Changes in Fatty Acids in Ripening Mango Pulp (Variety Alphonso)

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Oil extracts were isolated from five stages of ripening Alphonso mango pulp and the fatty acid compositions of the extracts were determined by gas–liquid chromatography. Ripening of pulp was found to be associated with an increase in glyceride content, followed by changes in fatty acid composition of the pulp. As the fruit ripens, a decrease in linoleic acid content and an increase in linolenic acid content, as well as reciprocal distribution of palmitic acid and palmitoleic acid, were seen. A correlation of organoleptically evaluated aroma and flavor characteristics with the ratio of palmitic acid to palmitoleic acid was observable.

Changes in carbohydrate and carotenoid contents of ripening mangoes (*Mangifera indica* L.) have been studied (John et al., 1970; Krishnamurthy et al., 1960). A study of the changes in fatty constituents of mango pulp and their metabolic role during ripening of mango has been lacking. In general, while the fruits are maturing, the degree of unsaturation of the fatty acids becomes greater (Braverman, 1963). Recent work on lipids in ripening banana fruit (Goldstein and Wick, 1969) indicated that unsaturated fatty acids, particularly linoleic and palmitoleic acids, decreased during ripening. The present investigation relates to changes in fatty acid composition of ripening pulp of Alphonso mango, a commercially important variety, and their correlation with the appearance of aroma and flavor.

EXPERIMENTAL SECTION

Freshly picked, unripe olive green Alphonso mangoes were purchased from a local market in three batches at different time intervals and kept at ambient temperature (25–30°) for ripening in a well-ventilated room. Each batch was subdivided into raw, half ripe, table-ripe, fully ripe, and over ripe groups of mangoes by six expert taste panelists based on aroma, flavor, and texture. Each group was further divided into three lots, each of which consisted of five mangoes having uniform organoleptic scores. In all cases the peel was removed and the pulp of five mangoes from each individual lot was subjected to subsequent analysis.

Pulp Oil Extracts. The pulp oil extracts of all samples were determined gravimetrically (Gholap et al., 1971). Each sample was de-peeled, cut into small pieces rejecting the seed, and extracted five times with sufficient volume of peroxide-free diethyl ether in a Waring blender. The ether layer was decanted off after each extraction, and finally the pulpy mass was centrifuged to obtain further residual ether extract. The total ether extract was dried over anhydrous sodium sulfate and filtered. The amount of oil extract was determined after removal of ether in vacuum under a nitrogen atmosphere at 50°.

Glyceride Content. Glyceride content of each extract was estimated colorimetrically (Boekenoogen, 1968) after splitting the oil extract with aldehyde-free ethanolic sodium hydroxide followed by oxidation of the liberated glycerol with sodium metaperiodate, and finally the color developed by addition of chromotropic acid was measured at 570 mμ by a Baush and Lomb spectrophotometer.

Phospholipid Content. The presence of phospholipid in all samples was estimated by acid digestion of a 50-mg sample followed by 1-amino-2-naphthol-4-sulfonic acid–molybdate color reagent (Fiske and Subbarow, 1925).

Thin-Layer Chromatography (tlc). Tlc separation of oil extracts was carried out essentially according to the meth-

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od described by Malins and Mangold (1960). Tlc plates were prepared by coating a slurry of silica gel (E. Merck) with water (1:2, w/v) with the aid of an applicator, dried at room temperature, and activated at 110° for 1 hr; 100 μg of each extract in chloroform (A.R. BDH) solution was dropped on the plate and the plate was developed using a petroleum ether–diethyl ether–acetic acid (80:20:1, v/v) solvent system. The chromatograms were visualized by spraying the plate with 50% sulfuric acid, followed by charring at 140° for 20 min. The nature and intensity of the spots representing the qualitative composition of the pulp oil extracts were noted. The triglyceride component of the oil extracts was identified by comparing the R_f value of an authentic reference sample of tripalmitin (Hormel Institute, Minn.).

Fatty Acid Methyl Esters. One-hundred milligrams of each pulp oil extract was refluxed for 1 hr with 15 ml N ethanolic KOH. The resulting solution was diluted with three times its volume of water and extracted repeatedly with diethyl ether to remove nonsaponifiables. Free fatty acids were recovered by acidification of the soap solution and multiple extraction with ether. The ether extract was demineralized by washing with water and the fatty acids were obtained by the removal of ether at a reduced pressure under nitrogen atmosphere. Methyl esters were prepared by reacting the fatty acids with diazomethane (Arndt, 1943).

Gas–Liquid Chromatography. A gas chromatograph used for the analysis of fatty acid methyl esters of each extract was a BARC Model equipped with a flame ionization detector and with a column of stainless steel (¼ in. o.d. × 6 ft) packed with 20% ethylene glycol succinate on acid washed 60/80 Chromosorb W. Nitrogen at a flow rate of 40 ml/min was used as a carrier gas. The temperature of the column and the injection port was maintained at 185 and 235°, respectively. The fatty acids were identified by comparing the retention times of reference standards (Analabs, Inc., Conn.). Gas chromatographic peak areas were determined by multiplying peak height by peak width at half height.

RESULTS AND DISCUSSION

The effect of the ripening of Alphonso mangoes on pulp oil constituents separated on a tlc plate is represented in Figure 1. The major spot below the solvent front has been characterized as triglyceride(s). As ripening proceeds, an increasing amount of triglyceride(s) has been observed from the nature and intensity of this spot on the tlc plate, while the minor spots at the base and up to the half height of the plate practically remain unchanged. According to the present method of separation, highly polar lipids do not migrate on the plate. Any change in polar lipid constituents of pulp oil extracts of ripening mangoes would have reflected on the tlc plate by showing a gradation in the nature and intensity of the base spot. In fact,
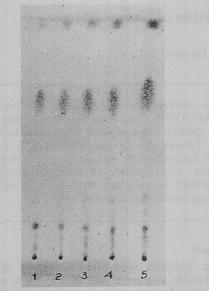
Figure 1. Tlc separation of pulp oil extracts from ripening mango pulp (variety Alphonso) on silica gel plate. See Experimental Section for tlc details. 1, raw. 2, half ripe. 3, table ripe. 4, fully ripe. 5, over ripe.

Table I. Pulp Oil Extract and Glyceride Content of Mango Pulp in Different Ripening States of Mango Stored at Ambient Temperatures (25–30°) (Mean Values in Nine Independent Lots)

Period of storage (no. of days)	State of ripeness, pulp color	Pulp oil extract (% wt of wet pulp)	Glyceride (% wt of pulp oil extract)
1	Raw, greenish white	0.17	46.2
6	Half ripe, dull yellow	0.29	56.5
12	Table ripe, yellow	0.46	70.8
15	Fully ripe, orange yellow	0.60	85.6
18	Over ripe, orange	0.95	87.5

Table II. Fatty Acid Composition (% wt) of Glyceride Component of Mango Pulp During Ripening (Mean Values in Nine Independent Lots)

Fatty acid	Raw	Half ripe	Table ripe	Fully ripe	Over ripe
$C_{12:0}$	0.3	0.2	0.3	0.3	0.4
$C_{14:0}$	0.9	2.0	2.8	2.9	2.9
$C_{16:0}$	28.9	23.9	23.6	26.8	25.6
$C_{16:1}$	17.7	22.1	26.3	31.3	30.7
Unknown	Trace	0.3	0.3	0.7	0.8
$C_{18:0}$	1.4	1.2	1.4	1.5	1.7
$C_{18:1}$	25.2	25.8	24.2	19.4	20.4
$C_{18:2}$	15.7	7.9	2.5	2.2	2.0
$C_{18:3}$	9.9	16.6	18.6	14.9	15.5

Table III. Correlation of Aroma and Flavor Characteristics of Ripening Mango Pulp with the Ratio of Palmitic Acid and Palmitoleic Acid

State of ripeness	Aroma and flavor[a]	$\dfrac{C_{16:0}}{C_{16:1}}$
Raw	Nil[b]	1.63
Half ripe	+	1.08
Table ripe	+++	0.89
Fully ripe	+++	0.86
Over ripe	++	0.83

[a] Organoleptic score: +, represents mild; ++, represents strong; +++, represents very strong. [b] With respect to aroma and flavor in ripe state.

only a trace amount of phospholipid has been found to occur in all samples.

Table I gives the results on the pulp oil extracts and glyceride content of ripening mango pulp. On the onset of ripening, both of these values increased up to fully ripe stage; thereafter, there is no appreciable change in glyceride content of the extract, whereas a steep rise in pulp oil content is seen. This could be attributed to changes in carotenoid content during ripening (Krishnamurthy et al., 1960).

The percentage composition of fatty acids in ether extracts of mango pulp at different ripening stages is given in Table II. Unsaturated fatty acids rather than saturated ones seem to be metabolically active during ripening. As the pulp ripens (Table II, ripe state) a considerable decrease in linoleic acid and a subsequent increase in linolenic acid is observed, while oleic acid remains unchanged during this period. The distributions of palmitic and palmitoleic acids in different ripening stages are reciprocal to each other.

The present studies show that the ripening of mango is associated with the changes in glyceride content as well as in fatty acid composition of the pulp. Irrespective of the changes in composition of highly unsaturated fatty acids in ripening mango pulp, the changes in distribution of palmitic and palmitoleic acids are presumably of interest, in view of strong aroma and flavor characteristics of mango. Table III shows the relationship between the intensity of aroma and flavor characteristics and the ratio of palmitic–palmitoleic acid in ripening mango pulp. As the mango ripens, the strong aroma and flavor of Alphonso mango appears in a table ripe state, when the ratio of palmitic–palmitoleic acid becomes less than 1 and remains practically constant in advanced stages of ripening. In the raw and half ripe state of mango having a very mild aroma and flavor, the ratio is always greater than 1. It seems likely that among the fatty acids, particularly, the ratio of palmitic–palmitoleic acid is an index of aroma and flavor of mangoes. Depending upon whether this ratio is greater or less than 1, the mango has mild or strong

aroma and flavor. Conclusive evidence for this correlation must, however, rest on biochemical studies of related enzyme systems. Relatively lower score in aroma and flavor of over ripe mango pulp (Table III) is probably due to deterioration of flavor compounds during prolonged storage.

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