Changing Lipid Class Patterns during Maturation of Sweet Oranges

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Hamlin, Pineapple, and Valencia sweet oranges were harvested monthly from trees between July 1971 and May 1972. Vesicular lipids were extracted from each cultivar and separated into neutral lipids (NL), resin acids and glycolipids (RAGL), and phospholipids and other polar lipids (PPL) by silica gel chromatography. The NL and RAGL fractions from each cultivar showed varying trends in their percentage distributions during the ripening period. The patterns of change for the PPL fraction, however, were similar: the percentage of PPL increased from July, reached a maximum during November and December, then declined until May. Phospholipid phosphorus declined at rates of 1.57, 1.02, and 0.66 μg of P (mg of lipid)$^{-1}$ (month)$^{-1}$ for Hamlin, Pineapple, and Valencia oranges, respectively. While ripening occurred fastest in Hamlin (early maturing fruit), the rate of senescence (as measured by phospholipid loss) also occurred at the fastest rate in this cultivar.

There is a wealth of information on morphology (Schneider, 1968; Albrigo and Carter, 1977), chemical composition (Sinclair, 1961; Kefford and Chandler, 1970; Nagy et al., 1977), and nutritional quality (Araujo, 1977; Ting, 1977) of sweet oranges. Limited data, however, exist on the types of biochemical changes that take place during growth and senescence of the sweet orange (Ting and Attaway, 1971). Two important factors for studying biochemical changes are: (1) to more accurately define the legal maturity of sweet oranges for commercial marketing and (2) to correlate specific compositions of the fruit with different maturation periods. Currently, five standards are used by USDA (1969) to legally define mature oranges, viz. (1) color break, (2) minimum juice content, (3) minimum percentage of total soluble solids, (4) minimum acid content, and (5) total soluble solids/acid ratio (degrees Brix/acid). The ratio of total soluble solids to total acid has been the single most important factor for the establishment of maturity standards. According to Harding et al. (1940) that ratio may be misleading as a measure of quality since a high ratio does not always mean superior quality fruit and, conversely, a low ratio does not always indicate inferior fruit. Chemical composition of sweet oranges depends on fruit variety, rootstock–scion interaction, tree health, soil type, fertilization, climate, geographical location, position of the fruit on the tree and above all, maturation period (maturation of citrus fruits is intimately related to seasonal changes) (Harding et al., 1940; Bitters, 1961).

The effects of maturation on the chemical components in the edible portion of sweet oranges have been studied the most because of the economical and nutritional importance of orange juice. Some of the earliest studies on compositional changes, as a function of maturation, involved total soluble solids, sugar contents (reducing, nonreducing, total), and total acid contents (Harding et al., 1940; Bartholomew and Sinclair, 1943; Roy, 1945). From these studies and others (Ting, 1954; Harding and Sunday, 1964; Hilgeman et al., 1967), the typical maturation pattern which emerged was that total soluble solids and total sugar levels increased while total acids decreased. Since sugars comprise 63 to 80% of the total soluble solids (Bartholomew and Sinclair, 1943), total sugars parallel total soluble solids during fruit development.

Other less extensive studies on changes in chemical constituents during sweet orange ripening were conducted on ascorbic acid (Harding and Winston, 1939; Harding et al., 1940; French and Abbott, 1940), total nitrogen and free amino acids contents (Cameron et al., 1936; Clements and Leland, 1962; Ting, 1967; Vandercook and Price, 1972; Zamorani et al., 1972), pectic substances (Gaddum, 1934; Sinclair and Jolliffe, 1958, 1961; Rouse et al., 1962, 1964), carotenoids (Miller et al., 1941; Pennisi et al., 1955; Higby, 1963; Rotstein et al., 1972; Gross, 1977), and ethanol (Davis, 1970).

Only one study has been conducted on the relationship of lipid changes to citrus fruit maturation (Nordby and Nagy, 1977). That study showed that the relative percentages of saturated and monounsaturated long-chain hydrocarbons in sweet orange juice sacs changed during fruit development. We now report changes in the distribution of lipid classes during sweet orange ripening.

EXPERIMENTAL SECTION

Hamlin, Pineapple, and Valencia sweet oranges were obtained from USDA Whitmore Experimental Farm (Crops Research Division, Orlando, Fla.). Fruit were collected monthly from mid July 1971 to mid May 1972; they were harvested from trees according to Sites and Reitz (1950, 1951). Total soluble solids (degree Brix) and acid values were determined on fruit of each cultivar by official analytical methods (AOAC, 1970). The preparation of juice sac powders and extraction of lipids were carried out according to methods previously described (Nagy and Nordby, 1970, 1971). Lipids were purified by the method of Wuthier (1966) and fractionated into neutral lipids, resin acids and glycolipids, and phospholipids and other polar lipids by silica gel column chromatography (Nagy et al., 1975). Completeness and purity of lipid class separation were monitored by thin-layer chromatography. Phospholipid phosphorus was determined on the purified lipid by the method of Bartlett (1959).

RESULTS AND DISCUSSION

In Florida, sweet oranges regularly bloom between February 15 and March 20 (Harding et al., 1940). Within 3 to 5 weeks of the blossoming, fruit have set and are less than 1 cm in diameter. From fruit-set to maturity, sweet oranges pass through three well-defined stages (Bain, 1958): (1) rapid cell division, (2) cell enlargement, and (3) maturation.

In our study, sampling of fruit began in mid-July when the fruit were essentially in the cell enlargement stage. The average equatorial diameters of fruit in July were 6.03 cm (Hamlin), 6.20 cm (Pineapple), and 5.72 cm (Valencia). Fruit from July 1971 to May 1972 were analyzed for pH, total acid, and total soluble solids contents (Table I). These three tests are commonly employed for assessing

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Table I. Chemical Indicators on Maturing Sweet Oranges

Sampling period	Hamlin pH	Hamlin Total acid, %	Hamlin Total soluble solids, %	Pineapple pH	Pineapple Total acid, %	Pineapple Total soluble solids, %	Valencia pH	Valencia Total acid, %	Valencia Total soluble solids, %
July 1971	2.87	2.89	8.16	2.79	3.63	7.54	2.73	4.11	7.37
August	3.04	1.72	8.49	3.02	2.35	7.64	2.86	3.90	7.66
September	3.22	1.38	9.37	3.04	1.95	8.74	2.89	2.57	7.96
October	3.53	1.03	10.20	3.42	1.13	9.37	3.15	1.96	7.74
November	3.62	0.84	11.20	3.56	0.89	10.14	3.20	1.59	9.31
December	3.74	0.68	11.14	3.51	0.91	9.97	3.47	1.00	8.44
January	3.92	0.66	11.87	3.86	0.68	10.16	3.29	1.21	11.37
February	4.08	0.47	9.11	3.72	0.75	11.38	3.30	1.11	11.86
March	4.23	0.52	12.04	3.98	0.60	10.59	3.79	0.81	11.25
April	4.08	0.55	11.92	3.96	0.68	12.17	3.87	0.68	10.79
May 1972	4.00	0.63	11.86	4.04	0.57	12.40	3.87	0.72	10.40

sweet orange maturity, and any new maturation test should always be compared to these three. In July, when the fruit were immature and green colored, the percentage of acid was highest and percentage of soluble solids the lowest for each cultivar. The earliest maturing orange (Hamlin) in this study had lower acid and higher soluble solids contents than the mid-season maturity orange (Pineapple) which, in turn, showed lower acid and higher soluble solids than the late maturity orange (Valencia). With all three oranges, maturation was accompanied by increased pH, decreased total acid, and increased soluble solids. Hamlin oranges generally mature (as measured by Brix/acid ratio) during October and November. By March and April, the juice vesicles of this cultivar showed extensive dehydration and the juice exhibited an insipid taste. The mid-season orange (Pineapple) matures during November and December. Extensive drying out of the juice vesicles was noticed in this orange during April and May. The late season cultivar (Valencia) generally matures during February and March; however, in our study this orange showed maturity (Brix/acid = 9.4) during January. There was no noticeable drying out of juice vesicles during Valencia's maturation period. Harding et al. (1940) noted, however, that the juice volume of Valencia (mL of juice/100 g of fruit) decreased during May through July.

The pulp of oranges is divided into segments (carpels, ca. 10) and each segment is composed of hundreds of juice sacs. The external proteolipid membrane (vesicular membrane) of the juice sac envelopes several cellular structures: large vacuoles, nuclei, mitochondria, leucoplasts, chromoplasts, and plastids. The juice of oranges consists of liquid expressed from the cytoplasm and from the vacuoles of internal cells within the sac (Albrigo and Carter, 1977). The expressed juice consists of soluble and insoluble substances of complex composition: celluloses, pectins, sugars, vitamins, minerals, essential oils, organic acids, lipids, flavonoids, proteins, amino acids, and other nutrients.

In our experiment, juice sacs were removed from the carpels of each cultivar and freeze-dried. The amounts of lipids (those within juice sacs and cuticular wax) extracted during the maturation periods are shown in Figure 1. Lipids extracted from Hamlin oranges showed minor fluctuations between July (10.3 mg/g of powder) and March (11.0 mg/g of powder) then increased sharply between March and May (17.2 mg/g of powder). Pineapple and Valencia oranges possessed high lipid contents in July and thereafter, showed noticeable decreases until October. In Pineapple, the lipid concentration varied slightly between October and January and then increased from 9.5 to 13.5 mg/g of powder between January and May. Valencia showed the least variation between October

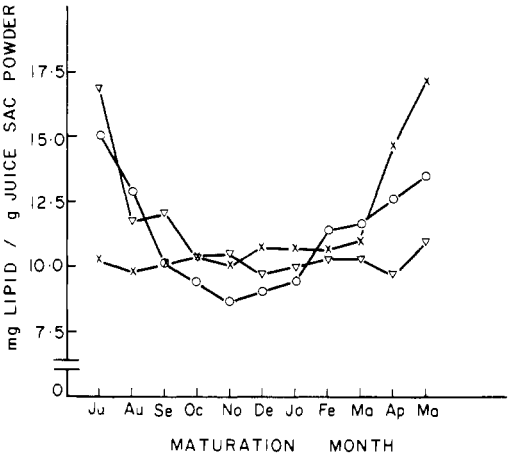

Figure 1. Amounts of lipids extracted from juice sac powders during maturation period: (X) Hamlin, (O) Pineapple, (▽) Valencia.

and April but a slight increase was evident during April and May. A lipid sample extracted from Valencia powder from a June sample (not shown in Figure 1) showed an increased concentration above the May period, thus, confirming the trend of increased lipid extractability with senescence.

The weight of juice sac powder was directly related to the concentrations of sugars and acids within the vacuolar sap. During intermediate stages of development (July to October, Figure 1), the amounts of lipids extracted were generally high owing to cellular lipid requirements (membrane and cuticular wax formation) and low contents of sugar. As sugar accumulated (Table I), the amounts of lipids extracted (based on an equal weight of sac powder) decreases. Between October and February, the amounts of extracted lipids from Hamlin and Valencia powders were relatively uniform; Pineapple showed wider fluctuations during this period. During March to May, extensive drying out of juice sacs occurred in Hamlin and Pineapple oranges. There were enhanced lipid contents from the extracted powders during this senescent period but we have no explanation as to why this should occur. We speculate that lipids which were once tightly bound to membranous structures and not extractable by $CHCl_3$–MeOH solvents became extractable due to a senescent activity which caused dissociation of tightly bound lipids from other chemical moieties.

Citrus lipids may be separated into three principal classes according to chemical structure and chromatographic resolution on silica gel columns (Rouser et al., 1967). In general, citrus lipids can be grouped into: (1) neutral lipids (NL); (2) resin acids and glycolipids (RAGL),

and (3) phospholipids and other polar lipids (PPL). The NL fraction of orange vesicular lipids includes monoglycerides, 1,2-diglycerides, 1,3-diglycerides, free sterols, free fatty acids, hydrocarbons, triglycerides, sterol esters, lipid pigments, and other components (Nagy and Nordby, 1970). The RAGL fraction consists of sterol glucosides, esterified sterol glucosides, cerebrosides, monogalactosyl diglycerides, digalactosyl diglycerides, resin acids, and other minor components (Nordby and Nagy, 1971). The PPL fraction is the largest and unquestionably the most complex. The major phospholipids are phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, and phosphatidic acid (Vandercook et al., 1970; Braddock, 1972).

The distribution of lipid classes during maturation of the three sweet orange cultivars is shown in Table II.

Maturation Patterns of Hamlin. The NL fraction showed an initial percentage of 27.7 in July and, thereafter, continuously dropped during the intervening months to a low of 22% in October. From October to May, NL percentages showed a reverse trend; i.e., a progressive increase from 22 to 34.7%. The RAGL fraction showed a minor percentage fluctuation of no statistical significance between July and October. From October to February there was a noticeable decrease in percentage with a low observed in February (18.5%). Between February and May, an upward percentage reversal occurred with a total increase of about 10%. The PPL fraction increased nonuniformly from July to a high of 56.7% in November and December. From December to May there was a marked decrease of about 19% for this fraction.

Maturation Patterns of Pineapple. An initial percentage of 25.8 was observed for the NL fraction in July and, thereafter, monthly percentages declined until a low of 21.7% was reached in November. From December to February, no drastic changes in NL percentages occurred; this was evident by a uniform percentage of about 24 for these 3 months. NL percentages increased after February; May showed 28.3% (about a 4% increase over February). The RAGL fraction showed an initial value of 25.3% in July. From August to November, no significant changes occurred in this fraction as evident by a uniform percentage of about 23. A nonuniform increase in RAGL percentages was observed between November and May (highest in May, 34.5%). The PPL fraction increased nonuniformly from July to a high of 54.3% for November and, thereafter, showed a continuous decline. From November to May, there was a total distribution loss of 17%.

Maturation Patterns of Valencia. In contrast to Hamlin and Pineapple oranges, Valencia's NL percentage was noticeably lower for July (18.5%). During August through November, no noticeable upward or downward percentage trend was evident; the percentages for these months were all about 20%. From December to February there was a rapid increase in NL percentages, i.e., from 22 to 29.5%. The February through May showed some minor erratic percentage fluctuations. In contrast to Hamlin and Pineapple, this period (February to May) was visibly static with no noticeable upward trend. The RAGL fraction of Valencia was noticeably higher than Hamlin and Pineapple during July through January. While there were two discernible high percentages during July and August, the September through May period showed only minor percentage fluctuations and no definitive upward or downward trend. The PPL fraction increased gradually from July (46%) to a high in November (47.3%). From November to May, there was a continuous decline in percentage. A

Table II. Distributions of Lipid Classes in Maturing Sweet Oranges (%)

Sampling period	Hamlin[a]			Pineapple[a]			Valencia[a]		
	Neutral lipids	Resin acids and glycolipids	Phospholipids and other polar lipids	Neutral lipids	Resin acids and glycolipids	Phospholipids and other polar lipids	Neutral lipids	Resin acids and glycolipids	Phospholipids and other polar lipids
July 1971	27.7 ± 0.3	22.0 ± 0.6	50.0 ± 1.0	25.8 ± 1.0	25.3 ± 1.0	49.0 ± 1.4	18.5 ± 0.7	35.8 ± 0.9	46.0 ± 1.4
August	23.3 ± 0.6	23.5 ± 0.5	53.3 ± 1.1	25.0 ± 1.4	23.3 ± 0.3	51.7 ± 1.2	20.5 ± 0.3	34.0 ± 0.4	46.3 ± 0.6
September	23.3 ± 0.8	21.3 ± 0.5	55.0 ± 0.9	24.0 ± 0.0	23.3 ± 0.6	52.7 ± 0.6	20.8 ± 0.3	32.3 ± 0.3	47.0 ± 0.0
October	22.0 ± 0.4	22.0 ± 0.6	55.8 ± 0.9	24.3 ± 0.3	23.3 ± 0.3	52.3 ± 0.3	19.7 ± 0.3	33.3 ± 0.9	47.0 ± 0.6
November	24.7 ± 0.3	19.3 ± 0.7	56.7 ± 0.7	21.7 ± 0.3	23.7 ± 0.9	54.3 ± 1.2	20.3 ± 0.3	32.5 ± 0.3	47.3 ± 0.3
December	24.7 ± 0.7	19.0 ± 0.6	56.7 ± 0.9	24.4 ± 1.1	27.0 ± 2.5	48.4 ± 1.7	22.0 ± 0.4	31.0 ± 0.8	46.8 ± 0.3
January	27.0 ± 0.7	18.8 ± 0.5	54.3 ± 0.6	24.0 ± 0.4	28.8 ± 0.3	47.3 ± 0.5	24.5 ± 0.9	32.0 ± 0.7	44.0 ± 0.9
February	29.5 ± 0.7	18.5 ± 0.3	51.8 ± 0.3	24.8 ± 0.3	29.5 ± 0.7	45.8 ± 0.3	29.5 ± 0.5	29.8 ± 1.0	40.5 ± 1.0
March	30.0 ± 1.1	23.5 ± 0.5	46.3 ± 1.3	27.7 ± 0.3	30.3 ± 0.3	42.3 ± 0.3	28.6 ± 1.7	29.6 ± 0.9	40.8 ± 0.9
April	32.8 ± 0.9	25.3 ± 1.0	42.3 ± 1.1	28.5 ± 0.7	29.8 ± 0.6	41.5 ± 0.5	30.7 ± 1.4	31.0 ± 1.0	38.3 ± 0.9
May 1972	34.7 ± 0.9	28.0 ± 0.6	37.0 ± 1.0	28.3 ± 0.5	34.5 ± 0.7	37.3 ± 0.5	29.5 ± 0.5	32.3 ± 0.6	38.2 ± 0.5

[a] Values represent mean ± standard error of four–seven composite fruit samples.

Table III. Phospholipid Phosphorus/Lipid Ratios in Maturing Sweet Oranges

Sampling period	μg of P/mg of lipid		
	Hamlin	Pineapple	Valencia
July 1971	20.0 ± 0.6	19.5 ± 0.4	17.1 ± 0.6
August	21.3 ± 0.8	20.7 ± 0.8	17.8 ± 0.4
September	21.7 ± 0.7	21.0 ± 0.3	18.8 ± 0.2
October	22.1 ± 0.5	21.0 ± 0.3	18.7 ± 0.4
November	22.2 ± 0.8	21.9 ± 1.0	18.9 ± 0.2
December	22.8 ± 0.6	19.3 ± 1.5	18.6 ± 0.4
January	21.7 ± 0.3	18.9 ± 0.4	17.5 ± 0.1
February	20.4 ± 0.5	18.2 ± 0.3	16.4 ± 0.6
March	19.2 ± 0.6	16.8 ± 0.3	16.5 ± 1.1
April	17.5 ± 0.5	16.6 ± 0.5	15.4 ± 0.2
May 1972	14.6 ± 0.6	14.9 ± 0.4	15.2 ± 0.3

total distribution loss of about 9% was evident over that period.

While relatively few studies have been conducted on the lipid components of ripening fruit, Galliard (1968) and Kalra and Brooks (1973) reported losses of polar lipids during ripening of apple and tomato fruits. Of the three lipid fractions we studied, only the PPL fraction declined consistently after the fruit had reached maturity (Table II). Examination of phospholipid phosphorus/lipid ratios (as μg of P/mg of lipid, Table III) also showed loss of phospholipids. After reaching a maximum ratio during November and December, a continuous decline in these ratios was observed for all three cultivars. Except for May, the ratios were highest for Hamlin, intermediate for Pineapple and lowest for Valencia. When compared to the ratio of Valencia oranges, the lower ratios observed in May for Hamlin and Pineapple oranges might be due to a more rapid breakdown of phospholipids during senescence. Regression analysis showed that for the interval between December (high) and May (low), the rate of loss of phospholipid phosphorus for Hamlin oranges was 1.57 μg of P (mg of lipid)$^{-1}$ month^{-1}. For Pineapple and Valencia oranges during the interval November (high) to May (low), rates of loss were 1.02 and 0.66 μg of P (mg of lipid)$^{-1}$ month^{-1}, respectively. The data in Tables I, II, and III confirm that ripening and senescence (measured by phospholipid loss) occurred fastest in the early season orange (Hamlin). In effect, rates of ripening and senescence show that Hamlin > Pineapple > Valencia.

We were unable to relate the legal maturity periods of the three orange cultivars with specific lipid metabolic conditions; albeit lipid-fruit ripening correlations might exist. The most important findings were: (1) even though the three cultivars mature over a wide period (November to March), phospholipid breakdown occurred in all three about the same time (December and January) and (2) senescence caused an increase in the extractability of lipids.

Lipids are important components of membranous systems. The chemical nature and quantitative distribution of lipid classes within the membrane imparts specific properties and functions to interfaces. During fruit ripening and senescence, cytoplasmic structures reorganize within the cells (Cocking and Gregory, 1963) and membrane permeability increases (Sacher, 1962). Cellular disorganization resulting from catabolism during senescence is accompanied by enzymatic breakdown of lipoprotein membranes (Galliard, 1975). Only limited information is available on the hydrolytic and oxidative lipid enzyme systems of citrus tissues. However, studies of these enzyme systems in other fruit and plant tissues have shown that after breakdown of lipoprotein membrane structures, fatty acids are released from many unbound polar lipids by lipolytic acyl hydrolases. In addition, the ubiquitous phospholipase D attacks polar groups of polar lipids by hydrolytic and transphosphatidylation mechanisms (Galliard, 1975). Fatty acid moieties liberated from these lipids are further subjected to oxidative breakdown by α- and β-oxidative systems, lipoxygenases, and fatty acid hydroperoxide degrading systems (Hitchcock and Nichols, 1971).

Wooltorton et al. (1965) showed that in ripening apples, increases in lipoxygenase activity were accompanied by increases in the evolution of ethylene. Although present evidence indicates that the major biochemical production of ethylene in higher plants is via the methionine pathway (Baur and Yang, 1972), Galliard et al. (1968) showed that apple fruit was able to convert linolenic acid to ethylene via a lipoxygenase-catalyzed reaction. Ethylene production in citrus fruit is considered responsible for color development and enhanced respiratory activity (Sinclair, 1961) and a stimulant of cellulase activity and the loosening of tree fruit (Rasmussen and Jones, 1969). In a more recent study, Rasmussen (1975) monitored ethylene production of freshly picked Hamlin, Pineapple, and Valencia oranges monthly for about half a year, starting from December. During that period, ethylene production tended to increase progressively and was greater for Hamlin and Pineapple than for Valencia. In our study, the percentage of the PL fraction declined steadily during those months, and the rates of decline were in the order Hamlin > Pineapple > Valencia. Also working with freshly harvested citrus, Davis (1970) found that ethanol concentration increased sharply during October–December in Hamlin and during December–January in Valencia. He reported that such sharp increases could be used as indicators of fruit maturity. Those periods of high ethanol production Davis reported correspond closely with the onset (November–January) of enhanced phospholipid breakdown in our study. In nonclimacteric fruit, such as citrus, the ripening and senescent stages are not readily delineated. Our study shows that these two stages might be distinguished on the basis of phospholipid content.

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Individual Lipids and Proximate Analysis of Various Foods. 4. Commercial Cake Mixes

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Commercially prepared cake mixes were purchased from several supermarkets. Samples were analyzed for water, protein, total fat, ash, fatty acids, sterols, and *cis,cis*-methylene interrupted polyunsaturated triglycerides. The data indicate that in the process of making cake mixes the manufacturer used either vegetable oil alone or a mixture of animal and vegetable fat. Animal fat alone was not used in the mixes tested. The amount of total fat ranged from 8.2 to 15.3 g/100 g of product and the cholesterol from 0 to 22 mg/100 g of product.

Many consumers now use commercially prepared cake mixes. Therefore, information concerning the content of cholesterol and saturated vs. polyunsaturated fatty acids would be helpful to the consumer in the selection of these mixes. Some consumers may add a small amount of fat or oil to enhance flavor or texture and/or use milk instead of the water which is specified in the instructions. However, these additions probably would not significantly alter the quantity of sterols or fatty acids found in the cake mixes as prepared by the manufacturer.

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MATERIAL AND METHODS

Eighteen cake mixes were selected from seven different brands for the analyses. The brands were: Betty Crocker, Duncan Hines, Mrs. Wright's, Giant Food, Grand Union, Ann Page, and Washington. The following types of cake mixes were included in the study: yellow (No's. 2, 5, 7, 9, 13, 14, and 16), white (No's. 4, 11, and 18), chocolate (No's. 1, 6, 8, 10, 12, 15, and 17), and spice (No. 3).

The samples were homogenized in a Waring blender using chloroform–methanol extractant (2:1, v:v), as previously described by Sheppard et al. (1974). The fatty acids were esterified using boron trifluoride–methanol according to the Association of Official Analytical Chemists (AOAC, 1975) procedure as modified by Solomon et al.