Variation in Lipid Composition of Apples in Relation to Watercore

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Abbreviations: CI, chemical ionization; DGDG, digalactosyldiglyceride; EI, electron impact; FAME, fatty acyl methyl esters; FID, flame ionization detector; GC-MS, gas chromatography-mass spectrometry; MDGD, monogalactosyldiglyceride; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; TLC, thin-layer chromatography.
Table 1. Glycolipid, phospholipid, and sterol content from skin and flesh tissues of normal and watercore-affected ‘Delicious’ apples.\(^z\)

| Constituent      | Normal fruit | Fruit with watercore |
|------------------|--------------|----------------------|
|                  | NS| NF| WS| WNF| WAF|
| Glycolipid       |      |      |     |     |      |
| MGDG             | 31.4 ± 0.4 | 19.7 ± 0.3 | 39.9 ± 0.7 | 24.9 ± 0.4 | 29.7 ± 0.6 |
| DGDG             | 132.4 ± 2.5 | 30.7 ± 1.1 | 162.5 ± 8.2 | 39.6 ± 1.0 | 50.7 ± 2.8 |
| Phospholipid     |      |      |     |     |      |
| PC               | 485.6 ± 7.7 | 253.4 ± 6.6 | 508.9 ± 12.3 | 293.5 ± 8.3 | 321.7 ± 6.3 |
| PE               | 269.1 ± 8.1 | 144.3 ± 7.4 | 320.7 ± 9.7 | 209.2 ± 5.4 | 240.0 ± 3.7 |
| PG               | 63.1 ± 0.2 | 16.8 ± 0.7 | 70.6 ± 0.8 | 20.3 ± 0.7 | 20.7 ± 0.1 |
| PI               | 189.1 ± 7.5 | 67.2 ± 0.5 | 209.7 ± 6.9 | 83.2 ± 0.7 | 86.9 ± 0.9 |

| Sterol           |      |      |     |     |      |
|------------------|      |      |     |     |      |
| Free sterols     |      |      |     |     |      |
| Cholesterol      | 1.7 ± 0.3 | 0.9 ± 0.1 | 1.7 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.1 |
| Campesterol      | 3.6 ± 0.2 | 2.0 ± 0.1 | 3.7 ± 0.4 | 2.0 ± 0.2 | 2.1 ± 0.1 |
| Sitosterol       | 66.3 ± 0.6 | 53.0 ± 0.5 | 78.4 ± 0.2 | 48.1 ± 0.5 | 54.2 ± 0.7 |
| Steryl esters    |      |      |     |     |      |
| Cholesterol      | 0.2 ± 0.05 | 0.1 ± 0.02 | 0.2 ± 0.03 | 0.1 ± 0.02 | 0.1 ± 0.02 |
| Campesterol      | 0.4 ± 0.06 | 0.2 ± 0.04 | 0.4 ± 0.10 | 0.2 ± 0.03 | 0.2 ± 0.03 |
| Sistosterol      | 8.0 ± 0.50 | 3.1 ± 0.20 | 9.2 ± 0.30 | 3.4 ± 0.10 | 3.8 ± 0.20 |

\(^{z}\)Mean of three replicates ± SE.

Abbreviations: NS, normal skin; NF, normal flesh; WS, skin from apples with watercore; WNF, apples with watercore, normal flesh; WAF, apples with watercore, affected flesh.

Table 2. Fatty acid composition (weight percentage of total) of glycolipids (MGDG and DGDG) from skin and flesh tissues of normal and watercore-affected ‘Delicious’ apple.\(^z\)

| Constituent fatty acids | Normal fruit | Fruit with watercore |
|-------------------------|--------------|----------------------|
|                         | NS| NF| WS| WNF| WAF|
| MGDG                    |      |      |     |     |      |
| 16:0                    | 4.4 ± 0.5 | 9.2 ± 0.8 | 6.6 ± 0.5 | 14.1 ± 0.7 | 17.6 ± 0.9 |
| 18:0                    | 2.8 ± 0.1 | 0.7 ± 0.1 | 3.5 ± 0.4 | 2.0 ± 0.1 | 2.8 ± 0.1 |
| 18:1                    | 3.7 ± 0.4 | 2.1 ± 0.2 | 4.2 ± 0.3 | 2.4 ± 0.2 | 2.9 ± 0.1 |
| 18:2                    | 33.5 ± 1.2 | 11.1 ± 0.6 | 36.7 ± 0.6 | 30.0 ± 1.3 | 36.7 ± 0.7 |
| 18:3                    | 55.6 ± 1.1 | 76.9 ± 1.5 | 49.0 ± 0.7 | 51.5 ± 1.2 | 40.0 ± 1.0 |
| DGDG                    |      |      |     |     |      |
| 16:0                    | 10.1 ± 0.4 | 16.6 ± 0.4 | 15.1 ± 0.1 | 16.8 ± 1.0 | 18.1 ± 0.6 |
| 18:0                    | 10.9 ± 0.3 | 6.9 ± 0.2 | 11.4 ± 0.8 | 7.0 ± 0.4 | 7.9 ± 0.5 |
| 18:1                    | 4.1 ± 0.2 | 2.3 ± 0.1 | 5.7 ± 0.3 | 3.1 ± 0.2 | 3.4 ± 0.5 |
| 18:2                    | 28.7 ± 1.0 | 18.7 ± 1.0 | 32.0 ± 1.1 | 25.4 ± 0.9 | 27.3 ± 1.0 |
| 18:3                    | 46.2 ± 1.5 | 55.5 ± 1.6 | 35.8 ± 1.2 | 47.7 ± 1.3 | 43.3 ± 1.1 |

\(^{z}\)Mean of three replicates ± SE.

Abbreviations: NS, normal skin; NF, normal flesh; WS, skin from apple with watercore; WNF, apples with watercore, normal flesh; WAF, apples with watercore, affected flesh.

Results and Discussion

The lipid composition in apple fruit was dominated by galacto- and phospholipid in skin and flesh tissues (Table 1). Digalactosyl diglyceride (DGDG) was the major galactolipid, with more than twice the amount of monogalactosyl diglyceride (MGDG) in the skin. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) represented a higher percentage of the lipids in the fruit.

Sterol composition was determined by FID-GC and further confirmed by GC-MS (Wang et al., 1988). Means of three replicate samples were reported.

A stream of N\(_2\) and redissolved in 2 ml of hexane. Silicic-acid column chromatography (100- to 200-mesh Bio Sil A, Bio Rad Laboratories) was used to separate the steryl ester and free sterols by elution with 10 hexane : 1 ether (v/v; steryl ester) and 2 hexane : 1 ether (v/v; free sterols). Steryl ester was cleaved to free sterol by saponification (1 M KOH in 80% ethanol for 1 h at 80°C under N\(_2\)) and hexane extraction. Lathosterol (cholest-7-\(\alpha\)-ol) was added to each sample as an internal standard. Free sterols were precipitated with digitonin (Sigma). The digitonides were collected on a glass fiber filter (Reeve Angel, Clifton, N.J.) and washed with 1 acetone : 2 diethyl ether (v/v). Free sterols were liberated from the digitonides by refluxing in 1 ml of pyridine for 30 min at 100°C. After the samples had cooled, 1 ml of deionized, distilled water was added, and the free sterols were recovered by extraction with 2 ml of hexane. Sterol composition was determined by FID-GC and further confirmed by GC-MS (Wang et al., 1988). Means of three replicate samples were reported.

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Table 3. Fatty acid composition (weight percentage of total) of phospholipids (PC, PG, PE, PI) from skin and flesh tissues of normal and watercore-affected ‘Delicious’ apple.\(^z,y\)

| Constituent | Normal fruit | Fruit with watercore |
|-------------|--------------|----------------------|
|             | NS           | NF                   | WS | WNF | WAF |
| PC          |              |                      |    |      |     |
| 16:0        | 12.0 ± 0.6   | 21.0 ± 1.1           | 12.9 ± 0.5 | 22.8 ± 1.0 | 24.0 ± 0.9 |
| 18:0        | 10.0 ± 0.3   | 4.3 ± 0.4            | 11.1 ± 0.3 | 4.4 ± 0.3  | 4.5 ± 0.2  |
| 18:1        | 3.3 ± 0.1    | 2.5 ± 0.2            | 3.8 ± 0.4  | 3.0 ± 0.4  | 3.1 ± 0.3  |
| 18:2        | 54.4 ± 1.1   | 57.6 ± 1.2           | 56.2 ± 1.2 | 59.0 ± 1.5 | 61.4 ± 1.2 |
| 18:3        | 20.3 ± 0.9   | 14.6 ± 0.7           | 16.0 ± 0.7 | 10.8 ± 1.1 | 7.0 ± 0.5  |
| PE          |              |                      |    |      |     |
| 16:0        | 12.9 ± 0.5   | 29.1 ± 0.9           | 13.9 ± 0.4 | 30.5 ± 0.8 | 30.4 ± 0.9 |
| 18:0        | 8.5 ± 0.2    | 2.9 ± 0.1            | 9.6 ± 0.3  | 3.2 ± 0.2  | 3.4 ± 0.1  |
| 18:1        | 1.3 ± 0.1    | 1.4 ± 0.1            | 1.8 ± 0.1  | 1.7 ± 0.1  | 1.9 ± 0.1  |
| 18:2        | 62.5 ± 1.6   | 57.4 ± 1.8           | 65.7 ± 1.5 | 60.3 ± 1.4 | 61.4 ± 1.2 |
| 18:3        | 14.8 ± 0.8   | 9.2 ± 0.6            | 9.0 ± 0.6  | 4.3 ± 0.2  | 2.9 ± 0.1  |
| PG          |              |                      |    |      |     |
| 16:0        | 42.8 ± 1.4   | 47.4 ± 1.6           | 43.2 ± 1.3 | 48.3 ± 1.7 | 49.4 ± 1.2 |
| 18:0        | 4.7 ± 0.2    | 2.3 ± 0.1            | 5.2 ± 0.1  | 3.2 ± 0.1  | 3.1 ± 0.1  |
| 18:1        | 19.0 ± 0.5   | 14.8 ± 0.3           | 20.0 ± 0.4 | 15.2 ± 0.1 | 16.3 ± 0.2 |
| 18:2        | 23.1 ± 0.7   | 23.7 ± 0.5           | 25.1 ± 0.7 | 26.1 ± 0.5 | 27.5 ± 0.6 |
| 18:3        | 10.4 ± 0.2   | 11.8 ± 0.4           | 6.5 ± 0.2  | 7.2 ± 0.3  | 3.7 ± 0.2  |
| PI          |              |                      |    |      |     |
| 16:0        | 23.9 ± 0.6   | 38.7 ± 0.7           | 26.1 ± 0.5 | 41.3 ± 1.1 | 42.5 ± 1.2 |
| 18:0        | 12.8 ± 0.3   | 1.9 ± 0.1            | 14.5 ± 0.4 | 7.1 ± 0.2  | 7.2 ± 0.1  |
| 18:1        | 1.6 ± 0.1    | 1.1 ± 0.1            | 1.8 ± 0.1  | 1.2 ± 0.1  | 1.1 ± 0.2  |
| 18:2        | 48.6 ± 1.4   | 40.1 ± 0.8           | 46.7 ± 1.2 | 47.1 ± 1.3 | 48.5 ± 1.3 |
| 18:3        | 13.1 ± 0.5   | 18.2 ± 0.3           | 10.9 ± 0.7 | 8.3 ± 0.6  | 5.7 ± 0.3  |

\(^z\)Mean of three replicates ± se.  
\(^y\)Abbreviations: NS, normal skin; NF, normal flesh; WS, skin from apples with watercore; WNF, apples with watercore, normal flesh; WAF, apples with watercore, affected flesh.

The fatty acid profiles of galacto- and phospholipids from apple skin and flesh were similar and relatively unsaturated (Tables 2 and 3). Palmitic (C 16:0), stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2), and linolenic acid (C 18:3) were the predominant fatty acids. Identification of these fatty acid esters was confirmed by GC-MS. The full electron impact (EI) mass spectra of fatty acid esters was similar to the authentic standard, as reported (Heller and Milne, 1978). The FAME of palmitate, stearate, oleate, linoleate, and linolenate derived from extracted phospholipid in apple. Apple skin contained more galacto- and phospholipids than flesh. Tissue with watercore contained more galacto- and phospholipid than normal tissue (Table 1).

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Table 4. Ratio of unsaturated to saturated fatty acids [(18:1 + 18:2 + 18:3)/(16:0 + 18:0)] in glycolipids and phospholipids from skin and flesh tissues of normal and watercore-affected ‘Delicious’ apple.\(^z\)

| Constituent | Unsat/saturated | Normal fruit | Fruit with watercore |
|-------------|-----------------|--------------|----------------------|
| Glycolipid  |                 | NS           | NF                   | WS | WNF | WAF |
| MGDG        | 12.89           | 9.10         | 8.90                 | 5.20 | 3.90 |
| DGDG        | 3.76            | 3.26         | 2.77                 | 3.20 | 2.80 |
| Phospholipid|                 |              |                      |    |      |     |
| PC          | 3.55            | 2.95         | 3.17                 | 2.68 | 2.51 |
| PE          | 3.67            | 2.13         | 3.26                 | 1.97 | 1.96 |
| PG          | 1.11            | 1.01         | 1.07                 | 0.94 | 0.90 |
| PI          | 1.72            | 1.46         | 1.30                 | 1.30 | 1.24 |

\(^z\)Abbreviations: NS, normal skin; NF, normal flesh; WS, skin from apples with watercore; WNF, apples with watercore, normal flesh; WAF, apples with watercore, affected flesh.

Table 5. Ratio of linolenic to linoleic plus oleic acids [(18:3)/(18:2 + 18:1)] in glycolipids and phospholipids from skin and flesh tissues of normal and watercore-affected ‘Delicious’ apples at harvest.

| Constituent | (18:3)/(18:2 + 18:1) | Normal fruit | Fruit with watercore |
|-------------|----------------------|--------------|----------------------|
| Glycolipid  |                      | NS           | NF                   | WS | WNF | WAF |
| MGDG        | 1.49                 | 5.83         | 1.20                 | 1.58 | 1.01 |
| DGDG        | 1.41                 | 2.64         | 0.95                 | 1.67 | 1.41 |
| Phospholipid|                      |              |                      |    |      |     |
| PC          | 0.35                 | 0.24         | 0.27                 | 0.17 | 0.11 |
| PE          | 0.23                 | 0.16         | 0.13                 | 0.07 | 0.05 |
| PG          | 0.25                 | 0.31         | 0.14                 | 0.17 | 0.08 |
| PI          | 0.26                 | 0.44         | 0.17                 | 0.17 | 0.11 |

\(^z\)Abbreviations: NS, normal skin; NF, normal flesh; WS, skin from apples with watercore; WNF, apples with watercore, normal flesh; WAF, apples with watercore, affected flesh.
Table 6. Ratios of free sterols/phospholipids, PC/PE and (cholesterol + campesterol)/sitosterol from skin and flesh tissues of normal and watercore-affected ‘Delicious’ apples.

| Constituent                                      | Normal fruit |   | Fruit with watercore |
|--------------------------------------------------|--------------|---|----------------------|
|                                                  | NS           | NF | WS                   | WNF | WAF |
| (Free sterols)(phospholipids)^{−1}                | 0.071        | 0.079 | 0.076               | 0.084 | 0.086 |
| (PC)(PE)^{−1}                                     | 1.800        | 1.770 | 1.590               | 1.400 | 1.340 |
| (Cholesterol + campesterol)(sitosterol)^{−1}      | 0.080        | 0.083 | 0.069               | 0.071 | 0.056 |

Abbreviations: NS, normal skin; NF, normal flesh; WS, skin from apples with watercore; WNF, apples with watercore, normal flesh; WAF, apples with watercore, affected flesh.

galacto- and phospholipids were also confirmed by selected ion-monitoring using the molecular ions \(M^+\) and quasimolecular ions \((M + 1)^+\) in EI and chemical ionization (CI), respectively. The molecular ions \(M^+\) in EI of palmitate, stearate, oleate, linolate, and linoleate were 270, 298, 296, 294, and 292, respectively. The quasimolecular ions \((M + 1)^+\) in CI of palmitate, stearate, oleate, linolate, and linoleate were 271, 299, 297, 295, and 293, respectively. The GC-MS relative retention time of these fatty acids were also identical to the relative retention times of authentic samples.

Linolenic acid was the predominant fatty acid in MGDG and DGDG (Table 2), while linoleic acid was the predominant fatty acid in PC, PE, and phosphatidylinositol (PI).

Phosphatidyglycerol (PG) and PI contained a relatively higher amount of the saturated fatty acid, palmitic acid (C 16:0), than of other phospholipids (Table 3). The degree of fatty acid unsaturation in MGDG was much higher than that in DGDG (Table 4). PC and PE were less saturated than PG and PI (Table 4). There was a corresponding increase in the percentage of the saturated lipids in the watercored tissue. The ratios of unsaturated (18:1 + 18:2 + 18:3) to saturated (16:0 + 18:0) (18:3) to (18:1 + 18:2) of galacto- and phospholipids were lower in watercore-affected tissue than in normal tissue (Tables 4 and 5). This result perhaps reflects the depletion of unsaturated fatty acids by lipid peroxidation (Hariyadi and Parkin, 1991) or changes in lipid metabolism in watercore-affected tissue. Different lipid components may have specific roles in maintaining membrane structure and function. Fatty acid unsaturation is one of the key factors regulating membrane function (Dickens and Thompson, 1982). The decrease of this ratio may contribute to a decrease in the fluidity of membranes in watercore-affected fruit.

A high content of sterols and sterol esters is characteristic for the plasma membrane of plant cells. These steryl lipids serve to stabilize the lipid bilayer. Changes in sterol level and composition have been shown to affect membrane permeability and development in higher plants (Goad, 1983). A large increase in the content of sterol lipids, as well as a dramatic change in their sterol composition, occurs with ripening in apple (Galliard, 1968) and tomato (Lycopersicon esculentum Mill.) (Chow and Jen, 1978). The free sterols in apple fruit are \(\beta\)-sitosterol, cholest erol, and campesterol. The identity of these sterols was established by gas-liquid chromatography (GLC) and mass spectrometry. The GLC-MS relative retention times, the fragmentation patterns, and the significant ions and relative intensities of the full EI-MS of these sterols matched the established standards, as reported (Heller and Mime, 1978; Sawai et al., 1978). \(\beta\)-Sitosterol is the major desmethylsterol in apple fruit, comprising > 90% of the sterol present. Cholesterol and campesterol01 are present in 0.2% and 4%, respectively. Stigmasterol was not detected. The major esterified sterol, sitosterol, was present in apple skin and flesh. Steryl esters of campesterol and cholesterol were also present in trace amounts (Table 1). Steryl esters may also modulate membrane properties (Goad, 1983; Grunwald, 1975). The apple skin contained higher amounts of free sterols and steryl esters than flesh (Table 1). There was a concentration difference in sterol content in skin or flesh between normal and watercore-affected apples. Tissue with watercore contained more sterol and steryl esters than normal tissue (Table 1). The lipid composition of the various membranes of plant cells greatly affects the fluidity of their lipid matrix (Brockerhoff, 1974). The interaction of free sterols with phospholipids may affect membrane function and permeability (Grunwald, 1975). The ratio of free sterols to phospholipids was higher, whereas the ratios of PC to PE and cholesterol + campesterol to sitosterol were lower in watercored tissue (Table 6). These results indicate a decrease in the fluidity of membranes in watercore-affected tissue. This phenomenon is similar to that which occurs in ripening or senescence (Legge et al., 1986). In this respect, apples with watercore may be regarded as more advanced physiologically than normal apples.

Taken together, our study showed that watercore-affected fruit contains more glycolipids, phospholipids, and sterols than normal fruit. It has a higher ratio of free sterols to phospholipids and lower ratios of PC to PE and cholesterol + campesterol to sitosterol. All of these factors contribute to a lower membrane fluidity in watercored tissue and may reflect accelerated senescence.

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