

Comparative Nutritional Quality of Palmstearin—Liquid Oil Blends and Hydrogenated Fat (vanaspati)

S. Ray and D.K. Bhattacharyya*

Department of Chemical Technology, University Colleges of Science and Technology,
Calcutta University, Calcutta - 700 009, India

ABSTRACT: An attempt was made to use high-melting low-digestible fat palmstearin as a vanaspati substitute by blending it with polyunsaturated fatty acid-rich liquid oils. This blending produced fat products of zero-*trans* fatty acid content and melting points below the human body temperature, so that they can be digested easily. The new blended products were fed to male albino rats (Charles Foster strain); the coefficients of digestibilities were 94.2% for palmstearin and rapeseed oil blend, 95.1% for palmstearin and sunflower oil blend, and 96.2% for palmstearin and soybean oil blend, which were somewhat better than the digestibility coefficient of conventional vanaspati (93.6%). Feeding experiments for three months showed comparable results in terms of serum lipid profiles. The blended products significantly increased the total cholesterol level but not the free cholesterol level in serum and liver of rats when compared with those of the conventional vanaspati group of rats. *JAOCS* 73, 617–622 (1996).

KEY WORDS: Cholesterol, digestibility, methyl ester, palmstearin, phospholipid, rapeseed oil, soybean oil, sunflower oil, total lipid, triglyceride.

Hydrogenated fats are used as edible products in such forms as shortening, cooking fat, and margarine. In India and many other countries, hydrogenated fats known as vanaspati are consumed as a substitute for ghee (anhydrous butterfat). Hydrogenated fats for the above kinds of edible uses are produced by partial and selective hydrogenation of liquid oils, such as soybean, sunflower, rapeseed, cottonseed, and rice bran oil. The hydrogenated fats are characterized by having slip melting points of 31–41°C and *trans* fatty acid contents at 30–60% levels, depending on the nature of the liquid oil.

Trans fatty acids of hydrogenated fat products have been reported to contribute to several health problems, including thrombogenesis leading to coronary heart disease (1–3). Interest has grown, therefore, on production of fat products that will simulate hydrogenated fat products but will not contain *trans* fatty acids. Such zero-*trans* fat products are known in commerce as vanaspati-like fats of the unhydrogenated type.

*To whom correspondence should be addressed at Department of Chemical Technology, Calcutta University, University Colleges of Science and Technology, 92, Acharya Prafulla Chandra Road, Calcutta - 700 009, India.

There are two distinct methodologies for making zero-*trans* vanaspati-like fat products, namely, interesterification and simple physical blending of oils and fats. The interesterification process involving randomization requires a saturated fatty acid-rich oil or oil fraction, such as palm oil or its fraction, called palmstearin. Palmstearin is produced by fractionating palm oil by one of three different processes, namely, dry fractionation, detergent fractionation, and solvent fractionation. The yield of palmstearin varies from 20 to 30%, depending on the process of fractionation adopted. Palmstearins vary in fatty acid composition and triglyceride composition. Palmstearins contain 1–2% myristic acid, 47–74% palmitic acid, 4–6% stearic acid, 16–37% oleic acid, and 3–10% linoleic acid. Palmstearins consist mainly of the triglycerides C₄₆ (0.5–3 mol%), C₄₈ (12–56 mol%), C₅₀ (34–50 mol%), C₅₂ (5–37 mol%), and C₅₄ (0–8 mol%) (4).

While work has been done to utilize palmstearin in interesterification with liquid oils to make vanaspati-like fat products (5), and the nutritional quality of these interesterified products also has been evaluated (6), the scope of making fat products that simulate the hydrogenated vanaspati products by simple physical blending of palmstearin with liquid oils does not appear to have been examined and evaluated. Digestibility of an oil can be improved by mixing with another oil, as was observed by Gottenbos and Vles (7). Although the stability of a blended product will be affected, with a lowered shelf life in view of its polyunsaturated fatty acid (PUFA) content, shelf life can be protected by appropriate food-grade antioxidants.

The present study deals with the nutritional evaluation of vanaspati-like fat products prepared from palmstearin by blending with liquid oils, such as soybean, sunflower, and rapeseed. The evaluation is used for comparison with conventional vanaspati (hydrogenated and rich in *trans* fatty acids) in experimental animals (rats) with respect to the coefficient of digestibility, growth response, food efficiency ratio, lipid profiles of serum and liver tissue.

EXPERIMENTAL PROCEDURES

Preparation of dietary fats. Refined, bleached, and deodorized (RBD) palmstearin was supplied by the Palm Oil Research In-

stitute of Malaysia (PORIM) (Kuala Lumpur, Malaysia). The dietary fats were prepared by simple blending of RBD palmstearin with liquid oils in a 40:60 (w/w) ratio. The liquid oils used were RBD proprietary brands of sunflower oil (Flora Brand, Lipton India Ltd., Bombay, India), rapeseed oil (Rasoi Ltd., Calcutta, India), and soybean oil (Vital Brand, Britannia Industries Ltd., Bombay, India). Three blended fat products are formed as follows: (i) palmstearin and sunflower oil blend, (ii) palmstearin and rapeseed oil blend, and (iii) palmstearin and soybean oil blend. Conventional vanaspati, a proprietary brand (Dalda, Hindustan Lever Ltd., Calcutta, India), was used as control fat.

Analysis of fat products. The slip melting points of the fat products were determined according to a method of the Indian Standards Institution (8). Gas-liquid chromatography was employed to determine the fatty acid composition of the dietary fat products (9) by converting the fats into methyl esters (10). *Trans*-fatty acid contents in conventional vanaspati and vanaspati-like fat products were determined by infrared spectrophotometry according to Allen (11).

Animal experiment. The animal experiments were designed and conducted according to previous reports from this laboratory (12,13). Male albino rats of the Charles Foster strain (selected for authenticity of the strain) were kept in individual cages and were fed the three blended fat products and conventional vanaspati and fresh water *ad libitum*. Daily food consumption and weekly body weight gain were recorded. Two separate feeding experiments were conducted. The goal of one feeding experiment was to determine the coefficient of digestibility of four dietary fats; the second feeding experiment was conducted to evaluate nutritional attributes of the different fat products.

Experiment 1. Determination of coefficient of digestibility of fat products. Rats ($n = 45$, body weight 155–160 g) were divided into five groups of equal average body weight. Three groups of rats were given three palmstearin-liquid oil blended fat products, one group of rats was given conventional vanaspati, and another group of rats was fed a fat-free glucose (71%) diet to determine the endogenous/metabolic fecal lipid, which consists of the lipid materials secreted into the intestinal tract from endogenous sources. After allowing the rats 2 d for orientation, they were kept for 10 d on diets that contained fat-free casein, 18%; glucose, 61%; yeast, 1%; experimental fat product, 20%; liver extract (Orheptal; Merck, Bombay, India, derived from fresh liver, rich in vitamin B₁₂ and other B vitamins), 3%; salt mixture, 7% [Hawk-Oser Salt Mixture No. 4. Stoichiometrically approximately equivalent to the Osborne-Mendel mixture: 100 g of salt mixture A contains FeNH₄ citrate, USP 91.36; CuSO₄ · 5H₂O, 5.97; NaF, 0.76; MnSO₄ · 2H₂O, 1.07; KAl(SO₄)₂ · 12H₂O, 0.54; KI, 0.24; and ZnSO₄ · H₂O, 0.06; 1000 g of Hawk-Oser Salt Mixture No. 4 contains salt mixture A, 16.7; Ca citrate · 4H₂O, 308.2; Ca(H₂PO₄)₂ · H₂O, 112.8; K₂HPO₄, 218.7; KCl, 124.7; NaCl, 77.0; CaCO₃, 68.5; 3 MgCO₃ · Mg(OH)₂ · 3H₂O, 35.1; and MgSO₄ (anhydrous), 38.3] (14). Every day, the exact amount of food consumption was recorded, and the feces of each rat

were collected, dried, and stored. Feces for 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 fecal fat. The coefficient of digestibility was calculated according to the standard method (15), with a correction being made for endogenous/metabolic fecal lipid. The results are expressed as means of three values, obtained from three rats of each group consisting of nine rats.

Experiment 2: Evaluation of nutritional characteristics of fat products. Rats ($n = 32$, body weight 80–90 g) were divided into four groups, each consisting of eight rats, having equal average body weight, and fed experimental diets composed of fat-free casein, 18%; starch, 55%; salt mixture, 4% [composition of salt mixture 12 (in g): NaCl, 292.5; KH₂PO₄, 816.6; MgSO₄, 120.3; CaCO₃, 800.8; FeSO₄ · 7H₂O, 56.6; KI, 1.66; MnSO₄ · 2H₂O, 9.35; ZnCl₂, 0.5452; CuSO₄ · 5H₂O, 0.9988; CoCl₂ · 6H₂O, 0.0476] (16); cellulose, 3%; one multivitamin capsule (vitamin A, I.P. 10,000 units; thiamine mononitrate, I.P. 5 mg; riboflavin, I.P. 5 mg; pyridoxin hydrochloride, I.P. 1.5 mg; vitamin B₁₂, I.P. 5 mcg., calcium pantothenate, USP 5 mg; niacinamide, I.P. 50 mg; ascorbic acid, I.P. 400 units; cholecalciferol, USP 15 units; menadione, I.P. 0.1 mg; folic acid, I.P. 1 mg; vitamin E, USP 0.1 mg) per kg of diet; and experimental fat, 20%. The diets were adequate in all nutrients.

Rats were maintained on the above diets *ad libitum* for 12 wk. The amounts of daily diet consumed by each rat and weekly body weight gains were noted. Rats were killed under anesthesia, 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/methanol mixture and then estimated gravimetrically (17). According to standard methods, the individual lipid components, such as total and free cholesterol (18), phospholipids (19), and triglycerides (20) in serum and liver tissue lipids were estimated colorimetrically. For statistical analysis of results, Student's *t*-test (21) was performed.

RESULTS AND DISCUSSION

The blended products made from palmstearin and individual liquid oils had no *trans* fatty acids but were rich in polyunsaturated fatty acids (PUFA), whereas the hydrogenated fat product vanaspati contained 47.5% *trans* fatty acids (Table 1). The slip melting points of the blended fats were slightly below the hydrogenated vanaspati. To some extent, the blended products showed (Table 2) greater digestibility than the conventional hydrogenated fat vanaspati. Among the blended products, the soybean oil blend exhibited the highest coefficient of digestibility. The increased digestibilities could be attributed, perhaps, to zero-*trans* content and high PUFA content in the blended products.

TABLE 1
Characteristics of Dietary Fats

| Dietary fat | Slip melting point (°C) | Fatty acid composition (wt%) | | | | | % <i>Trans</i> |
|-------------------------------------|-------------------------|------------------------------|-------------------|-------------------|-------------------|-------------------|----------------|
| | | C _{16:0} | C _{18:0} | C _{18:1} | C _{18:2} | C _{18:3} | |
| Palmstearin and sunflower oil blend | 34.0 | 28.32 | 5.52 | 30.88 | 34.00 | 1.28 | 0.00 |
| Palmstearin and rapeseed oil blend | 36.0 | 24.72 | 2.88 | 48.28 | 16.36 | 7.76 | 0.00 |
| Palmstearin and soybean oil blend | 35.5 | 28.80 | 4.08 | 26.06 | 36.46 | 4.40 | 0.00 |
| Conventional vanaspati | 37.0 | 29.60 | 11.00 | 49.70 | 9.70 | — | 47.50 |

(trans monoene)

The rat group raised on the palmstearin–soybean oil blend product displayed maximum gain in weight in the early stages of growth compared with the conventional (hydrogenated) vanaspati control group (Fig. 1). This trend was reflected apparently, but not statistically, in the palmstearin and sunflower oil blend group. The palmstearin and rapeseed oil blend group showed no significant change in body weight, compared with the conventional vanaspati group, as evident from Figure 1.

The overall growth-promoting effect of the palmstearin blends in the early phase of feeding might be ascribed to the composition pattern of the total unsaturated fatty acids, including PUFA content as contributed by the liquid oils (Table 1). The conventional vanaspati control group showed a satisfactory growth-promoting effect, which agreed well with the observation of Mattson (22), who suggested that *trans* fatty acid dietary fat had little effect on growth.

The variation in food efficiency ratios (calculated by dividing the gain in body weight by food intake) of the different dietary fats was examined (Fig. 2). The best food efficiency ratio (FER) was observed with the palmstearin and soybean oil blend. In fact, a significant increase in the FER at

the end of the first, fourth, eighth, and twelfth weeks was noted, compared with conventional vanaspati control group. The FER of the palmstearin and sunflower oil blend increased significantly at the end of the first week, and thereafter at the end of the twelfth week, in comparison with conventional vanaspati. The palmstearin and rapeseed oil blend, however, produced no significant changes in the FER in comparison with conventional vanaspati.

Statistically there was no significant change in the total lipid content of serum of rats from the four dietary groups (Table 3). It is evident from Table 3 that free cholesterol content in serum of rats did not significantly change for the three dietary palmstearin blends in comparison with the vanaspati group. However, the total cholesterol content in serum of rats that ingested the three blended fats became significantly higher than that of the conventional vanaspati-fed group. This may be due to the high PUFA content of the liquid oils present in the three blends and also to their having zero-*trans* fatty acid content. *Trans* fatty acids had been found to lower the activity of the enzyme lecithin-cholesterol acyl transferase (LCAT) (23,24). This enzyme is responsible for converting

TABLE 2
Metabolic Lipid and Coefficient of Digestibility of the Dietary Fats Fed Rats at 10% Level

| | Metabolic lipid | Dietary fat group | | | |
|--|-----------------|-------------------------------------|------------------------------------|-----------------------------------|--------------------------|
| | | Palmstearin and sunflower oil blend | Palmstearin and rapeseed oil blend | Palmstearin and soybean oil blend | Conventional vanaspati |
| Number of rats | 9 | 9 | 9 | 9 | 9 |
| Average weight of rats (g) | 157.330 ± 0.817 | 157.600 ± 0.681 | 157.200 ± 0.461 | 157.500 ± 0.427 | 157.400 ± 0.300 |
| Average weight gain (g) | 19.1 ± 0.1 | 30.1 ± 0.5 | 29.6 ± 0.3 | 33.3 ± 0.3 | 29.3 ± 0.3 |
| Average food eaten (g/rat for 10 d) | 100.890 ± 0.548 | 114.406 ± 0.266 | 114.833 ± 0.517 | 113.833 ± 0.088 | 110.500 ± 0.435 |
| Average fat intake (g/rat for 10 d) | 0.000 | 11.440 ± 0.026 | 11.483 ± 0.051 | 11.383 ± 0.008 | 11.050 ± 0.043 |
| Average fat excreted (mg/rat for 10 d) | 210.000 ± 0.763 | 769.333 ± 0.600 | 870.666 ± 0.666 | 632.000 ± 0.866 | 911.330 ± 0.440 |
| Metabolic fecal lipid (g for 10 d) | 0.210 | — | — | — | — |
| Coefficient of digestibility | — | 95.1 ± 0.03 ^a | 94.2 ± 0.09 ^a | 96.2 ± 0.08 ^a | 93.6 ± 0.77 ^a |
| Level of significance ^b | — | P < 0.001 | P < 0.01 | P < 0.001 | — |

^aStandard error of mean for three groups of rats, each group consisting of three rats for each fat.

^bStatistical comparison with conventional vanaspati group.

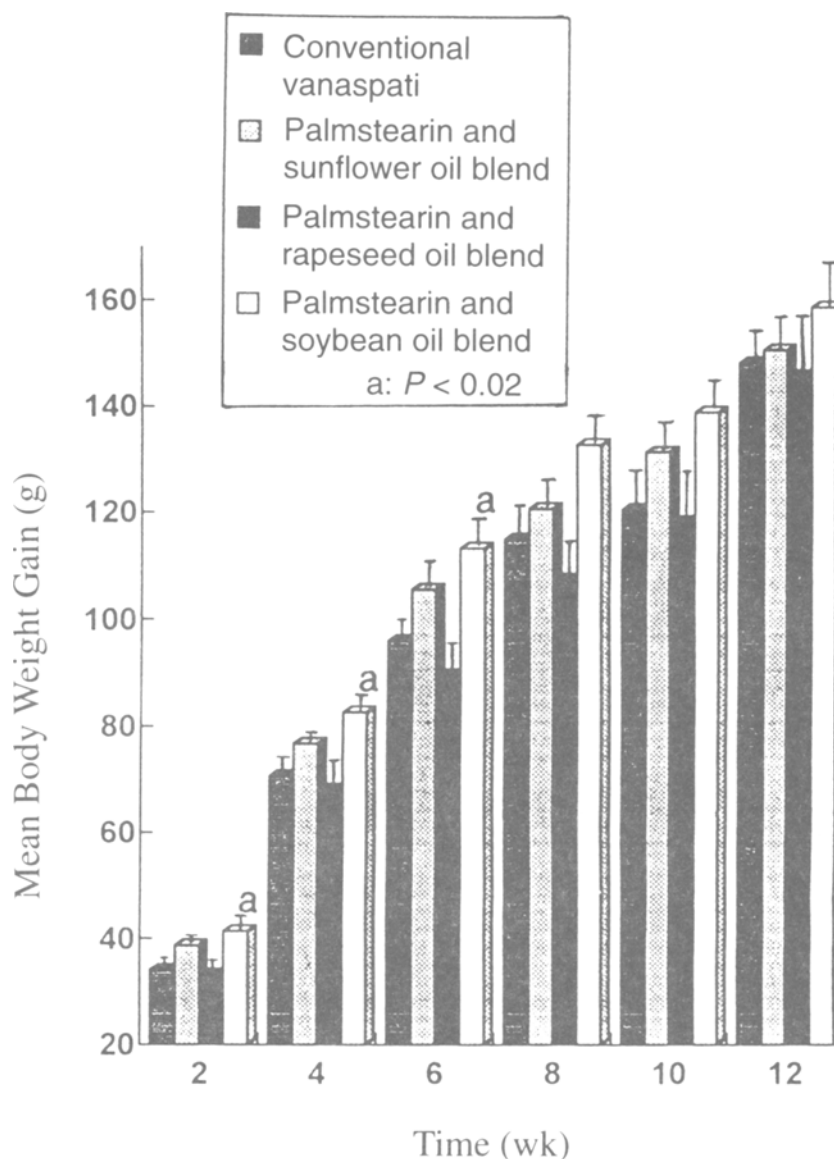


FIG. 1. Growth response of rats fed dietary fats at 20% level at the end of different weeks.

cholesterol to cholesterol ester. The presence of *trans* fatty acid in conventional vanaspati significantly lowered LCAT activity for this fat-fed group; the effect was seen in low total cholesterol levels due to reduced formation of cholesterol ester. For dietary palmstearin-liquid oil blends, cholesterol ester increased, a result which might be helpful for plasma membrane functions of permeability and fluidity (25).

Triglyceride (TG) levels showed a decreasing trend in serum of rats fed all three blended products (Table 3). The increase in TG level in the conventional vanaspati group may be due to the presence of much less linoleic acid and high *trans* fatty acid (Table 1). A recent experiment showed that oleic acid and linoleic acid had a TG-lowering effect in serum (26) of human beings. This supports the present observation in mammalian systems also.

The amounts of phospholipid in the serum of rats, fed the three dietary blends, increased significantly when compared with that of the serum of rats of the conventional vanaspati group. The increase in phospholipid content could be due to zero-*trans* fatty acid and high PUFA content in the blends.

The total lipid content of rat liver increased significantly for the rapeseed oil and soybean oil blends when compared with the conventional vanaspati group (Table 4). The total cholesterol level rose significantly for the three blend groups when compared with the conventional vanaspati group, but free cholesterol levels did not show any significant change. The increase in total cholesterol was due to an increase in cholesterol ester formation (Table 4). The observations corroborated earlier reports (27–29). According to statistical significance level, the phospholipid con-

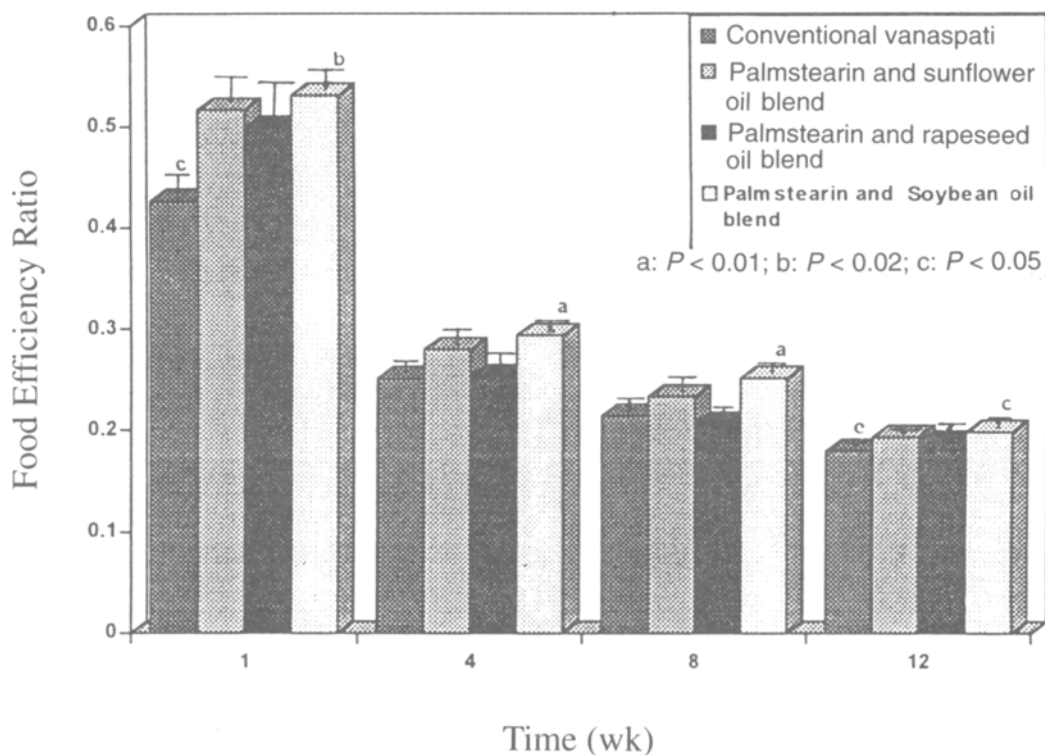


FIG. 2. Food efficiency ratio of rats fed dietary fats at 20% level at the end of different weeks.

tent decreased for the palmstearin and sunflower oil blend and increased for the palmstearin and soybean oil blend compared with the conventional vanaspati (Table 4). TG contents in liver tissues were almost identical for the dietary blended products in comparison with conventional vanaspati.

Fat products similar to hydrogenated vanaspati but having zero-*trans* fatty acid and substantial PUFA content can be prepared by the simple process of blending palmstearin with liquid oils rich in PUFA. The blended fat products are nutritionally equivalent or, to some extent, better, than the *trans* fatty acid-rich and essential fatty acid (EFA)-deficient vanaspati product (made by partial and selective hydrogenation of liquid oils) with respect to their coefficients of digestibility and in the contents of total and free cholesterol in serum and liver tissue of rats.

ACKNOWLEDGMENT

The authors thank PORIM (Kuala Lumpur, Malaysia) for RBD palmstearin.

REFERENCES

1. Mensink, R.P., and M.B. Katan, Effect of Dietary *Trans* Fatty Acids on High Density and Low Density Lipoprotein Cholesterol Levels in Health Subjects, *N. Engl. J. Med.* 323:439–445 (1990).
2. Zock, P.L., and M.B. Katan, Hydrogenation Alternatives: Effects of *Trans* Fatty Acids and Stearic Acid Versus Linoleic Acid on Serum Lipids and Lipoproteins in Humans, *J. Lipid Res.* 33:399–410 (1992).
3. Willett, W.C., M.J. Stampfer, J.E. Manson, G.A. Colditz, F.E. Speizer, B.A. Rosner, L.A. Sampson, and C.H. Hennenkens, In-

TABLE 3
Lipid Spectrum of Serum (mg/dL) of Rats Fed Dietary Fats at 20% Level^a

| Group | Total lipid | Total cholesterol | Free cholesterol | Cholesterol ester | Triglyceride | Phospholipid |
|-------------------------------------|----------------|----------------------------|------------------|----------------------------|---------------------------|----------------------------|
| Palmstearin and sunflower oil blend | 400.93 ± 7.94 | 152.69 ± 8.83 ^b | 11.62 ± 1.01 | 141.07 ± 7.88 ^b | 26.40 ± 3.40 ^c | 116.11 ± 2.59 ^b |
| Palmstearin and rapeseed oil blend | 385.18 ± 17.98 | 109.90 ± 4.93 ^d | 12.10 ± 0.65 | 97.79 ± 4.66 ^d | 17.53 ± 1.16 ^b | 122.92 ± 8.54 ^b |
| Palmstearin and soybean oil blend | 416.43 ± 21.67 | 123.38 ± 4.67 ^b | 10.54 ± 0.52 | 112.83 ± 4.70 ^b | 28.51 ± 0.93 ^d | 138.50 ± 6.89 ^b |
| Conventional vanaspati (control) | 400.04 ± 29.87 | 86.09 ± 5.01 | 11.17 ± 0.44 | 74.92 ± 5.04 | 35.93 ± 2.03 | 66.51 ± 8.16 |

^aResults are means ± SE. ^b $P < 0.001$, ^c $P < 0.05$, ^d $P < 0.01$.

TABLE 4
Lipid Spectrum of Liver (mg/g) of Rats Fed Dietary Fats at 20% Level^a

| Group | Total lipid | Total cholesterol | Free cholesterol | Cholesterol ester | Triglyceride | Phospholipid |
|-------------------------------------|---------------------------|---------------------------|------------------|--------------------------|--------------|---------------------------|
| Palmstearin and sunflower oil blend | 68.83 ± 9.99 | 10.76 ± 0.81 ^b | 2.81 ± 0.22 | 7.95 ± 0.72 ^b | 4.65 ± 1.20 | 12.68 ± 1.87 ^c |
| Palmstearin and rapeseed oil blend | 76.71 ± 7.60 ^d | 9.99 ± 1.18 ^e | 3.07 ± 0.33 | 6.91 ± 1.30 ^d | 3.07 ± 0.80 | 19.51 ± 2.18 |
| Palmstearin and soybean oil blend | 86.88 ± 7.43 ^e | 9.98 ± 0.90 ^e | 3.51 ± 0.43 | 6.47 ± 0.81 ^e | 4.50 ± 1.00 | 21.00 ± 0.65 ^b |
| Conventional vanaspati (control) | 50.34 ± 3.91 | 5.32 ± 0.51 | 2.79 ± 0.27 | 2.89 ± 0.34 | 2.12 ± 0.60 | 16.96 ± 0.39 |

^aResults are means ± SE. ^b*P* < 0.001, ^c*P* < 0.05, ^d*P* < 0.02, ^e*P* < 0.01.

- take of *Trans* Fatty Acids and Risk of Coronary Heart Disease Among Women, *The Lancet* 341:581–585 (1993).
- Pantzaris, T.P., *Pocketbook of Palm Oil Uses*, Palm Oil Research Institute of Malaysia, Kuala Lumpur, 1988, p. 13.
 - Majumdar, S., and D.K. Bhattacharyya, *Trans Free Vanaspati from Palmstearin and Vegetable Oils by Interesterification Process*, *Oleagineux* 41:235–240 (1986).
 - Majumdar, S., and D.K. Bhattacharyya, Nutrition of Hydrogenated Vanaspati and Interesterified Vanaspati Made from Palmstearin and Selective Oils, *Ibid.* 41:393–399 (1986).
 - Gottenbos, J.J., and R.Q. Vles, The Nutritive Value of Palm Oil, ISF/AOCS World Congress, New York, Abstract No. 19, April 27–May 1, 1980.
 - Indian Standard I.S. Methods of Sampling and Test for Oils and Fats (Revised)*, Fourth Reprint, May 1975, Indian Standard Institution (ISI), IS:548 (Part 1), 1964, p. 33.
 - Ghosh Chaudhuri, P., M.M. Chakrabarty, and D.K. Bhattacharyya, Modification of Some Tree-Borne Seed Fats for the Preparation of High Priced Confectionary Fats, *Fette Seifen. Anstrichm.* 85:224–227 (1983).
 - Metcalfe, L.D., and A.A. Schmitz, Rapid Preparation of Fatty Acid Esters for Gas-Chromatographic Analysis, *Anal. Chem.* 33:363–364 (1961).
 - Allen, R.R., A Rapid Method for Determination of *Trans* Unsaturation in Fats and Derivatives, *J. Am. Oil Chem. Soc.* 46:552–553 (1969).
 - Sarkar, S., and D.K. Bhattacharyya, Nutrition of Rice Bran Oil in Relation to Its Purification, *Ibid.* 68:956–962 (1991).
 - Ray, S., and D.K. Bhattacharyya, Comparative Nutrition Study of Enzymatically and Chemically Interesterified Palm Oil Products, *Ibid.* 72:327–330 (1995).
 - Hawk's Physiological Chemistry*, 14th edn., edited by L. Oser, TATA McGraw-Hill Publishing Company Ltd., New Delhi, 1976, p. 1376.
 - Angur, V., H.S. Rollman, and H.J. Duel Jr., The Effect of Crude Lecithin on Coefficient of Digestibility and Rate of Absorption of Fats, *J. Nutr.* 33:177–186 (1947).
 - Jones, J.H., and C. Foster, A Salt Mixture for Use with Basal Diets Either Low or High in Phosphorous, *Ibid.* 24:245–256 (1942).
 - Kates, M., *Techniques of Lipidology*, American Elsevier Publishing Co., Inc., New York, 1972, pp. 349 and 351.
 - Zlatkis, A., B. Zak, and A. Boyle, A Method for the Determination of Serum Cholesterol, *J. Lab. Clin. Med.* 41:486–490 (1953).
 - Chen, P.S., T.Y. Toribara, and H. Warner, Microdetermination of Phosphorus, *Anal. Chem.* 28:1756–1758 (1956).
 - Van Handel, E., and D.B. Zilversmit, Micromethod for the Direct Determination of Serum Triglyceride, *J. Lab. Clin. Med.* 50:152–157 (1957).
 - Biometrika Tables for Statisticians, Vol. 1*, edited by E.S. Pearson, and H.O. Hartley, Cambridge, 1966, Table 12.
 - Mattson, F.H., An Investigation of the Essential Fatty Acid Activity of Some of the Geometrical Isomers of Unsaturated Fatty Acids, *J. Nutr.* 71:366–370 (1960).
 - Takatori, T., F.C. Phillips, H. Shimasaki, and O.S. Privett, Effects of Dietary Saturated and *Trans* Fatty Acids on Tissue Lipid Composition and Serum LCAT Activity in the Rat, *Lipids* 11:272–280 (1976).
 - Moore, C.E., R.B. Alfin-Slater, and L. Aftergood, Effect of *Trans* Fatty Acids on Serum Lecithin: Cholesterol Acyl Transferase in Rats, *J. Nutr.* 110:2284–2290 (1980).
 - Achaya, K.T., Nutrition and Health Aspects of Fats, in *Treatise on Fats, Fatty Acids and Oleochemicals*, Vol. 1, edited by O.P. Narula, Industrial Consultants, New Delhi, 1994, pp. 0-1/2–0-1/12.
 - Sen, D.P., Nutritional Qualities of Fats and Oils, *Ibid.*, pp. 0-3/1–0-3/11.
 - Klein, P.D., Linoleic Acid and Cholesterol Metabolism in the Rat, I. The Effect of Dietary Fat and Linoleic Acid Levels on the Content and Composition of Cholesterol Esters in Liver and Plasma, *Arch. Biochem. and Biophysics* 76:56–64 (1958).
 - Whitney, J.E., and S. Roberts, Influence of Previous Diet on Hepatic Glycogenesis and Lipogenesis, *Am. J. Physiol.* 181:446–450 (1955).
 - Avigan, J., and D. Steinberg, Effects of Saturated and Unsaturated Fat on Cholesterol Metabolism in the Rat, *Proc. Soc. Exp. Biol. Med.* 97:814–816 (1958).

[Received May 26, 1995; accepted January 19, 1996]