Comparative Nutritional Study of Enzymatically and Chemically Interesterified Palm Oil Products

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ABSTRACT: A comparative evaluation of the nutritional value of palm oil, which was interesterified by lipozyme and by chemical catalysis, has been done with albino rats (Charles Foster strain). The two interesterified fats have identical coefficients of digestibility, and rats fed the two fats exhibit similar patterns of growth response and food efficiency ratio. The total lipid and total cholesterol levels in serum of rats of the two interesterified fats are nearly the same, but there is a significant difference in free cholesterol level. The lipozyme-catalyzed product creates a higher level of free cholesterol. Liver lipids of rats are identical in amount for the two groups, but the total cholesterol level of the lipozyme-catalyzed group is significantly less than the chemically catalyzed group. The positional distribution of fatty acids differs between the two interesterified products. The nutritional attributes of interesterified oils can vary depending on the specificity of the catalyst employed. *JAOCS 72,* 327–330 (1995).

KEY WORDS: Cholesterol, digestibility, interesterification, lipozyme, *Mucor miehei,* palm oil, total lipid.

Interesterification processes by either chemical catalysis or lipozyme-catalyzed methodologies are gaining commercial importance for making specific edible fat products, such as shortenings and margarine bases (1–5). The lipozyme-catalyzed interesterification process has a number of advantages over chemical interesterification. An important feature is that the lipozyme-catalyzed interesterified fat product retains the fatty acids almost intact in the 2-position (6), and the 2-position is generally rich in essential unsaturated fatty acids (EFA). This finding is useful from a nutritional point of view, because the 2-monoglycerides produced by pancreatic lipase digestion are the main carriers of fatty acids through the intestinal wall. The chemically-catalyzed interesterified product has the fatty acids randomly distributed, and, as a result, the 2-position becomes deficient in EFA. The 2-monoglycerides will therefore transport different kinds of fatty acids through the intestinal wall.

To what extent the difference in the fatty acid compositions of the 2-monoglycerides between the lipozyme and chemically-catalyzed interesterified fat products will influence their dietary effects is a matter of immense biochemical and nutritional importance. A literature survey did not reveal any report on the assessment of the nutritional quality of lipozyme-catalyzed interesterified products.

The present study investigates the dietary effects of lipozyme-catalyzed interesterified palm oil and chemically-catalyzed interesterified palm oil on a comparative basis in rats on the coefficient of digestibility, growth, food efficiency ratio and total lipid and cholesterol levels in serum and liver.

EXPERIMENTAL PROCEDURES

Dietary fat samples. Refined, bleached and deodorized (RBD) palm oil was supplied by Palm Oil Research Institute of Malaysia (PORIM) (Kuala Lumpur, Malaysia). The palm oil was interesterified by a chemical catalyst and by lipozyme catalyst, and the two interesterified products were used as dietary fats.

Chemically-catalyzed interesterification. Chemically-catalyzed interesterification of RBD palm oil was carried out with 0.2% sodium methoxide catalyst (30% wt/vol solution in dry methanol) at 90°C in nitrogen atmosphere for 45 min. High-vacuum steam distillation of the interesterified product was done to remove methyl esters (7).

Lipozyme-catalyzed interesterification. Mucor miehei lipase (Lipozyme IM 20) was supplied by Novo Nordisk (Bagsveard, Denmark). The lipozyme-catalyzed reaction was done by following published methods (8,9). The *Mucor miehei* enzyme was added to the RBD palm oil at 10% level by weight and stirred at 60°C for 4 h until it reached a melting point of 37°C.

Analysis of interesterified products. The slip melting point of each interesterified product was determined according to a method of the Indian Standards Institution (10). Pancreatic lipase hydrolysis of the two interesterified products was carried out by standard methods (6,11) with porcine pancreatic lipase (Sigma, St. Louis, MO). Gas–liquid chromatography was employed for the determination of fatty acid composition of original palm oil and the two interesterified fat products (12), as well as for their 2-monoglycerides by converting them into methyl esters (13).

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Animal experiment. Male albino Charles Foster rats were housed individually and were fed the two interesterified diets and water *ad libitum* daily. Daily food consumption and weekly body weight gain were recorded. Two separate feeding experiments were conducted. One feeding experiment was aimed at determining the coefficient of digestibility of the two interesterified fat products. The second feeding experiment was conducted to evaluate nutritional attributes of the two kinds of interesterified palm oil.

Experiment 1. To determine the coefficient of digestibility of the two interesterified dietary fat products, 27 rats (body weight, 130–135 g) were divided in three groups of equal average body weight. Two groups of rats were given the two interesterified products, and one group was given fat-free glucose (71%) diet to determine the metabolic lipid that consists of the lipid materials secreted into the intestinal tract from endogenous sources. After allowing the rats two days for orientation, they were kept for ten days on diets that contained: fat-free casein, 18%; glucose, 61%; yeast, 1%; liver extract (Orheptal, Merck, Bombay, India; derived from fresh liver rich in vitamin B_{12} and other B vitamins), 3%; salt mixture, 7% (14); interesterified fat, 10%. The exact amount of diet consumption was recorded daily, and the feces of each rat were collected, dried and stored daily. Feces of the 10-d period were pooled and powdered, and fecal fat was extracted by Soxhlet extraction with petroleum ether (b.p. 60–80°C, reagent quality) for a few hours. The fecal fat remaining as soap in the residual feces was hydrolyzed overnight with dilute HCl (1:4) digestion, and then Soxhlet extraction was repeated for complete extraction of fat. The coefficient of digestibility was calculated according to the standard method (15), with a correction being made for metabolic lipid.

Experiment 2. To evaluate the nutritional attributes of the two interesterified palm oil products, two groups of eight rats each (80–90 g body weight) were fed two interesterified fat diets that contained: fat-free casein, 18%; starch, 65%; salt mixture, 4% (16); cellulose, 3%; one multi-vitamin capsule per kg of diet; fat, 10%. The diets were adequate in all nutrients.

Rats were maintained on the above diets *ad libitum* for 28 d. The amount of daily diet consumed by each rat and weekly weight gain were noted. Rats were sacrificed under anaesthesia, blood was collected, and liver tissues were excised and stored at deep freeze temperature (–20°C) for analysis. The total lipids were extracted from serum and liver tissue with chloroform and methanol mixture and then estimated gravimetrically (17). The total and free cholesterol in serum and liver lipids were determined by the method of Zlatkis *et al.* (18). For statistical analysis of results, Student's *t*-test (19) was performed.

RESULTS AND DISCUSSION

The two interesterified fats are digestible to the same extent, as indicated by the coefficient of digestibility (97%), as evident from Table 1.

Rats fed the two fats exhibit similar patterns of growth response and food efficiency ratio (Tables 2 and 3). No significant difference was found in total lipid levels in serum and liver of rats fed the two fats (Table 4).

It is also evident (Table 4) that the free cholesterol level in the serum of rats was significantly higher with the enzymatically (lipozyme) interesterified group than with the chemically interesterified group, although the total cholesterol level was identical for the two dietary groups. This observation was not expected, as the lipozyme-interesterified product contains more EFA, such as linoleic acid, in the 2-monoglycerides (Table 5).

The difference in free cholesterol level between the two dietary fats can probably be attributed to the difference in nature of fatty acids in the free state and in the monoglycerides that are invariably formed *in vivo* by pancreatic lipolysis. The difference in the positional distribution of fatty acids originally in the triglycerides of the two types of interesterified fats

TABLE 1
Metabolic Lipid and Coefficient of Digestibility of the Dietary Fats Fed Rats at 10% Level

		Dietary fat group	
	Metabolic lipid	Catalytic interesterification	Enzymatic interesterification
Number of rats	9	9	9
Average weight of rats (g)	133.600 ± 0.230	133.500 ± 0.288	133.500 ± 0.115
Average weight gain (g)	16.700 ± 0.180	23.500 ± 0.173	23.300 ± 0.173
Average food eaten (g/rat for 10 d)	90.000 ± 1.120	98.690 ± 0.049	98.030 ± 0.260
Average fat intake (g/rat for 10 d)	0.000	9.869 ± 0.004	9.803 ± 0.026
Average fat excreted (mg/rat for 10 d)	180.000 ± 0.721	428.000 ± 11.010	427.660 ± 19.200
Metabolic lipid (g for 10 d)	0.180 ± 0.0007	—	—
Coefficient of digestibility	—	97.120 ± 0.269[a]	97.470 ± 0.197[a]
Level of significance	—	NS[b]	NS

[a]Standard error of mean of three groups of rats, each consisting of three rats for each fat. [b]NS, not significant ($P > 0.05$).

TABLE 2
Growth Response[a]

Group	Initial weight (g)	First week (7 d) g	Second week (14 d) g	Third week (21 d) g	Fourth week (28 d) g
Catalytically-interesterified palm oil fed	85.000 ± 2.180	114.375 ± 2.290	140.875 ± 2.770	158.000 ± 2.210	184.125 ± 4.960
Enzymatically-interesterified palm oil fed	85.000 ± 2.020	113.500 ± 2.810	140.375 ± 2.280	154.000 ± 1.180	183.500 ± 2.440
Level of significance	NS[b]	NS	NS	NS	NS

[a]Results are mean ± S.E. [b]NS, not significant ($P > 0.05$).

TABLE 3
Food Efficiency Ratio of Diets Containing Dietary Fats at 10% Level[a]

Group	First week (7 d)	Second week (14 d)	Third week (21 d)	Fourth week (28 d)
Catalytically-interesterified palm oil fed	0.382 ± 0.013	0.324 ± 0.015	0.214 ± 0.014	0.271 ± 0.038
Enzymatically-interesterified palm oil fed	0.373 ± 0.026	0.324 ± 0.013	0.165 ± 0.017	0.327 ± 0.022
Level of significance	NS[b]	NS	NS	NS

[a]Results are mean ± S.E. [b]NS, not significant ($P > 0.05$).

(Table 5) brings out these changes in composition of free fatty acids and 2-monoglycerides.

The lipozyme-interesterified fat product contains predominantly palmitic acid in the 1- and 3-positions of triglycerides and the unsaturated fatty acids in the 2-positions, whereas the chemically-interesterified product shows palmitic acid in almost equal proportion in the 1-, 2- and 3-positions of triglycerides. The unsaturated fatty acids, namely oleic and linoleic, are also distributed in the 1-, 2- and 3-positions at random.

The ratio of palmitic acid and unsaturated fatty acids is lower in the 2-position and higher in the 1,3-positions for the lipozyme-interesterified fat in comparison with the chemically-interesterified fat.

The positional distribution pattern of fatty acids in the triglycerides of the two interesterified fats suggests that palmitic acid is likely to be released more into the free state from the lipozyme-interesterified fat. It can be hypothesized that the increased palmitic acid permeates through the intestinal cell wall, undergoes β-oxidation to form more acetyl CoA, which may be transformed to more cholesterol.

The free cholesterol levels in the liver lipids of the two dietary groups of rats show no significant differences, but the total cholesterol level is significantly higher ($P < 0.05$) for the catalytically-interesterified group than for the lipozyme-interesterified group.

The observation that the liver total cholesterol level of the chemically-interesterified group is higher than that of the lipozyme-interesterified group, while free cholesterol remains identical in both groups, suggests that more cholesterol ester is formed in the chemically-interesterified group. Because pancreatic lipase is 1,3-specific, oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) are formed *in vivo* in case of catalytically-interesterified palm oil. In the presence of acetyl-CoA and adenosine triphosphate, oleic and linoleic acids are preferred substrates to palmitic acid ($C_{16:0}$) in rat liver for the enzyme acetyl-CoA cholesterolacyl transferase. Thus, more cholesterol ester is formed in the liver of rats fed the catalytically-interesterified fat.

It is known that plasma cholesterol level does not necessarily reflect the liver tissue cholesterol concentration in the

TABLE 4
Total Lipid, Free and Total Cholesterol in Serum and Liver Tissue[a]

Group	Total lipid in serum (mg/dL)	Total lipid in liver tissue (mg/g)	Free cholesterol in serum (mg/dL)	Total cholesterol in serum (mg/dL)	Free cholesterol in liver tissue (mg/g)	Total cholesterol in liver tissue (mg/g)
Catalytically-interesterified palm oil fed	503.130 ± 63.470	40.590 ± 1.350	23.300 ± 3.330	99.430 ± 5.110	2.006 ± 0.220	4.500 ± 0.310
Enzymatically-interesterified palm oil fed	511.800 ± 64.370	42.720 ± 2.740	41.070 ± 6.800	105.400 ± 13.600	2.100 ± 0.220	3.340 ± 0.240
Level of significance	NS[b]	NS	$P < 0.01$	NS	NS	$P < 0.05$

[a]Results are mean ± S.E. [b]NS, not significant ($P > 0.05$).

TABLE 5
Characteristics of Dietary Fats

Dietary fat	Slip melting point (°C)	Composition (w/w%)			
		Fatty acid	Total triglycerides	2-Position (based on 2-monoglyceride)	1,3-Positions
Original palm oil	34.5	$C_{16:0}$	46.6	17.1	61.3
		$C_{18:0}$	3.5	2.0	4.2
		$C_{18:1}$	40.9	61.2	30.7
		$C_{18:2}$	8.9	19.0	3.8
Chemically-interesterified palm oil	41.0	$C_{16:0}$	46.1	44.9	46.7
		$C_{18:0}$	3.6	3.2	3.8
		$C_{18:1}$	41.2	40.0	41.8
		$C_{18:2}$	8.9	10.7	8.0
Enzymatically-interesterified palm oil	37.0	$C_{16:0}$	45.6	28.8	54.0
		$C_{18:0}$	4.0	3.9	4.0
		$C_{18:1}$	41.5	52.8	35.6
		$C_{18:2}$	8.9	13.8	6.4

rate of hepatic synthesis of cholesterol because they involve the influence of plasma levels of phospholipids, triglycerides, cholesterol-combining protein and other factors (20).

Lipozyme-interesterified palm oil behaves similarly to palm oil with respect to cholesterol in serum and liver (21), presumably due to their similar positional distributions of fatty acids.

The chemically-catalyzed interesterified palm oil appears to have an edge over the lipozyme-interesterified product in forming relatively less free cholesterol in serum of rats. The positional distribution of fatty acids in the triglyceride molecules, it may be hypothesized, appears to play an important role in regulating the synthesis of free cholesterol in serum.

ACKNOWLEDGMENTS

The authors thank PORIM (Kuala Lumpur, Malaysia) for RBD palm oil, and Novo (Bagsveard, Denmark) for lipozyme IM 20.

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[Received March 25, 1994; accepted December 14, 1994]