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Ripening and Solar Exposure Alter Polar Lipid Fatty Acid Composition of 'Honey Dew' Muskmelons

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Additional index words. *Cucumis melo*, chilling tolerance

Abstract. **Polar lipids were extracted from immature through overripe 'Honey Dew' muskmelons (*Cucumis melo* L.) that were exposed to high or low levels of solar radiation. Fatty acid composition of the polar lipids changed and the percentage of unsaturated fatty acids increased as fruit ripened. The percentage of monounsaturated fatty acids palmitoleic and oleic acid as a percent of total fatty acids increased from 8% in melons of minimum maturity to >50% in overripe melons. Also, the ratio of unsaturated to saturated fatty acids increased from 2.2 to 5.0. Total polar lipid fatty acid compostion from middle mesocarp tissue (flesh) did not change as much during ripening as the polar lipid composition from the epidermis (peel). Peel tissue from the top of melons relative to the ground had unsaturation ratios of C18 fatty acids and C16 fatty acids 33% and 62% greater, respectively, than peel from the bottom of the melon. Melons of minimum maturity exposed to solar radiation had significantly more unsaturated C18 fatty acids than shaded melons. Increase in the percentage of unsaturated polar lipid fatty acids in 'Honey Dew' melons may relate to increases in chilling tolerance reported to occur with ripening and solar exposure.**

'Honey Dew' melons may develop chilling injury (CI) if they are held for >2 weeks at 5C or below (Lipton, 1978; Wiant, 1938). CI greatly limits the export and marketability of 'Honey Dew' melons (Lipton and Mackey, 1984). The occurrence of this disorder is often variable, and several factors have been identified that influence the sensitivity of melons to CI. These include the degree of melon ripeness (Lipton, 1978; Lipton et al., 1979) and the exposure of the fruit to solar radiation during development (Lipton and Aharoni, 1979).

The fluidity of the cellular membranes at chilling temperatures has been related to the susceptibility of the plant to CI (Lyons, 1973). This fluidity is related to the relative portion of saturated and unsaturated fatty acids of the membrane lipids (Lyons et al., 1964, Lyons and Raison, 1970). Some treatments that increase the resistance of plant tissues to CI also increase the percentage of unsaturated fatty acids in membrane lipids. This response has been demonstrated with intermittent warming (Wang and Baker, 1979; Wang and Anderson, 1982), temperature conditioning (Wilson and Crawford, 1974), and various chemical treatments (Wang and Baker, 1979; Waring et al., 1976).

The mechanism by which 'Honey Dew' melons increase their tolerance to chilling temperatures during ripening or solar exposure is not known. The objective of this study was to determine whether changes in polar lipid fatty acid saturation occur and could explain changes in chilling tolerance in 'Honey Dew' melons associated with ripening and/or solar exposure.

'Honey Dew' melons were grown on the horticultural field station in Fresno, Calif. Fruit were tagged at anthesis and four fruit each were harvested after 10, 20, 25, 35, 40, 46, 50, or 55 days post anthesis (DPA). At 35, 40, 46, 50, and 55 DPA, fruit were at minimum horticultural maturity, full maturity, initial ripening, ripe, and overripe stages of maturity, respectively, according to the criteria described by Pratt et al. (1977).

In addition to harvesting fruit at different developmental stages, vines were moved at 21 DPA to expose four melons to solar radiation while four comparable melons were covered with vines to minimize solar exposure. These melons were harvested 35 DPA, i.e., at minimum horticultural maturity.

At the time of harvest, two 4 × 4-cm plugs were cut through to the seed cavity on each melon midway between the stem and blossom ends on the top and bottom of the melon relative to the ground. These cores of tissue were divided into the outer 1- to 2-mm epidermis and hypodermal mesocarp (peel) and middle mesocarp 2 cm below the peel tissue (flesh). Tissue slices were frozen in liquid N_2, freeze-dried, and stored at 20C under N_2.

Lipids were extracted and recovered from 1-g samples of freeze-dried melon by the procedure of Folch et al. (1957). The extract was dried at 40C under reduced pressure, dissolved in 5 ml of petroleum ether, and polar lipids were separated from neutral lipids by passing the extract through silica gel Sep-Pak cartridges. Cartridges were conditioned with 5 ml of methanol followed by 5 ml of chloroform and 5 ml of petroleum ether. The extract was passed through the cartridge, and neutral lipids were eluted with 20 ml of chloroform followed by polar lipids eluted with 20 ml of methanol. The polar lipid fraction was dried at 40C under reduced

Received for publication 3 Nov. 1989. Mojtaba Massoudi is gratefully acknowledged for his technical assistance in lipid analysis. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Table 1. Influence of maturity and location on the melon on fatty acid composition and degree of unsaturation of polar lipids in the peel of 'Honey Dew' melons.

Ripeness class[z]	Days post-anthesis	Fatty acid composition (%)						Unsaturation ratios[y]			
		16:0	16:1	18:0	18:1	18:2	18:3	I	II	III	IV
A	10	32.0	0.1	3.5	1.4	20.7	41.7	1.68	1.8	18.5	0.0
B	20	26.5	0.0	4.8	7.7	21.3	38.7	1.66	2.2	14.4	0.0
C	25	25.0	0.0	5.2	11.4	19.7	37.7	1.64	2.3	13.6	0.0
1	35	25.2	0.2	5.9	8.0	20.2	39.8	1.68	2.2	12.0	0.01
1–2	40	24.2	1.7	5.6	12.3	17.0	37.0	1.59	2.3	12.6	0.07
2	46	20.7	4.7	3.2	22.3	13.3	35.0	1.59	3.2	24.8	0.24
3	50	20.7	10.8	3.3	27.7	8.3	28.7	1.41	3.3	27.9	0.54
4	55	15.8	23.0	1.2	34.9	4.2	20.5	1.28	5.0	55.3	1.50
Location on melon											
Top		23.4	5.8	3.9	16.2	14.1	35.9	1.58	2.9	24.8	0.34
Bottom		23.4	3.7	4.7	15.7	17.1	34.3	1.57	2.7	18.6	0.21
Level of significance											
Ripeness								**	***	***	***
Immature (A–C) vs. mature (1–4)								***	***	***	***
Location on melon								NS	NS	**	**
Ripeness × location								NS	NS	NS	*

[z]Ripeness classes were: A, B, and C = immature; 1 = minimum horticultural maturity; 1–2 = full maturity; 2 = initial ripening; 3 = ripe; and 4 = overripe.
[y]I = unsaturation index = (%16:1 + %18:1 + 2(%18:2) + 3(%18:3))/100; II = ratio of unsaturated/saturated fatty acids; III = ratio of 18:1 + 18:2 + 18:3/18:0; IV = ratio of 16:1/16:0.
[NS,*,**,***]Nonsignificant or significant at P = 0.05, 0.01, or 0.001, respectively, according to F test.

Table 2. Influence of maturity and location on the melon on fatty acid composition and degree of unsaturation of polar lipids in the flesh of 'Honey Dew' melons.

Ripeness class[z]	Days post-anthesis	Fatty acid composition (%)						Unsaturation ratios[y]			
		16:0	16:1	18:0	18:1	18:2	18:3	I	II	III	IV
A	10	35.5	0.0	3.9	1.4	26.5	32.6	1.52	1.5	15.8	0.0
B	20	36.2	0.8	3.6	1.4	26.9	30.8	1.48	1.5	17.4	0.02
C	25	35.9	0.0	3.3	1.5	26.6	32.5	1.52	1.6	14.2	0.0
1	35	32.0	0.5	3.8	2.3	26.5	33.9	1.58	1.8	17.5	0.02
1–2	40	33.5	6.2	8.4	4.3	17.9	28.8	1.33	1.4	7.7	0.18
2	46	31.1	7.4	8.1	4.1	17.7	31.1	1.40	1.6	9.4	0.26
3	50	36.2	15.1	6.5	10.6	6.8	24.8	1.14	1.3	7.9	0.42
4	55	33.0	14.8	3.0	16.2	8.4	24.6	1.22	1.8	15.3	0.45
Location on melon											
Top		33.5	4.3	5.0	4.2	21.4	30.9	1.44	1.6	12.7	0.12
Bottom		32.9	6.3	5.8	5.5	19.0	30.3	1.41	1.6	13.6	0.20
Level of significance											
Ripeness								*	NS	*	**
Immature (A–C) vs. mature (1–4)								***	NS	***	***
Location on melon								NS	NS	NS	*
Ripeness × location								NS	NS	*	NS

[z]Ripeness classes were: A, B, and C = immature; 1 = minimum horticultural maturity; 1–2 = full maturity; 2 = initial ripening; 3 = ripe; and 4 = overripe.
[y]I = unsaturation index = (%16:1 + %18:1 + 2(%18:2) + 3(%18:3))/100; II = ratio of unsaturated/saturated fatty acids; III = ratio of 18:1 + 18:2 + 18:3/18:0; IV = ratio of 16:1/16:0.
[NS,*,**,***]Nonsignificant or significant at P = 0.05, 0.01, or 0.001, respectively, according to F test.

pressure, and polar lipids were saponified with methanolic NaOH (ASTM standard). Methyl esters of the resulting fatty acids were prepared using boron trifluoride in methanol (Metcalfe and Schmitz, 1961). The esterified acids were analyzed using a Varian 2730 (Varian, Sugar Land, Texas) gas liquid chromatograph equipped with a flame ionization detector and a 1.8-m stainless steel column packed with 10% SP-2330 on 100-120 Supelcoport. The oven was at 200C, the injector and detector were at 225C, and the flow rate of carrier gas was 20 ml·min^{-1}. Identification of individual fatty acid methyl esters was made using known standards.

The fatty acid composition of polar lipids from the peel of 'Honey Dew' melons changed slightly from 10 DPA to 35 DPA when fruit reached maturity (Table 1). After 35 DPA, melons began to ripen and the composition of fatty acids changed rapidly. The monounsaturated fatty acids palmitoleic acid and oleic acid increased from 8% of the fatty acids 35 DPA to >50% in overripe fruit 55 DPA. During this time, the unsaturation index decreased slightly, but the ratio of unsaturated to saturated fatty acids doubled and the saturation of both C16 and C18 fatty acids increased.

Composition of the polar lipid fatty acids isolated from flesh tissue did not change during ripening as much as those in the peel tissue. The monounsaturated fatty acids increased from 3% to 30% of the fatty acids (Table 2), and the degree of saturation of C16 fatty acids changed similarly to those in the peel tissue. However, the ratios of unsaturated to saturated fatty acids and the degree of saturation of C18 fatty acids did not increase during fruit development as in peel tissue.

In addition to ripeness, position of the melon peel tissue relative to the ground influenced polar lipid fatty acid composition. Peel tissue from the top of the melon had an average of 33% and 62% greater unsaturation ratios of C18 and C16 fatty acids, respectively, than were found in the bottom peel (Table 1).

Exposure of the melons to solar radiation during fruit growth and development significantly increased the unsaturation index and the ratio of unsaturated to saturated fatty acids (Table 3). The unsaturation of C18 fatty acids was increased by solar exposure but not that of C16 fatty acids. Solar exposure also induced the production of a yellow pigment in all four exposed melons.

The unsaturation of polar lipid fatty acids increased as 'Honey Dew' melons ripened, primarily due to the increase of palmitoleic and oleic acids. In mitochondria isolated from mango fruit, the molar ratio of palmitoleic

Table 3. Influence of solar exposure on fatty acid composition and degree of unsaturation of polar lipids in the peel of 'Honey Dew' melons of minimum horticultural maturity (Class 1).

Solar exposure	Fatty acid composition (%)						Unsaturation ratios[z]			
	16:0	16:1	18:0	18:1	18:2	18:3	I	II	III	IV
Yes	24.6	0.0	5.0	7.7	20.6	41.7	1.74	2.4	14.4	0.00
No	25.0	0.3	6.9	8.2	19.7	37.9	1.62	2.0	9.9	0.01
F test							*	*	**	NS

[z]I = unsaturation index = (%16:1 + %18:1 + 2(%18:2) + 3(%18:3))/100; II = ratio of unsaturated/saturated fatty acids; III = ratio of 18:1 + 18:2 + 18:3/18:0; IV = ratio of 16:1/16:0.
NS,*,**Nonsignificant or significant at P = 0.05 or 0.01, respectively.

to palmitic acid also increased with ripening while decreasing in fruit held at chilling temperatures (Kane et al., 1978). In apple flesh, however, the degree of unsaturation of fatty acids from phospholipids decreased as fruit ripened (Lurie and Ben-Arie, 1983).

Polar lipid C18 and C16 fatty acids extracted from the top peel tissue were more unsaturated than those from the bottom of the melon. Higher temperatures increase the rate of ripening and may explain this difference in fatty acid composition. The temperature of fruit tissue on the top of the melon has been shown to be elevated due to solar exposure (Lipton et al., 1987), while tissue in contact with the ground would tend to be cooler due to shading and the influence of the soil temperature. This difference in maturity between the top and the bottom of the melon may explain the differences in fatty acid composition that were observed. Light may also have a direct effect on the control of fatty acid synthesis and composition (Browse et al., 1981). Either of these mechanisms may also explain the differences in polar lipid composition observed in melons of minimum maturity exposed or not exposed to solar radiation.

The degree of unsaturation in the membrane fatty acids has been reported to influence chilling sensitivity of plant tissues (Lyons et al., 1964; Lyons and Raison, 1970). Chilling injury of 'Honey Dew' melons decreases as melons ripen (Lipton, 1978; Lipton et al., 1979), is usually found on the lower half of the melon (Lipton et al, 1987), and is less in melons exposed to the sum during maturation (Lipton and Aharoni, 1979; Lipton et al, 1987). In this study, the degree of unsaturation in polar lipid fatty acids of 'Honey Dew' melons increased coincidently with the decrease in chilling sensitivity that has been associated with these factors. In peaches and nectarines, controlled atmosphere storage and intermittant warming, which reduce low-temperature-induced internal breakdown, also increased the ratio of unsaturated 18-carbon fatty acids and decreased the ratio of linoleic to linolenic acid (Wang and Anderson, 1981). Intermittent warming had a similar effect on cucumbers and peppers (Wang and Baker, 1979). Acclimation of cotton and snap bean seedlings at 12C for 1 or 2 days decreased chilling sensitivity and increased the percentage of unsaturated fatty acids in the phospholipids isolated from leaf tissue (Wilson and Crawford, 1974). Treatment of cucumber seedlings with paclobutrazol, however, imparted chilling tolerance without changing leaf membrane lipid composition (Whitaker and Wang, 1987). Murata and Yumaya (1984) have suggested that the degree of fatty acid saturation in specific classes of phospholipids, especially in the phosphytidyl glycerols, may be a better indicator of chilling sensitivity than total phospholipid fatty acids.

In 'Honey Dew' melons, the degree of unsaturated fatty acids from polar lipids isolated from the peel appears to be inversely related to chilling sensitivity of the melon. This supports the hypothesis that membrane properties may determine the chilling response of a plant tissue. To better understand the involvement of membranes in chilling sensitivity of 'Honey Dew', additional studies need to be done to determine how fatty acids are distributed among the different phospholipids and within different membrane systems of the cell. Identification of the mechanism controlling chilling sensitivity would lead to new methods to prevent CI during storage and marketing of 'Honey Dew' melons.

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