Detection of Interesterified Fats in Hydrogenated Fats

S. Adhikari and **J. Adhikari***
Ganesh Scientific Research Foundation, New Delhi, 110 015, India

Interesterification of fats is being used increasingly as an alternative to hydrogenation in preparing shortening and margarine bases. The detection of interesterified fats in vanaspati (a hydrogenated fat) is relevant because of possible adulteration problems. Either palmitic acid-rich or stearic acid-rich interesterified fats were blended with 13 market samples of hydrogenated fat (vanaspati) and examined by on-plate lipase hydrolysis of glycerides, gas chromatographic determination of fatty acids of the isolated 2-monoglycerides and calculation of two emperical indices. These were R_1, the ratio of the amounts of palmitic acid present in the 2-position to that in the total glyceride, and R_2, the ratio of saturated acid present in the 2-position to total saturated fatty acid in the fat. The vanaspati, R_1 was always below 10 and R_2 was always below 20. The presence of 5–10% interesterified fat raised both figures and offered a suitable basis for the detection of interesterified fats in hydrogenated fats.

KEY WORDS: Detection, hydrogenated fats, interesterified fats, lipase hydrolysis, thin-layer chromatography, vanaspati.

Hydrogenation and interesterification are both means of hardening fats. The presence of *trans* fatty acids, easily estimated by infrared spectroscopy, can be used to detect straight hydrogenated fats upon admixture with interesterified products. Silroy and Bhattacharyya (1) showed that hydrogenated fats gave cooling curves with a characteristic hump, absent in interesterified products, which made it possible to distinguish an interesterified fat product from a hydrogenated fat. The detection of an interesterified fat in a hydrogenated fat is more difficult but is required nevertheless. The latter is becoming more relevant because interesterification is permitted in many countries as a means of producing hard fats.

In interesterified fats, fatty acids are distributed randomly, which implies that the proportion of any fatty acid at the 1,3- or the 2-position of glycerol is the same and is equal to that of the particular fatty acid in the total triglycerides. In interesterified fats with melting points of 35°C or more, the proportion of saturated fatty acids, both in the fat as a whole and in the 2-position, is in the range of 30–35% or greater (2). On the other hand, in straight hydrogenated fats, only a small proportion of the total saturated fatty acids is in the 2-position (Adhikari, S., and J. Adhikari, unpublished data).

The pancreatic lipase (a 1,3-specific lipase) hydrolysis has been used to obtain a 2-monoglyceride fraction from fats. The latter is carried out in a reaction medium of a buffer solution, calcium chloride and bile salt. Subsequent analysis of the 2-monoglyceride by gas-liquid chromatography (GLC) yields information on the fatty acid composition at the 2-position (3). Dutta *et al.* (4) carried out the lipase hydrolysis directly on the thin-layer chromatography (TLC) plate without using calcium chloride and bile salt. This procedure, which takes about 2 h, has been employed in the present work to isolate 2-monoglycerides which were analyzed by gas chromatography as fatty acid methyl esters after recovery.

*To whom correspondence should be addressed at J. Adhikari, Ganesh Scientific Research Foundation, 64-65, Najafgarh Road, New Delhi, 110015, India.

EXPERIMENTAL PROCEDURES

Fats employed. Randomly interesterified fats (IF) were prepared by the method of Chakrabarty *et al.* (5) from blends of equal quantities of the following oils: (i) palm (P) and cotton seed (C) [IF(PC)]; (ii) ricebran (R) and mowrah (M) (*Madhuca latifolia*) [IF(RM)]; (iii) ricebran and sal (*Shora robusta*) (S) [IF(RS)]; and (iv) sunflower (Su) and sal [IF(SuS)]. The first blend was rich in palmitic acid, whereas the latter three blends were rich in stearic acid. All interesterified fats had melting points between 35 and 38°C. Ricebran and cottonseed oils were supplied by Hindustan Vegetable Oils Corporation Ltd. (New Delhi, India), and sal and mowrah oils were from K.N. Oils Industries (Mahasamund, India). Thirteen packs of branded vanaspati were purchased from three major cities in India (Delhi, Kanpur and Amritsar) and coded with a V symbol, *e.g.*, VRT, VPG, VMF, *etc.* (VRT, Rath vanaspati, manufactured by Shriram Industrial Enterprises, New Delhi, India; VPG, Panghat vanaspati, manufactured by Shriram Industrial Enterprises; VMF, Punjab Markfed vanaspati, Markfed vanaspati and Allied Industries, Punjab, India). Tests were made mostly on 90:10 and 80:20 mixtures of vanaspati and interesterified fats, as well as a few on 95:5 mixtures to test the lower limits of detection.

Lipase hydrolysis on TLC Plates (4). A solution of 2–5 mg lipase (triacyl glycerol lipase, Type II, prepared for sigma [pfs]-grade Steapsin from hog pancreas, stated selectivity 35–70 units per mg; Sigma Chemical Co., St. Louis, MO) was prepared in 0.3–0.4 mL of tris buffer. This was applied evenly on a silica gel G (BDH Chemicals, Poole, U.K.) TLC plate (20 × 20 cm, 0.5 mm thick). The plate was pre-developed with diethyl ether (reagent grade, as were all other solvents) to move any organic contaminants to the top, where they were removed by scraping off a narrow band.

The triglyceride blends (1–4 mg) in *n*-hexane were applied as evenly as possible over the enzyme band. The plate was immediately placed, with the coated side down, in an incubator (40°C) for 10 min. The reaction was then stopped by exposing the plate to HCl vapor for 1 min in a closed TLC chamber, and the acid fumes were blown away from the plate under a fan. The plate was next developed in an ascending manner three times in succession with diethyl ether up to 2 cm from the line of application. The ether on the plate was removed in a stream of air, and the plate was then developed to 14 cm with a solvent mixture of *n*-hexane/diethyl ether/acetic acid (80:20:1.5) for resolution of the partial glycerides.

After removal of the solvents from the chromatogram by a stream of air, different bands were located with iodine vapor. The monoglyceride band was identified (by means of standard samples plotted alongside) and scraped off. The 2-monoglycerides were extracted with diethyl ether and converted to methyl esters in sodium methoxide-

methanol by the method of Luddy *et al.* (6). Original oil samples were similarly converted to methyl esters.

Gas chromatography analysis. Fatty acid compositions were determined on a Varian 3700 gas chromatograph (Varian, Palo Alto, CA) equipped with dual flame ionization detectors (FID) and a 6 ft × 1/8 in. stainless steel column packed with 3% DEGS-X supported on Aeropak (80:100 mesh; both supplied by Varian). The column, injector and FID temperatures were 180, 200 and 230°C, respectively. The nitrogen carrier gas flow rate was 30 mL/min, and the fatty acid composition was recorded with a computer data system CDS III (Varian Instruments, Walnut Creek, CA).

Calculation of indices. Two indices were computed from the data. R_1 represents the proportion of palmitic acid in the 2-monoglyceride expressed as a percentage of its proportion in the total triglyceride. R_2 represents the ratio of total saturated fatty acids in the 2-monoglycerides to total saturated fatty acids in the blended fats.

RESULTS AND DISCUSSION

In India, vanaspati is currently obtained by straight hydrogenation of selected vegetable oils by using supported nickel catalysts to obtain a hardened fat with a capillary slip point of up to 41°C. In practice, a figure of 37°C is most commonly attained. Oils currently in common use are cottonseed oil, ricebran oil (both rich in palmitic acid) and soybean oil, with lesser quantities of palm olein, rapemustard oil, sunflower oil and sal fat. Final addition of 5% sesame oil as a marker for vanaspati is mandatory. Because so many oils can be used, and slip point is the main product criterion, the fatty acid composition of vanaspati can be expected to vary widely. In the present

TABLE 1

Fatty Acids Present in Total Triglycerides (TG) and in 2-Monoglycerides (MG) of Vanaspati, Interesterified Palmitic-Rich Fat and of Their Mixtures in Various Proportions

Fat[a]	Position	Fatty acids					Indices[b]	
		16.0	18.0	18.1	18.2	Others	R_1	R_2
IF(PC)	TG	36.9	3.1	27.4	29.5	3.1	—	—
	MG	36.0	2.8	28.5	28.5	4.2		
VRT	TG	24.6	4.5	50.1	18.9	1.9	2.0	12.0
	MG	0.5	3.0	68.6	25.6	2.8	—	—
VRT + IF(PC) (95:5)	TG	25.3	4.4	50.5	18.3	1.5	11.5	19.8
	MG	2.9	3.0	67.3	25.8	1.0	—	—
VRT + IF(PC) (90:10)	TG	26.0	4.3	50.3	17.9	1.5	20.0	26.7
	MG	5.2	2.9	64.9	26.0	1.0	—	—
VRT + IF(PC) (80:20)	TG	27.3	4.2	50.3	16.4	1.8	36.2	40.3
	MG	9.9	2.8	59.5	26.8	1.0	—	—
IF(RM)	TG	29.2	11.7	37.7	21.2	0.2	—	—
	MG	30.3	11.9	37.8	20.0	—	—	—
VRT + IF(RM) (95:5)	TG	24.8	4.0	49.5	19.9	1.9	8.0	19.0
	MG	2.0	3.5	67.1	26.3	1.1	—	—
VRT + IP(RM) (90:10)	TG	25.0	5.2	48.9	19.1	1.5	14	25.1
	MG	3.5	3.9	65.5	25.0	2.1	—	—
VRT + IF(RM) (80:20)	TG	25.5	5.9	47.6	19.4	1.6	25.1	35.7
	MG	6.4	4.8	62.4	24.5	1.9	—	—
IF(RS)	TG	17.2	23.1	40.2	15.4	4.1	—	—
	MG	19.2	23.5	39.5	14.9	2.9	—	—
VRT + IF(RS) (95:5)	TG	24.2	5.6	49.6	18.7	1.9	5.9	21.5
	MG	1.4	4.0	59.2	25.0	0.4	—	—
VRT + IF(RS) (90:10)	TG	23.8	6.8	49.2	15.8	4.8	14.2	23.8
	MG	3.4	4.8	67.7	24.5	0.6	—	—
VRT + IF(RS) (80:20)	TG	23.1	8.2	48.1	18.4	2.2	18.1	36.1
	MG	4.2	7.1	62.9	23.5	2.3	—	—
IF(SuS)	TG	5.5	22.8	36.5	31.5	3.7	—	—
	MG	5.9	22.6	36.4	32.0	3.1	—	—
VRT + IF(SuS) (95:5)	TG	23.6	5.4	49.4	19.5	3.6	3.9	21.8
	MG	0.9	3.9	67.0	25.9	2.5	—	—
VRT + IF(SuS) (90:10)	TG	22.7	6.4	48.7	20.2	2.5	4.4	20.3
	MG	1.0	4.9	65.4	26.2	2.0	—	—
VRT + IF(SuS) (80:20)	TG	24.2	3.6	49.8	21.4	1.0	6.6	30.5
	MG	1.6	6.9	62.2	26.9	2.4	—	—

[a]IF(RS), interesterified fat made from rice bran and sal fat; IF(SuS), interesterified fat made from sal fat and sunflower oil; IF(PC), interesterified fat made from palm and cottonseed; IF(RM), interesterified fat made from rice bran and mowrah. VTR, coded Vanaspat.

[b]Example: For fat No. 2:

$$R_1 = \frac{\text{Conc. of palmitic acid at 2-position} \times 100}{\text{Conc. of palmitic acid in total fat}} = \frac{0.5 \times 100}{24.6} = 2.0$$

$$R_2 = \frac{\text{Conc. of total saturated acid at 2-position} \times 100}{\text{Conc. of saturated acid in total fat}} = \frac{(0.5 + 3.0) \times 100}{(24.6 + 4.5)} = 12.0$$

study of 13 market samples, palmitic acid content varied from 13.3 to 30.9%, C_{18} monoene acid (which include both positional and geometric isomers) content ranged from 50.1 to 69.3% and C_{18} diene acid content (including both positional and geometric isomers) ranged from 2.7 to 22.3%.

Despite this wide range in fatty acid composition, the 2-monoglycerides from all the vanaspati samples showed only 0.5–1.6% of palmitic acid at the 2-monoglyceride position. Addition of a palmitic acid-rich interesterified fat (made from palm-cottonseed oil containing 36.9% palmitic acid) to the various vanaspati samples raised this proportion to 2.2–3.5% at the 5% level, 4.0–5.0 at the 10% level and 6.5–9.0% at the 20% level (Table 1). Since interesterified fats have to contain at least 35% saturated acid to reach the desired slip point, blends of all palmitic acid-rich IF fats are reasonably expected to show this increase in palmitic acid content.

For vanaspati-IF(RM) and vanaspati-IF(RS) blends, palmitic acid percent at the glyceride 2-position was 1.4–2.4% for 95:5 blends and higher when larger amounts of IF fats were added. However, for vanaspati-IF(SuS) blends, $C_{16:0}$ at the 2-glyceride position amounted to only 1.6%, even for 80:20 blends, thus indicating that palmitic acid concentration alone is not a sufficient criterion for the detection of stearic acid-rich interesterified fats.

Accordingly, for the purpose of detection, an index (R_1) was developed which is 100 times the ratio of palmitic acid at the 2-position to that in the intact fat. In the pure vanaspati samples studied, R_1 was in the range of 2–6.8. This value increased to higher levels upon incorporation of 5% interesterified fats IF(RM), IF(PC) or IF(RS). As shown in Table 1, out of the 23 95:5 blends, R_1 was greater than 10 in 15 cases, and for the others R_1 was 8–10. The overall range was 8–16.8. For 90:10 blends of vanaspati-IF(PC), vanaspati-IF(RM) and vanaspati-IF (RS), R_1 was always well above 10 (average range 13.3–24). However, for vanaspati-IF(SuS) blends, R_1 was at or below 10, even for the 90:10 blends. Thus, the determination of R_1 alone was considered insufficient for the purpose of detecting IF blends.

A second index (R_2) was calculated, which represented 100 times the ratio of total saturated acids present in 2-position to that present in the total triglyceride. For all pure vanaspati, it was equal to or less than 16.5. For 95:5 blends of vanaspati with IF(PC), IF(RM) or IF(RS), R_2 was 19–40 or higher, 22–66.8 for 90:10 blends, and even higher for 80:20 blends. For IF(SuS) blends, R_2 was 21–24, even for 95:5, and higher for 90:10 and 80:20 blends, thus offering a useful indication of the presence of IF fats.

From this discussion it can be summarized that for detection of interesterified fats in vanaspati, palmitic acid concentration at the 2-monoglyceride position can be adopted as the primary screening test. If its concentration at the 2-position is greater than 2%, it can be assumed that an IF fat is present. When it is below 2%, a second confirmation can be made by calculating R_1 or R_2. When R_2 is higher than 16.5, adulteration by interesterified fat is indicated.

The plate lipolysis procedure described, followed by gas chromatography analysis of the fatty acids, is simple and useful for the purpose of detecting IF in hydrogenated fats, such as vanaspati.

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