found in nickel-catalyzed, hydrogenated oil. Only 9 ppm of palladium metal was required for effective hydrogenation. Commercially, nickel catalysts are employed in the range of 200–500 ppm. Much higher *trans*-isomer contents were found in soybean oil hydrogenated with homogeneous palladium catalyst than those normally found with nickel catalysts. Although the higher activity and greater selectivity might provide improved stability of the hydrogenated oil, the increased *trans* content will reduce the yield of liquid oil. The catalyst in this study formed higher *trans*-isomers than heterogeneous palladium-on-carbon catalyst (Table I, Expts. 4 and 5). This unusual property might be useful in production of products such as shortenings and margarines, where the high *trans* content imparts desirable plasticity.

As little as 1 ppm palladium effectively catalyzed hydrogenation of soybean oil at 170 C; at this high temperature the catalyst appeared to decompose. Some black insoluble material was collected when the hydrogenated sample was filtered. At 150 C, some decomposition was visible, but at 120 C and below there was no decomposition of the catalyst. Soybean oil and hydrogenated soybean oil containing palladium acetylacetonate (Table I, Expt. 4) were submitted to a commercial testing laboratory for analysis after filtration. The initial oil contained 6 ppm Pd and the hydrogenated oil showed 5 ppm Pd. These values indicate that the catalyst was not decomposed during hydrogenation. Nickel acetylacetonate also decomposes at high temperatures, and the decomposed precipitate is catalytically inactive (2).

Heterogeneous catalysts have the advantage of ease of separation. With homogeneous catalysts such as palladium acetylacetonate, an extra step, e.g., an acid wash, is necessary for its removal. Alternatively, homogeneous catalysts could be heterogenized (12) by bonding them to carriers such as silica. Whether such a heterogenized catalyst would still be as active and selective as the homogeneous catalyst remains to be determined. The high linolenate selectivity and activity of palladium acetylacetonate demonstrated in this study warrants further investigation for possible commercial application.

REFERENCES

1. Koritala, S., and H.J. Dutton, JAOCS 43:86 (1966).
2. Emken, E.A., E.N. Frankel and R.O. Butterfield, JAOCS 43:14 (1966).
3. Fedeli, G., A. Favini and A. Balsamo, Riv. Ital. Sostanze Grasse 53:374 (1981).
4. Christopherson, S.W., and R.L. Glass, J. Dairy Sci. 52:1289 (1969).
5. Butterfield, R.O., and H.J. Dutton, JAOCS 44:549 (1967).
6. Koritala, S., JAOCS 45:197 (1968).
7. Allen, R.R., in Bailey's Industrial Oil and Fat Products, D. Swern, ed. Vol. 2, 4th ed., Wiley, New York, 1982, pp. 1–95.
8. Riesz, C.H., and H.S. Weber, JAOCS 41:400 (1964).
9. Riesz, C.H., and H.S. Weber, JAOCS 41:464 (1964).
10. Johnston, A.E., D. Macmillan, H.J. Dutton and J.C. Cowan, JAOCS 39:273 (1962).
11. Mukherjee, K.D., I. Kiewitt and M. Kiewitt, JAOCS 52:282 (1975).
12. Awl, R.A., and E.N. Frankel, JAOCS 58:557 (1981).

[Received May 14, 1984]

Variations in Fat Content and Lipid Class Composition in Ten Different Mango Varieties

M.A. ALI, M.A. GAFUR, M.S. RAHMAN and **G.M. AHMED**, Bangladesh
Council of Scientific and Industrial Research Laboratories, Rajshahi, Bangladesh

ABSTRACT

The kernels of 10 different mango varieties were extracted. The physico-chemical characteristics and lipid class composition of fats were studied. The fat content of mango kernels grown under the soil and climatic conditions of Bangladesh varied from 7.1% to 10%, depending on the variety. The total lipid extracts were fractionated into lipid classes by a combination of column and thin layer chromatography (TLC). The hydrocarbon and sterol esters varied from 0.3% to 0.7%, triglycerides from 55.6% to 91.5%, partial glycerides from 2.3% to 4% and free sterol from 0.3% to 0.6%. Free fatty acids amounted to 3.0-37% as oleic; glycolipids were 0.6-1.2% and phospholipids 0.11-0.8%. The fatty acid composition of triglyceride (TG) fractions was analyzed by gas liquid chromatography (GLC). Palmitic acid varied from 7.9 molar % to 10.0 molar %, stearic from 38.2% to 40.2%, oleic from 41.1% to 43.8%, linoleic from 6.0% to 7.6%, linolenic from 0.6% to 1.0% and arachidic acid from 1.7% to 2.6%. TLC revealed the presence of lyso-phosphatidylcholine, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and phosphatidic acid in the phospholipid fraction.

INTRODUCTION

Bangladesh occupies an important position among the mango growing countries in the world. A wide variety of mango (*Mangifera indica* L.) are extensively cultivated in Bangladesh with an annual production of ca. 500 thousand tons (1). It is considered the king of all indigenous fruits of the tropics. The ripe stone fruit is widely used for eating directly and for the manufacture of jam and jelly. The mango seeds are a waste product and are thrown away after being separated from the pulp. The seed represents up to 24% of the weight of the fruits and contains 7.1-10% fat depending on the variety.

The characteristics of mango kernel fat as reported by several workers (2-6) show that the fat is edible and has some resemblance to cocoa butter. Little attention (7) has been given to individual lipid components in mango kernel fat, although some researchers have suggested that they play a significant role in the stability and quality of vegetable fats (8-11).

Because the average fat content of mango seed is ca. 10%, the total annual production of mango fat in Bangladesh is ca. 10,000 tons. Thus, if properly collected and used, mango fat could be an abundant potential source of vegetable fat. The composition of fat varies with the source (12) and depends on factors such as climatic conditions, soil type, maturity of plant and variety (13,14).

The objective of this study is to evaluate the variations in fat content and lipid class composition in different mango varieties grown in Bangladesh.

MATERIALS AND METHODS

Ten varieties of ripe mango (Brindaboni, Fazli, Kalabau, Kanchamitha, Kuipahari, Lakhanvoge, Lengra, Mohanvoge, Misrakanta and Ranipasand) were collected from mango growers in Rajshahi.

The mango stones were separated and decorticated manually within 12 hours of depulping. After shelling, the kernels were immediately crushed and dried in the sun for 2-3 days to ca. 4-8% moisture. The moisture content in the fresh kernel was determined by IUPAC method I.B.1 (15).

The sun-dried kernel was ground with a micromiller and dried again in an oven at a temperature of 60-65 C to ca. 4% moisture just before extraction. The fat was extracted in a soxhlet with n-hexane for ca. 8 hours. The solvent was removed under reduced pressure and the percentage of fat content was calculated. The free fatty acids (FFA) were determined by AOCS method Ca 5a-40, iodine value by Cd 1-25, saponification value by Cd 3-25, melting point by Cc 3-25, unsaponifiable matter by Ca 6a-40 and peroxide value by Cd 8-53 (16).

Separation of Lipid Classes by Column and Thin Layer Chromatography

The total lipids were fractionated into lipid classes on a silicic acid column. The silicic acid (E. Merck, Darmstadt, W. Germany, 70-230 mesh) was washed with water and methanol to remove fines and impurities. It was activated at 120 C overnight and again for 1 hr immediately before the column was prepared. For each column, 25 gm silicic acid was washed with 250 ml of chloroform/methanol (7:1, v/v), 120 ml chloroform/methanol (15:1, v/v) and 160 ml chloroform. A slurry of 25 gm of silicic acid in chloroform was poured into the column (2.2 cm). After the column was washed with 100 ml diethyl ether and 325 ml 4% diethyl ether in petroleum ether (b.p. 60-70 C), 300 mg total lipids were dissolved in 5 ml eluting solvent and quantitatively transferred to the column.

The solvent systems used to elute the column were similar to those described by Hirsch and Ahrens (17). In a typical fractionation, 60 ml of 4% diethyl ether in petroleum ether eluted the hydrocarbons and sterol esters, 600 ml of the same solvent system removed the triglycerides and an additional 55 ml FFA. Sterols were eluted with 250 ml of chloroform and partial glycerides with an additional 350 ml of chloroform. The polar lipids, i.e., glycolipids and phospholipids, were eluted with 700 ml of acetone and 300 ml of methanol, respectively. The elution was controlled with a flow rate of 1.5-2 ml/min.

The elution of each fraction was monitored by microslide thin layer chromatography (TLC) to ensure uniformity of separation of each lipid class during silicic acid chromatography and the eluted solvents were collected in a weighed flask. The fractions thus obtained were evaporated in a rotary vacuum evaporator and were dried under reduced pressure before being weighed. The purity of the lipid classes was further checked by TLC on 20 × 20 cm plates coated with a layer (0.5 mm) of silica gel. The lipid classes were identified by R_f comparison with standard references. The neutral lipid classes were developed with petroleum ether/diethyl ether/acetic acid (90:10:1) (18). The weight percentage of each lipid class was based on total lipid recovered, which averaged 98.5% of the total lipid applied. Phospholipid obtained by column chromatography was estimated as total phosphorus content by AOCS Official Method Ja 5-55 (16).

The phospholipids were separated into individual phospholipid classes by TLC on 20 × 20 cm plates coated with a layer (0.5 mm thick) of silica gel H (without calcium sulphate as binder) made in a slurry of 1 mM sodium carbonate solution. The plates were developed with chloroform/methanol/acetic acid/water (25:15:4:2) (19). Phospholipids were identified by comparison with standards. The glycolipids were also separated by TLC on Silica gel G plates with acetone/acetic acid/water (100:2:1) (20) and were identified by comparison with known standards. Iodine vapor (21) was used as detecting agent. Specific spray reagents were also used: Zinzade's reagent (22) to detect phospholipid, Dragendorff reagent (23) to detect choline-containing lipids, α-napthol for glycolipids and ninhydrin for amino groups.

Fatty Acid Composition of TG Fraction

The fatty acid composition of the triglyceride (TG) fraction of each variety was analyzed as their methyl esters, which were prepared by the sodium methoxide method (24) and carried out by gas liquid chromatography (GLC) equipped with a flame ionization detector (FID). Nitrogen carrier gas was used at a flow rate of 25 ml/min. Fatty esters were separated on a 1.8 m × 2 mm i.d. glass column packed with 6% BDS (Butanediol succinate polyesters) on solid support Anakrom ABS 100/120 mesh. Analysis was carried out at isothermal column temperature of 190 C, injector and detector temperature for all GLC analysis was 200 C. The peaks were identified by comparison with standard methyl esters for retention times, by plotting the log of retention times against equivalent carbon length (ECL). The peak areas were determined by multiplying peak height by peak width at half height. The percentage of each peak was calculated as the percentage of the total area of all the peaks.

The sterols were separated from the unsaponifiables by IUPAC method II.C.8 (15) and gas chromatographed on a glass column, packed with 3% OV-17 on solid support Gas-Chrom Q 100/120 mesh with nitrogen as the carrier gas at a flow rate of 25 ml/min. The analysis was carried out at an isothermal column temperature of 230 C. Detector and injector temperatures were 240 C and 265 C. The peaks were identified with relative retention time (RRT) to cholesterol as described by method II.C.8 (15) as well as by comparison with the retention times of pure compounds. The percentage of each peak was calculated as the percentage of the total area of all peaks.

RESULTS AND DISCUSSION

The initial moisture content in kernels of variety Fazli was found to be the highest (68.0%) (Table I). The fat obtained from dried kernels was yellowish white and solidified at 25 C. The fat content was lower, and the iodine value was higher for seeds from variety Fazli than all other varieties. The data also show that the content of fat was higher and FFA and iodine value were lower for seeds from Lakhanvoge than other varieties. The fat content of Brindaboni, Kalabau, Misrakanta and Mohanvoge were liquid at ambient temperature (29 C). Fats from the other varieties were solid at the same temperature. The FFA levels in extracted fats of Fazli, Lengra, Kuipahari and Mohanvoge increased abnormally high during storage at 5 C. This result, however, is similar to the reported results of Osman (25) and Gafur et al. (7,26). The levels of FFA were 3-5% in all the fats immediately after extraction.

The triglycerides of mango fat appear to be hydrolyzed, which result in high percentages of FFA.

Total lipids were fractionated into lipid classes by silicic acid column chromatography (Table II). The percentage of TG was found to be abnormally low in the fats of Fazli, Lengra and Kuipahari compared with Lakhanvoge (91.5%). Slight variation of phospholipid in the methanol fraction was observed among the 10 varieties. The relative weight percentage of phospholipid (0.1-0.8%) agrees with the values (0.1-0.8%) obtained from total phosphorus content (16). However, these values were found to be lower than reported results (5). Variation was also observed in glycolipid fractions (0.6-1.2%).

TABLE I

Physical and Chemical Characteristics of Mango Fats[a]

Variety	Moisture in fresh kernel (%)	Fat content (%)	Iodine value	Melting point (°C)	Saponification value	Unsaponifiable matter (%)	Peroxide value
Brindaboni	62.0	9.3	51.2	27-28	195	1.7	0.1
Fazli	68.0	7.1	55.0	38-39	190	1.8	0.5
Kalabau	50.0	9.0	44.0	27-28	192	1.8	0.1
Kanchamitha	50.0	7.5	50.4	26-27	194	1.8	0.1
Kuipahari	61.0	8.0	50.3	44-45	193	1.7	0.5
Lakhanvoge	52.6	10.0	46.8	28-29	191	1.5	0.1
Lengra	48.5	9.7	46.0	46-47	195	1.5	0.5
Mohanvoge	50.0	7.8	55.0	25-26	188	1.9	0.2
Misrakanta	48.5	8.9	50.6	25-26	191	1.7	0.1
Ranipasand	56.0	9.4	51.2	34-35	197	1.9	0.1

[a]Mean results of 3 experiments.

TABLE II

Weight Percentage of Lipid Classes in Mango Fats[a]

Sl. no.	Variety	Hydrocarbons and sterol esters	Triglycerides	Free fatty acids	Free sterols	Partial glycerides	Glycolipid	Phospholipid
1.	Brindaboni	0.6	88.3	4.2	0.3	3.5	1.2	0.7
2.	Fazli	0.5	55.6	37.0	0.4	4.0	0.9	0.6
3.	Kalabau	0.4	89.7	4.1	0.6	3.0	0.6	0.3
4.	Kanchamitha	0.6	83.5	9.2	0.6	3.5	0.6	0.2
5.	Kuipahari	0.5	61.4	31.4	0.6	3.3	1.1	0.5
6.	Lakhanvoge	0.3	91.5	3.0	0.5	2.5	0.7	0.1
7.	Lengra	0.3	62.3	30.0	0.5	3.2	0.6	0.6
8.	Mohanvoge	0.6	83.0	9.0	0.6	3.9	1.2	0.8
9.	Misrakanta	0.4	88.5	5.5	0.5	2.3	0.7	0.4
10.	Ranipasand	0.7	87.4	5.9	0.5	3.1	0.8	0.5

[a]Mean results of 3 experiments.

TABLE III

Fatty Acid Composition in Triglyceride (TG) Fraction of Mango Fat (Molar %)[a]

Sl. no.	Variety	16:0	18:0	18:1	18:2	18:3	20:0
1.	Brindaboni	9.2	39.1	41.1	7.6	0.7	2.3
2.	Fazli	8.4	39.4	42.5	7.3	0.7	1.7
3.	Kalabau	8.1	40.2	41.5	7.3	0.6	2.2
4.	Kanchamitha	9.1	38.2	43.0	6.3	0.9	2.5
5.	Kuipahari	10.0	38.3	41.3	7.1	0.9	2.5
6.	Lakhanvoge	9.7	38.3	41.7	6.9	0.7	2.6
7.	Lengra	8.5	39.3	42.4	6.8	0.7	2.2
8.	Mohanvoge	9.0	38.6	41.4	7.6	0.9	2.4
9.	Misrakanta	7.9	39.2	43.8	6.0	1.0	2.0
10.	Ranipasand	7.9	39.9	42.0	7.4	0.6	2.2

[a]Mean results of 3 experiments.

The triglycerides and FFA were not well separated in the samples of Fazli, Lengra and Kuipahari. So the column load was reduced to 200 mg. Complete separation of free sterol and partial glycerides was also difficult to monitor as free sterol and diglycerides overlapped slightly. The FFA level of Fazli was higher (37%) and the partial glycerides were also higher than those of other varieties. The weight percentage of free sterol isolated by silicic acid column chromatography was found to be higher in Kalabau, Kuipahari and Mohanvoge than the values obtained by the digitonin procedure (27).

The fatty acid compositions of triglyceride fractions varied with the variety (Table III). The content of 16:0 (palmitic acid) was considerably higher in Kuipahari, Lakhanvoge and Brindaboni, and 18:1 (oleic acid) was lower than in the other varieties. The fat contained ca.

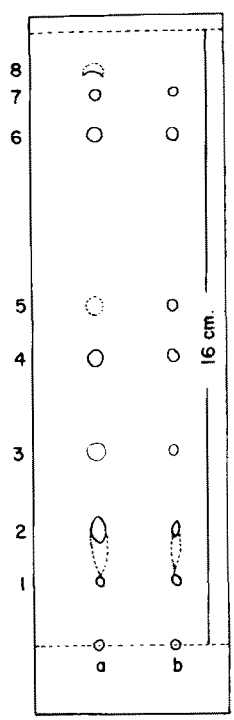

FIG. 1. TLC tracing of phospholipids of mango fat. Solvent system: chloroform/methanol/acetic acid/water (25:15:4:2). In (a) 1 = lysophosphatidylcholine, 2 = unidentified, 3 = phosphatidylcholine, 4 = phosphatidylinositol, 5 = unidentified-PS?, 6 = phosphatidylethanolamine, 7 = phosphatidic acid and 8 = unidentified. Adsorbent: Silica Gel H. (a) Phospholipids of mango fat. (b) Soybean phosphatides. Indicator: iodine vapor. Dotted spots indicate trace.

50% saturated acid, and the ratio of stearic/oleic acids varied according to variety from 0.88% to 0.96%. GLC of the sterol fraction showed that β-sitosterol (73%), stigmasterol (16.5%) and campesterol (10.5%) were the main components in mango fat (7). TLC (Fig. 1) revealed that lyso-phosphatidylcholine, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and phosphatidic acid were the major phospholipids of mango fat. Monogalactosyl diglyceride and digalactosyl diglyceride (trace) were also identified by TLC in the glycolipid fraction of mango fat.

ACKNOWLEDGMENT

The work was supported by a research grant from the International Foundation for Science, Sweden. Rezaul Haque determined fat contents.

REFERENCES

1. Mollah, M.R.I., and M.A. Miyan, B.J., Agric. Sci. I 2:90 (1974).
2. Narshima Char, B.L., B.R. Reddy and S.D. Thirunala Rao, JAOCS 54:494 (1977).
3. Bandyopadhya, C., and A.S. Gholap, Curr. Sci. India 48:935 (1979).
4. Dunn, H.C., and Hilditch, T.P., J. Sci. Chem. Ind. London 66:209 (1947).
5. Vanpee, W.M., L.E. Boni, M.N. Foma and A. Hendrikx, J. Sci. Food Agric. 32:485 (1981).
6. Tiscornia, E., V. Paganuzzi and E. Leoni, La Rivis Itali, Delle Sost. Grass. 56:332 (1979).
7. Gafur, M.A., and B. Töregård, unpublished results, 1980.
8. Fore, S.P., N.J. Morris, C.H. Mack, A.F. Freeman and W.G. Bickford, JAOCS 30:298 (1953).
9. Parkhurst, R.M., W.A. Skinner and P.A. Sturm, JAOCS 45, 641 (1968).
10. Brown, D.F., C.M. Carter and K.F. Mattil, JAOCS 51:502 (1974).
11. Sanders, T.H., JAOCS 57:8 (1980).
12. Lakshminarayana, G.J., Oil Technol. Assoc. India 10:75 (1977).
13. Sallans, H.R., JAOCS 41:215 (1964).
14. Fick, G.N., and Zimmerman, JAOCS 50:529 (1973).
15. International Union of Pure and Applied Chemistry, Standard Methods for the Analysis of Oils, Fats and Soap, 5th edn., Pergamon Press, Paris, 1976.
16. Official and Tentative Methods of the American Oil Chemists' Society, 3rd edn., Champaign, IL, 1980.
17. Hirsch, J., and E.H. Ahrens, Jr., J. Biol. Chem. 233:311 (1958).
18. Mangold, H.K., JAOCS 38:707 (1961).
19. Skipski, V.P., R.F. Peterson and M. Barclay, Bio-chem. J. 90:374 (1964).
20. Gardner, H.W., J. Lipid Res. 9:139 (1968).
21. Sims, R.P.A., and J.A.G. Larose, JAOCS 39:232 (1962).
22. Dittmer, J.C., and R.L. Lester, J. Lipid Res. 5:126 (1964).
23. Beiss, U., J. Chromatog. 13:104 (1964).
24. Glass, R.L., J. Dairy Sci. 48:1106 (1965).
25. Osman, S.M., World Congress Abst. Paper ISF, 425 (1980).
26. Gafur, M.A., M.S. Huq, B.K. Mandol and K. Ahmed, Bulletin Published by IFST, Dhaka, 1982.
27. Curchaine, A.J., W.H. Miller and D.B. Stein Jr., Clin. Chem. 5:609 (1959).

[Received December 15, 1983]

♣Oxidative Stability of High Oleic Sunflower and Safflower Oils

RICHARD H. PURDY, Richard H. Purdy Inc., 16 Josefa Ct., Novato, CA 94947

ABSTRACT

High oleic sunflower seed progenies derived from normal seed by chemical mutagenesis were extracted and their oils refined by standard laboratory procedures. Oxidative stability was related directly to linoleic acid content with an AOM value of 100 hr obtained at 1% linoleate. Data is presented comparing linoleate concentration and oxidative stability of oils obtained from normal sunflower seed and high linoleic (normal) and high oleic (naturally induced mutations) varieties of safflower seed.

INTRODUCTION

The ratio of oleic and linoleic acids in sunflower oil triglycerides is to a large extent dependent upon environmental conditions, particularly moisture and temperature during seed development (1,2). Cool northern climates yield high linoleic acid content, whereas warm southern areas result in high oleic acid content. Robertson et al. (3) developed excellent correlations between climate, latitude and fatty acid composition. Although saturates varied little, oleic acid contents ranged from 14% in Idaho to 50% in Texas, and linoleic acid from 41% in Arkansas to 75% in Manitoba. Oxidative stability of crude oils derived from seed grown in southern climates has been shown to be almost twice that of crude oils extracted from northern seed (4).

The fatty acid composition of the oil from safflower seed grown commercially in the United States is relatively consistent, varying little with climate or location (5). Linoleic acid contents range primarily from 76% to 79% with occasional values of 75% to 76% in Arizona and 80% in Montana. Oleic acid may vary from 12% to 15% in most locations. In 1957, Horowitz et al. (6) reported a natural mutant which revealed a reversal of the linoleate-oleate ratio. Breeding research resulted in stable varieties with oleic acid values of 77% to 80% and linoleic acid values as low as 12% to 13%. The oil derived from the high oleic seed exhibited excellent resistance to oxidative deterioration (7).

Soldatov (8) developed high oleic sunflower seed by treating "normal" planting seed with a solution of 0.5% of the mutagen dimethyl sulfate. Selected breeding resulted in some plants containing seeds with as high as 80% to 90% oleic acid. Whereas the "normal" seed increased in linoleic acid from 21% to 54% during the process of seed formation and ripening, with a subsequent reduction in oleic acid from 62% to 36%, the new cultivars showed a decrease in linoleic acid from 26% to 15% and an oleic acid increase from 64% to 79%.

Kharachenko (9) studied both "normal" (Peredovik) and a progeny derived from mutagen treated seed (Pervenets) under controlled environmental conditions. High temperature conditions promoted rapid oleic acid development during the initial stages of triglyceride synthesis in both varieties. However, biosynthesis of linoleic subsequently intensified at the expense of oleic in the Peredovik variety, whereas the Pervenets' oleic content continued to rise. Seeds of the high oleic Pervenets variety apparently contain a change of the seed genotype responsible for an irreversible blockage of the desaturating enzyme system.

Fick (10) developed progenies from the Pervenets cultivar that varied only 4% to 5% in their oleic content when