16:0	C18:0	C20:0	C22:0	C18:1	C18:2	C18:3	C20:1	C22:1
Before interesterification										
Whole triglycerides	0.8	39.5	3.4	0.3	0.2	26.3	9.6	5.0	0.2	14.6
2-Monoglycerides	—	31.1	1.3	—	—	47.1	15.5	2.5	0.4	2.0
Proportion in 2-position	—	26.2	12.7	—	—	59.7	53.8	16.6	66.6	4.6
After catalytic transesterification										
2-Monoglycerides after catalytic interesterification	—	50.4	5.1	—	—	29.2	6.6	—	—	8.6
Proportion in 2-position	—	42.5	50.0	—	—	37.0	22.9	—	—	19.6
After enzymatic (Lipozyme) interesterification										
2-Monoglycerides	—	35.7	0.9	—	—	44.1	14.9	2.6	—	1.8
Proportion in 2-position	—	30.1	8.8	—	—	55.9	51.7	22.8	—	4.1

the 2-position also distinguished the two kinds of interesterified fats.

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