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CHANGES IN LIPIDS OF TOMATO FRUIT STORED AT CHILLING AND NON-CHILLING TEMPERATURES

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato fruit; free fatty acids; phospholipid; phytosterols; steryl lipids; chilling; postharvest.

Abstract—Mature green tomato fruits (*Lycopersicon esculentum*) were stored at non-chilling or chilling temperature (15 or 2°, respectively) for 2, 4, 8 or 12 days after harvest. Fruit stored at 15° had progressed to the pink stage of ripening after 12 days, whereas fruit stored at 2° had not ripened significantly. On a fresh weight basis, the phospholipid content of tomato pericarp tissue changed very little during storage at either 15 or 2°. Free fatty acids increased three-fold in fruit held at 15° and 1.2-fold in fruit held at 2°. Total sterol content increased during storage at both 15 and 2°. The increase in free sterols was greater in chilled compared to unchilled fruit, whereas the reverse was true for steryl esters. Chilling had no effect on the amounts of free and acylated steryl glycosides. The stigmasterol:sitosterol ratio in all steryl lipids increased dramatically with storage at 15°, but only slightly with storage at 2°.

INTRODUCTION

Tomato fruits are subject to chilling injury when stored at low but non-freezing temperatures in the range 0–10° [1, 2]. The two major consequences of chilling injury are failure to ripen properly and increased susceptibility to postharvest decay [3–5]. Membrane dysfunction (e.g. loss of semi-permeability or enzymatic activity) resulting from a change in the physical properties of cell membranes at low temperature is thought to be the primary event which leads to chilling injury [1, 6].

Tomato fruits are most susceptible to chilling injury at the mature green stage [7, 8]. Partial ripening of tomatoes and several other fruits of tropical or subtropical origin results in decreased sensitivity to chilling temperatures [2, 3, 9]. In a previous study, changes in membrane lipid content and composition during ripening of tomato fruits were examined. By far the greatest change with ripening of the pericarp tissue occurred among steryl lipids [10]. There were large increases in the amounts of free sterols (FS), steryl glycosides (SG) and particularly steryl esters (SE), and also a dramatic increase in the ratio of stigmasterol to sitosterol (the two predominant sterols) in all steryl lipids. The present study was undertaken to determine the effects of postharvest storage at chilling compared to non-chilling temperature on membrane lipid metabolism in mature green tomato fruits. Attention was focused on changes in steryl lipids, but total phospholipid (PL) and free fatty acid (FFA) concentrations were also monitored over 12 days of storage at either 15 or 2°.

RESULTS

Tomato fruits harvested at 36 days postanthesis (mature green) progressed to the pink stage of ripening when stored at the non-chilling temperature of 15° for 12 days, but fruit stored at the chilling temperature of 2° did

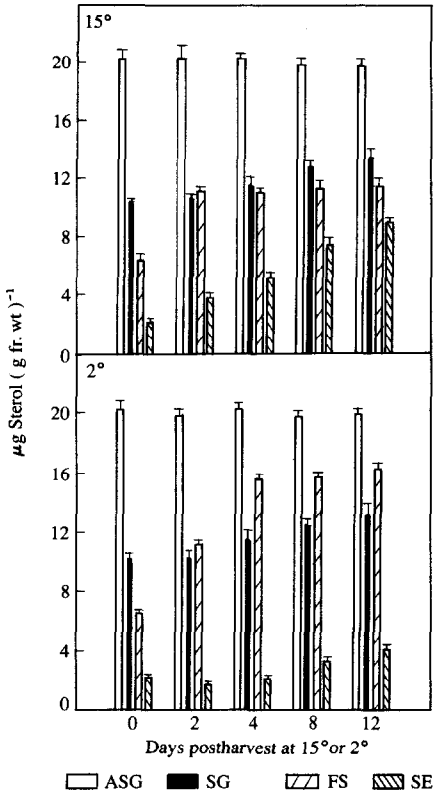

Fig. 1. Steryl lipid content of pericarp tissue from Rutgers tomato fruit harvested at 36 days postanthesis (mature green) and stored at either 15 or 2° for 0–12 days. Steryl lipids included acylated steryl glycosides (ASG), steryl glycosides (SG), free sterols (FS) and steryl esters (SE). Vertical bars represent one s.d. ($n \geq 4$).

not ripen appreciably. Changes in steryl lipid content of tomatoes stored at 15° for 12 days were similar to those previously reported for fruit ripened on the plant between 36 and 48 days postanthesis [10]; there were large increases in SE and FS, a moderate increase in SG and a slight decline in acylated steryl glycosides (ASG) (Fig. 1). The total steryl lipid content in fruit stored at 2° increased to a similar extent over 12 days, but the increase in SE was over three-fold less and the increase in FS two-fold more than those observed in fruit held at 15°. Regardless of storage temperature, there was a sharp rise in FS during the first two days postharvest. This rise quickly tapered off after two days at 15°, but continued through day four of storage at 2°.

Changes in sterol composition of steryl lipids from tomatoes stored at 15° for 12 days (Table 1) were also similar to those reported for fruit ripened on the plant between 36 and 48 days postanthesis [10]. There was a continuous increase in the stigmasterol:sitosterol ratio in each of the steryl lipids and this increase was most pronounced in the FS fraction (Table 2). The ratio of stigmasterol to sitosterol in steryl lipids of fruit held at 2° increased far less over 12 days of storage (Table 2), but the increase was proportionate in each steryl lipid fraction, that is, greatest in FS, intermediate is ASG and SG, and least in S.E.

On a fresh weight basis, the PL content of pericarp tissue from tomato fruits declined only slightly over 12 days of storage, regardless of storage temperature (data not shown). Thus, the ratio of steryl lipid to phospholipid increased during storage even when ripening was inhibited by low temperature. The mole ratios of FS to PL and of total membrane sterols (TMS = FS + SG + ASG) to PL in tomato fruit held at 15 or 2° for 0 to 12 days postharvest are shown in Table 3. After 12 days in storage, the FS:PL ratio had increased two-fold in fruit at 15° and 2.5-fold in fruit at 2°, the difference being due to the greater increase in FS content in the chilled fruit. In both chilled and unchilled fruit, the mole ratio of membrane steryl lipid to phospholipid (TMS:PL) increased from *ca* 1:4 to 1:3 over the 12 days of storage, much of this increase occurring during the first four days.

The FFA content in tomato fruit held at 15° rose gradually over the first four days of storage, increased sharply between four and eight days, then levelled off during the final four days (Fig. 2). Linoleic plus linolenic acid (18:2 + 18:3), which together composed the majority of the total FFA (*ca* 55–70%), showed the same trends. In fruit held at 2°, the FFA content declined over the first two days of storage, rose slightly between two and four days, increased more rapidly over four to eight days, then levelled off between eight and 12 days. In general, 18:2

Table 1. Sterol composition of steryl lipids from pericarp tissue of Rutgers tomato fruit harvested at 36 days postanthesis (mature green) and stored at either 15 or 2° for 0–12 days

	Days in storage at 15°					Days in storage at 2°				
	0	2	4	8	12	0	2	4	8	12
ASG										
Cholesterol	3.0	2.6	3.2	2.4	2.5	3.0	2.5	2.6	2.7	3.3
Campesterol	5.7	6.8	6.4	7.3	7.0	5.7	7.0	6.1	7.3	6.9
Stigmasterol	14.9	15.2	19.3	24.6	34.9	14.9	16.3	16.9	18.0	21.7
Sitosterol	60.2	59.8	57.2	52.2	40.9	60.2	59.6	59.6	57.3	53.1
Others	16.2	15.6	13.9	13.5	14.7	16.2	14.5	14.8	14.7	15.0
SG										
Cholesterol	4.5	2.8	2.5	1.8	1.8	4.5	2.8	2.8	2.3	2.5
Campesterol	6.1	6.8	6.1	6.1	5.4	6.1	7.0	6.3	7.0	6.2
Stigmasterol	14.1	14.3	16.3	26.9	38.7	14.1	13.0	14.2	14.5	21.2
Sitosterol	57.8	57.1	56.9	50.4	39.1	57.8	59.1	59.0	58.9	53.6
Others	17.5	19.0	18.2	14.8	15.0	17.5	18.1	17.7	17.3	16.5
FS										
Cholesterol	4.2	2.5	3.4	2.7	2.9	4.2	2.2	2.2	2.1	2.1
Campesterol	5.8	6.1	5.4	6.0	5.0	5.8	5.4	4.2	4.9	4.1
Stigmasterol	23.6	28.1	33.1	50.7	60.4	23.6	22.9	22.8	26.8	31.0
Sitosterol	50.8	48.5	45.3	26.8	18.1	50.8	55.2	54.8	48.4	44.2
Others	15.6	14.9	12.8	13.8	13.6	15.6	14.3	16.0	17.8	18.6
SE										
Cholesterol	14.9	8.0	5.5	3.0	3.7	14.9	12.7	13.0	6.2	7.0
Campesterol	7.6	6.5	5.7	6.6	6.6	7.6	6.7	5.3	6.5	5.9
Stigmasterol	5.0	6.3	7.1	12.0	23.7	5.0	6.0	6.6	6.8	8.2
Sitosterol	53.5	59.9	58.0	57.5	40.7	53.5	55.8	45.5	54.8	46.0
Others	19.0	19.3	23.7	20.9	25.3	19.0	18.8	29.6	25.7	32.9

Steryl lipids included acylated steryl glycosides (ASG), steryl glycosides (SG), free sterols (FS), and steryl esters (SE). Values represent the weight per cent of total sterol in each steryl lipid.
Standard deviations were generally less than 10% ($n \geq 5$).

Table 2. Changes in stigmasterol:sitosterol ratio in steryl lipids from pericarp tissue of Rutgers tomato fruit during storage at chilling (2°) and non-chilling (15°) temperatures

Steryl lipid	Storage temp.	Days postharvest 0	2	4	8	12
ASG	15°	0.25±0.01	0.25±0.01	0.34±0.02	0.47±0.02	0.85±0.03
	2°	0.25±0.01	0.27±0.01	0.28±0.03	0.31±0.02	0.41±0.01
SG	15°	0.24±0.01	0.25±0.01	0.29±0.01	0.53±0.02	0.99±0.05
	2°	0.24±0.01	0.22±0.01	0.24±0.01	0.25±0.01	0.40±0.01
FS	15°	0.46±0.02	0.58±0.02	0.73±0.03	1.89±0.09	3.34±0.10
	2°	0.46±0.02	0.42±0.02	0.42±0.02	0.55±0.04	0.70±0.03
SE	15°	0.09±0.02	0.11±0.01	0.12±0.02	0.21±0.04	0.58±0.03
	2°	0.09±0.02	0.11±0.01	0.13±0.03	0.14±0.03	0.17±0.02

Steryl lipids included acylated steryl glycosides (ASG), steryl glycosides (SG), free sterols (FS) and steryl esters (SE).

Values represent the mean ±s.d. ($n \geq 5$).

Table 3. Changes in the free sterol (FS) and total membrane sterol (TMS) to phospholipid (PL) mole ratios in pericarp tissue of Rutgers tomato fruit during storage at chilling (2°) and non-chilling (15°) temperatures

Storage temp.	Days postharvest 0	2	4	8	12
FS:PL mole ratio					
15°	0.044±0.002	0.078±0.001	0.078±0.002	0.082±0.003	0.081±0.002
2°	0.044±0.002	0.079±0.002	0.110±0.003	0.111±0.003	0.116±0.003
TMS:PL mole ratio					
15°	0.254±0.005	0.294±0.006	0.302±0.005	0.320±0.007	0.318±0.007
2°	0.254±0.005	0.291±0.007	0.335±0.008	0.338±0.008	0.353±0.012

TMS include free sterols, acylated steryl glycosides and steryl glycosides.

Values represent the mean ±s.d. ($n \geq 5$).

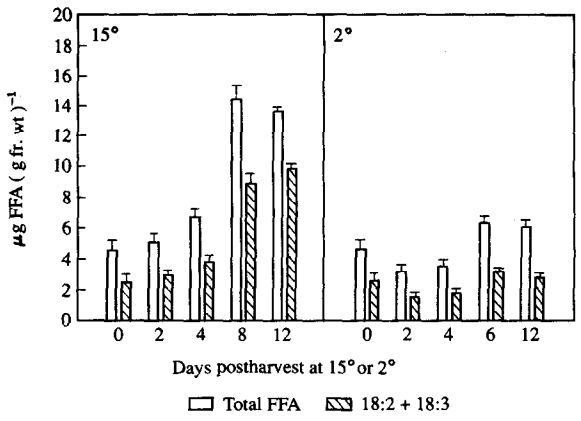

Fig. 2. Free fatty acid (FFA) content of pericarp tissue from Rutgers tomato fruit harvested at 36 days postanthesis (mature green) and stored at either 15 or 2° for 0–12 days. Values in $\mu g\,g^{-1}$ fr. wt are given for both total FFA and linoleic plus linolenic acid (18:2+18:3). Vertical bars represent one s.d. ($n \geq 4$).

+18:3 constituted a smaller portion of the total FFA (*ca* 50–60%) in chilled compared to unchilled fruit. At eight days postharvest, when FFA content peaked in both chilled and unchilled fruit, the total FFA content was over two-fold higher, and the amount of 18:2+18:3 three-fold higher, in fruit held at 15° compared to those held at 2°.

DISCUSSION

The steryl lipid content and composition of tomato pericarp tissue change dramatically with ripening [10, 14]. In this study, the PL content was found to decline by *ca* 5 to 10% by the pink stage of ripening (data not shown). There is a wide disparity in other reports on phospholipid changes associated with ripening of tomato, including: (i) very little change [15], (ii) a sharp increase through the turning stage with a rapid decline thereafter [16], (iii) a sharp decline between mature green and breaker [17] and (iv) a steady decline throughout [18]. These discrepancies lead to the conclusion that PL metabolism during ripening of tomato varies considerably from one cultivar to another [18]. Regardless of PL changes, the profound steryl lipid changes should have a

marked effect on the physical properties and hence the function of cell membranes [19]. A study by Legge *et al.* [20] has shown a large increase in the viscosity of plasma membranes with ripening of tomato fruit pericarp tissue. In the present study it was shown that the total steryl lipid content increased to the same extent in fruit stored at either 15 or 2° for 12 days, despite the fact that ripening was inhibited at the lower, chilling temperature.

This is surprising, particularly in light of the observation that the steryl lipid content of non-ripening mutant tomato fruits did not increase as it did in normal fruits between 30 and 52 days postanthesis [10]. However, a recent report has shown that by the mature green stage tomato fruits already contain all of the necessary precursor (mevalonic acid) for subsequent carotenoid and steryl lipid synthesis [21]. Thus, perhaps only the final steps of the isopentenoid biosynthetic pathway are required for sterol synthesis in fruit held at 2°.

After four days of storage, the steryl lipid composition of tomatoes held at 2° did differ significantly from that of tomatoes at 15°. Specifically, the FS content was higher while the SE content was lower in chilled fruit. This difference could be important in terms of membrane function, as FS are structural components of the plasmalemma and other cell membranes, whereas SE usually are not [22]. Perhaps of equal or greater importance, the stigmasterol:sitosterol ratio in each of the steryl lipids increased to a much greater extent in fruit stored at 15° compared to those stored at 2°. The difference in the side chain of these two predominant phytosterols (a double bond at Δ^{22} in stigmasterol) is thought to result in substantially different packing and fluidity of bilayer lipids [23].

In addition to changes in the steryl lipid and PL content of tomato fruit during storage at chilling and non-chilling temperatures, the present study included analyses of changes in FFA levels. A current hypothesis is that chilling injury is essentially an accelerated form of senescence in which the adverse effects of low temperature on lipid metabolism and membrane function elicit a cascade of deteriorative reactions, ultimately resulting in cell death [24]. The cleavage of membrane lipids by acyl hydrolases yielding FFA is thought to be one of the early events in senescence. Free 18:2 and 18:3 then serve as substrates for lipoxygenases, which generate lipid peroxides and free radicals [25]. The results of the present study provided no evidence that this mechanism plays a key role in the chilling injury of tomato fruit. There was no increased loss of PL in chilled compared to unchilled fruit. Also, the quantity of FFA, particularly 18:2 + 18:3, was consistently higher in unchilled fruit. One explanation for the latter observation is that the increase in FFA in fruit at 15° may be largely due to hydrolysis of thylakoid lipids during plastid transformation. At odds with this suggestion is the recent report by Nguyen and Mazliak [18] that galactolipid concentrations actually increase slightly during ripening. These authors also found a close correlation between galactolipid loss (particularly monogalactosyldiacylglycerol, MGDG) and the induction of chilling injury in pericarp tissue of tomato. Unfortunately, galactolipids were not analysed in this study, as they coeluted with the ASG and SG glycolipid column fractions and were lost in the course of saponification and recovery of steryl glycosides. However, preliminary data on crude chloroplasts isolated from pericarp tissue of mature green tomato fruit (cv 'Pik Red') stored for four or 12 days at either 15 or 2° support their finding that loss of galactolipids occurs with chilling. Preliminary results with pericarp from Rutgers fruit are less clear.

It should be noted that this study did not include an interval of post-chilling storage at ambient temperature, which is generally required before visual symptoms of chilling injury appear [26]. It is possible that membrane lipid degradation is greatly accelerated when chilled fruits are rewarmed. Studies of changes in lipid composition of subcellular membrane fractions, particularly the plasma membrane and chloroplasts, will be undertaken in the future to determine what roles steryl lipid synthesis and glycerolipid degradation play in chilling injury of tomato fruit.

EXPERIMENTAL

Plant material. Tomato plants (*L. esculentum* Mill.) cv Rutgers were grown in a greenhouse in 30 cm pots filled with a commercial peat moss/perlite-based potting mixt. Lighting consisted of natural daylight supplemented by rows of 400 W high-pressure Na lamps, which provided PAR of 400–500 μmol m^{-2} sec^{-1} for 12 hr per day. Flowers were tagged at anthesis and hand pollinated. Fruits of uniform size (103 $\pm$17 g fr. wt) were hand harvested at the mature green stage (36 days postanthesis). Assessment of maturity was based on visual inspection of the locular tissues, which generally showed some gel formation while the seeds remained firm and white. One group of five fruits was prepd for lipid analysis immediately after harvest. Additional groups of four or five fruits were stored at either 15 or 2° for 2, 4, 8 or 12 days after harvest. Fruits were quartered following excision of the stem scar region and locular tissue removed. Outer pericarp sections were blotted and immersed in liquid N_2. Frozen pericarp quarters from each fruit were fractured and stored at $-70°$ prior to lipid extraction. At least four tissue samples were analysed for each storage condition (temp. × duration).

Lipid extraction and fractionation. Frozen pericarp tissue samples (20 g fr. wt) were lyophilized overnight and homogenized in 50 ml of acidified (0.1% HOAc) $CHCl_3$–MeOH (2:1) with 3 × 15 sec bursts of a Polytron tissue homogenizer. FS (20 μg cholest-7-en-3β-ol) and FFA (50 μg *n*-heptadecanoic acid) int. standards were added to the homogenate which was filtered through a sintered glass funnel. Tissue residues were re-extracted with 40 ml of $CHCl_3$–MeOH and the combined extracts washed with 0.8% NaCl then MeOH–H_2O (1:1). The $CHCl_3$ phase containing total lipids was evapd under a stream of N_2 and lipids dissolved in 2 ml of $CHCl_3$ prior to silicic acid CC. Neutral lipids, including SE, FS and FFA, were eluted with $CHCl_3$–Me_2CO (20:1), ASG with $CHCl_3$–Me_2CO (2:1), SG with Me_2CO and PL with MeOH–H_2O (10:1). After drying under N_2, neutral lipids were dissolved in hexane and FFA extracted by partitioning with MeOH–H_2O (1:1) containing 25 mM Tris base (pH 9). FFA were recovered from the Tris buffer phase by hexane extraction after acidification with 6 M HCl. SE and FS were sepd by sequential elution from silica gel Sep-Pak with hexane–Et_2O (10:1) then hexane–Et_2O (2:1). SE were cleaved by alkaline saponification (1 M KOH in 80% EtOH), while ASG and SG were acid hydrolysed in dioxane plus 2 M TFA (1:1). Sterols freed by hydrolysis were recovered by hexane extraction after addition of 20 μg of cholest-7-en-3β-ol as int. standard.

Lipid analysis and quantification. FFA were converted to their Me esters (FAME) by heating at 70° for 10 min (sealed under N_2) with 12% (w/v) BF_3 in MeOH. FAME were extracted with

hexane, then sepd and quantified by FID GC as reported previously [11]. Total PL were quantified using the organic phosphate assay described in ref. [12]. Sterols derived from the four steryl lipid frs were pptd with digitonin, regenerated and quantified by FID GC using a 0.25 mm × 30 m SPB-1 fused silica capillary column [10, 13]. Quantification of steryl glycosides recovered following saponification of ASG and SG frs was also performed by FID GC after acetylation of the hexose moiety with Ac_2O as previously described [13].

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