Detection of Interesterified Fat in Hydrogenated Fat by Lipase Hydrolysis and by Cooling Curve Analysis

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Porcine pancreatic lipase hydrolysis and Jensen's cooling curve methods can be adopted for detecting interesterified fat products in a mixture with hydrogenated fats. Lipolysis of interesterified fats shows a relatively greater amount of saturated fatty acids in the 2-monoglycerides in comparison with hydrogenated fats. A similar trend of the distribution pattern of fatty acids also can be noted in a mixture. Interesterified fats do not show any rise in temperature in Jensen's cooling curve experiment, whereas hydrogenated fats show a distinct rise in temperature. A gradual increase in temperature is obtained for an interesterified fat when it is mixed with increasing content of a hydrogenated fat. On the other hand, hydrogenated fat shows a lowering in the rise of temperature when it is mixed with an interesterified fat. Simultaneous determination of the fatty acid profiles of the 2-monoglycerides and Jensen's cooling curve characteristics can be utilized in detecting the occurrence of one modified fat in another.

KEY WORDS: Cooling curve, hydrogenated palm, hydrogenated rice bran, hydrogenated soybean, interesterified palm, interesterified products, lipase hydrolysis.

Interesterification and hydrogenation are two important fat modification processes. Interesterified fats generally differ from hydrogenated fats in triglyceride and in fatty acid compositions (1). The two modified fats also differ in their cooling curve characteristics (2,3). However, it often becomes necessary to examine various methods to ascertain whether a product has been made by the interesterification process or by hydrogenation. The porcine pancreatic lipase, which possesses near absolute specificity for hydrolysis of the *sn*-1,3 positions in triglycerides, shows a difference in the distribution pattern of fatty acids in the resulting 2-monoglycerides of interesterified and hydrogenated fats. The porcine pancreatic lipolysis method, followed by examination of the fatty acids of the 2-monoglycerides, is a method for detecting an interesterified fat (4).

It also often becomes necessary to ascertain if an edible plastic fat product (*e.g.*, shortening) is actually a blend of a hydrogenated fat and an interesterified fat.

In the present study, the porcine pancreatic lipase hydrolysis and cooling curve methods have been examined for detecting an interesterified fat in a hydrogenated fat product.

EXPERIMENTAL PROCEDURES

Hydrogenated products were supplied by Rasoi Industries (Calcutta, India), and the interesterified palm products were prepared in this laboratory (5).

All these products were hydrolyzed by pancreatic lipase. Lipolysis was carried out by the method of Luddy *et al.* (6) with Steapsin (a pork panreatic lipase obtained from Sigma Chemicals Co., St. Louis, MO).

About 50 mg of triglyceride was taken into a small stoppered conical flask along with sufficient pancreatic lipase (about 50 mg). Then, 1.0 mL of 1 M tris-hydroxymethyl methylamine buffer (adjusted to pH 8.0), 0.1 mL of 22% $CaCL_2$ solution and 0.25 mL of 1% bile salt solution were added to the contents of the flask. The flask was warmed in a water bath at 40°C for 1 min without shaking. The flask was then stoppered and shaken vigorously transferred to a separatory funnel and extracted with diethyl ether. The ether layer was washed with water until neutral, dried over anhydrous sodium sulfate and filtered, and the ether was evaporated. The products were quickly isolated by preparative thin-layer chromatography on silica gel with a solvent system of hexane and diethyl ether (60:40, vol/vol). The monoglyceride layer was scooped from the plate and extracted with chloroform and then converted to the corresponding methyl esters according to the method of Metcalfe and Schmitz (7).

The hydrogenated fat products and interesterified palm oil were blended in different proportions by simple mixture, and their solidification behavior was studied by Jensen's cooling curve method (8,9). Jensen's apparatus consists of a 6 in. × 3/4 in. test tube fitted with a thermometer and a ring-shaped stirrer inside the test tube. The apparatus was clamped centrally into another 3-in. diameter outer tube. Melted fat (10 g) at 60°C temperature was poured into the inner tube and allowed to cool to a temperature of 40°C in air. This arrangement was then immersed into a water bath of appropriate temperature (usually 0, 5 and 17°C) up to the level of the fat in the inner tube. After the fat attained *ca.* 35°C, stirring was started with one upward and downward motion at intervals of 5, 20, 35 and 50 s, in such a manner that the surface of the fat remained intact. The temperature at the end of each minute was recorded. The stirring was discontinued when the rise in temperature ceased to be as high as 0.1°C. The temperature *vs.* time curve was plotted to obtain the solidification characteristics of the fat.

RESULTS AND DISCUSSION

The fatty acid compositions of the 2-monoglycerides of two interesterified fats are almost the same as for the whole triglycerides (Table 1). Table 2 reveals that the fatty

TABLE 1

Fatty Acid Composition of Total Triglyceride (TG) and of 2-Monoglyceride (MG) of Interesterified Palm Fats

Fat sample	Fatty acid (% w/w)				
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Total TG of interesterified palm oil[a]	44.8	1.3	42.4	11.4	—
2-MG	44.3	1.2	42.6	10.9	—
Proportion in 2 position	33.0	30.8	33.5	31.9	—
Total TG of interesterified palm oil[b]	45.9	5.7	40.0	8.3	—
2-MG	45.4	5.1	40.8	8.6	—
Proportion in 2 position	33.0	29.8	34.0	34.5	—

[a]Slip point at 41°C.
[b]Slip point at 42.8°C.

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TABLE 2

Fatty Acid Composition of Total Triglyceride (TG) and of 2-Monoglyceride (MG) of Hydrogenated Fats

Fat samples	Fatty acids (% w/w)						
	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{20:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Total TG of hydrogenated palm oil	1.4	44.2	3.6	—	45.5	5.3	—
2-MG	3.4	20.3	—	—	61.8	14.4	—
Proportion in 2 position	80.9	15.3	—	—	45.3	90.6	—
Total TG of hydrogenated soybean oil	0.2	16.6	1.8	1.9	74.2	5.3	—
2-MG	—	6.1	—	—	88.8	5.1	—
Proportion in 2 position	—	12.2	—	—	39.9	32.1	—
Total TG of hydrogenated rice bran oil	—	19.2	1.2	—	73.6	6.0	—
2-MG	—	15.0	—	—	78.2	6.8	—
Proportion in 2 position	—	26.0	—	—	35.4	37.7	—

acid compositions of the 2-monoglycerides of the hydrogenated fats are different from those of the corresponding whole triglycerides. In the hydrogenated fats the amount of $C_{16:0}$ decreases and that of $C_{18:1}$ increases in the 2 positions as revealed by lipolysis.

The important observation that emerges from these two tables is that the interesterified palm oil (slip point 41°C) and the hydrogenated palm oil (slip point 42.8°C) differ in the fatty acid composition of the 2-monoglycerides derived from them.

It appears, therefore, that these two kinds of modified fat products can be differentiated by lipase hydrolysis followed by determination of the composition of the individual fatty acids, such as palmitic and oleic, in the 2-monoglycerides derived from the products.

The proportion of an interesterified fat and a hydrogenated fat in a mixture can be obtained by the cooling curve method. Table 3 and Figure 1 display the solidification behavior of the blended products of hydrogenated palm oil and interesterified fat, prepared by randomizing a mixture of palm oil (80 parts) and rice bran oil (20 parts) in different proportion. Table 3 shows that an increase in the proportion of hydrogenated palm oil in the blend results in a rise in temperature upon solidification. This observation, together with high palmitic acid content, can be utilized in detecting the presence of hydrogenated palm oil in an interesterified fat product containing a high content of palm oil (80%).

Table 4 and Figure 1 show the solidification behavior of blends of hydrogenated soybean and interesterified product prepared from 70 parts of palm oil and 30 parts of rice bran oil.

Table 4 shows that there is a steady increase in the rise of temperature above 40% (by weight) of hydrogenated soybean oil in the blend with the above interesterified product. The gradual increase in the rise of temperature (Table 4) and the corresponding fatty acid profiles (Table 5) can serve as a method for detecting hydrogenated soybean oil in the interesterified product prepared from a palm and rice bran oil mixture.

TABLE 3

Solidification Behavior of Blends of Hydrogenated Palm Fat and Interesterified Fat Prepared from Palm and Rice Bran Oil (RBO) (80 + 20)

Fat blends	Super cooling temperature (°C)	Time for super cooling (in min)	Solidification temperature (°C)	Time for solidification (in min)	Rise in temperature (°C)
Interesterified fat (palm + RRO, 80 + 20) + hydrogenated palm					
(80 + 20)	25.70	12	25.80	14	0.10
(70 + 30)	25.95	14	26.15	16	0.20
(60 + 40)	25.95	13	26.20	16	0.25
(50 + 50)	26.45	11	26.90	15	0.45
(40 + 60)	26.25	11	27.25	15	0.95
(30 + 70)	26.60	9	27.45	14	0.85
(20 + 80)	26.85	10	28.55	16	1.70
(10 + 90)	27.15	11	29.30	17	2.15
Hydrogenated palm	27.37	8	29.80	13	2.55

TABLE 4

Solidification Behavior of Blended Products of Hydrogenated Soybean and Interesterified Fat Prepared from Palm and Rice Bran Oil Blend (70 + 30)

Fat blends	Super cooling temperature (°C)	Time for super cooling (in min)	Solidification temperature (°C)	Time for solidification (in min)	Rise in temperature (°C)
Interesterified (palm + RBO, 70 + 30) + hydrogenated soybean oil					
(60 + 40)	23.84	14	24.15	18	0.30
(50 + 50)	23.45	18	23.92	22	0.47
(40 + 60)	23.2	16	23.9	21	0.70
(30 + 70)	22.75	16	23.85	22	1.10
(20 + 80)	22.65	16	24.15	23	1.50
(10 + 90)	22.4	14	24.4	21	2.0
Hydrogenated soybean oil	25.4	9	28.85	14	3.45

TABLE 5

Fatty Acid Compositions of Some Blends of Interesterified (palm + RBO, 70 + 30) and Hydrogenated Soybean Oil[a]

Fat blends (% by wt)	Fatty acids (% w/w)						
	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
60 + 40	0.51	28.2	3.0	55.0	12.2	0.3	0.8
50 + 50	0.5	26.3	2.8	58.2	12.0	0.2	1.0
40 + 60	0.4	24.4	2.6	61.3	9.9	0.2	1.1
30 + 70	0.4	22.4	2.4	64.5	8.7	0.1	1.3
20 + 80	0.3	20.5	2.2	67.8	7.6	0.1	1.5
10 + 90	0.3	18.5	2.0	71.0	6.5	0.0	1.7

[a]RBO, rice bran oil.

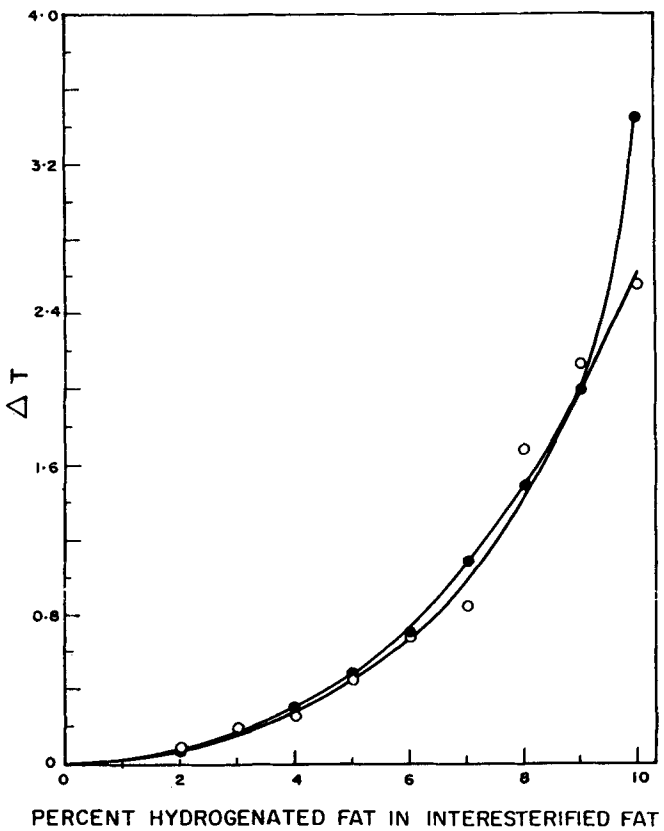

FIG. 1. Solidification behavior of blends of hydrogenated fats and interesterified fats in different porportions. ▼, Interesterified (palm + rice bran oil, 80 + 20) + hydrogenated palm oil; ●, interesterified (palm + rice bran oil, 70 + 30) + hydrogenated soybean oil.

Lipase hydrolysis and cooling curve analysis are suitable for distinguishing interesterified fats from hydrogenated fats and also offer a means of detecting interesterified fat products in a mixture containing hydrogenated fat products.

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