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LIPID CHANGES IN MICROSOMES AND CRUDE PLASTID FRACTIONS DURING STORAGE OF TOMATO FRUITS AT CHILLING AND NONCHILLING TEMPERATURES

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Abstract—Mature-green tomato fruits (*Lycopersicon esculentum*) were stored for four or 12 days at chilling (2°) or nonchilling (15°) temperature. Fruits stored for 12 days at 15° ripened to the turning stage, whereas fruits at 2° did not ripen. Lipids of plastid and microsomal membrane fractions from pericarp tissue were analysed at harvest and after four or 12 days of storage. After 12 days at either 15° or 2°, the ratio of phospholipids (PL) to protein in microsomes declined, with a concomitant increase in the ratios of total membrane sterols (TMS) and cerebrosides (CB) to PL. The TMS:PL and CB:PL ratios also increased in crude plastid fractions. In both microsomes and plastids, free sterols (FS) increased more at 2° than at 15°, and hence accounted for a larger percentage of the TMS (FS + acylated steryl glycosides + steryl glycosides). The ratio of stigmasterol to sitosterol in all steryl lipids, but particularly in FS, increased more at 15° than at 2°. The unsaturation index of fatty acids in PL and galactolipids generally increased slightly during storage at both 15° and 2°. The ratio of phosphatidylethanolamine to phosphatidylcholine increased in both membrane fractions at both temperatures. In plastids, the ratio of mono- to digalactosyldiacylglycerols declined substantially at 2° but not at 15°.

INTRODUCTION

Tomato fruits are prone to the physiological disorder known as chilling injury when stored at low, nonfreezing temperatures in the range 0–10° [1–3]. The major consequences of chilling injury in tomato are failure to ripen properly and increased postharvest decay [4, 5]. Dysfunction of one or more cell membranes at chilling temperature is thought to be the primary event which ultimately leads to injury [6–8]. Studies of electrolyte leakage and other physiological parameters [4, 5, 8, 9] have indicated that chilling adversely affects the plasma membrane in pericarp tissue of tomato, whereas ultrastructural and biophysical studies [10, 11] have provided evidence that the tonoplast is also affected.

Tomato fruits are very susceptible to chilling injury at the mature green stage of development [8, 12], and partial ripening of tomatoes as well as other fruits of tropical or subtropical origin is reported to reduce their sensitivity to chilling temperatures [3, 4, 13]. Ultrastructural studies of pericarp tissue from mature green tomatoes have shown that the internal lamellae of chloroplasts become disorganized after seven to 10 days of chilling [11, 14]. In leaf chloroplasts from several wild and domestic tomato species, a correlation between galactolipase activity and chilling sensitivity has been demonstrated [15]. It has also recently been reported that selective loss of MGDG, the major galactolipid in thylakoid membranes, is associated with chilling injury of mature green tomato fruits [16]. Thus, disruption of chloroplast membranes during chilling may interfere with the transformation to chromoplasts after rewarming of the fruits [14].

Studies comparing the effects of chilling and ripening on the lipid content of tomato fruit pericarp have shown that loss of PL and increase in free fatty acids are relatively slight at chilling temperature [16, 17]. It was also found, in pericarp from Rutgers tomato fruit [17], that an increase in total sterol occurred during storage at both 'ripening' (nonchilling) and chilling temperatures (15° and 2°, respectively), but the composition and distribution of sterols in FS, SE, ASG and SG differed in

Abbreviations: ASG, acylated steryl glycoside; CB, cerebroside; DGDG, digalactosyldiacylglycerol; DPG, diphosphatidylglycerol; FS, free sterol; GL, galactolipid (DGDG + MGDG); LPC, lysophosphatidylcholine; LPE; lysophosphatidylethanolamine; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, phospholipids; SE, steryl ester; SG, steryl glycoside; SQDG, sulphoquinovosyldiacylglycerol; TMS, total membrane sterol (ASG + SG + FS).

partially ripened and chilled fruits after 12 days. The present study was undertaken to determine whether the lipid changes observed in whole pericarp tissue are also evident in two subcellular membrane fractions (plastids and microsomes), with the objective of identifying specific changes during chilling which could result in subsequent membrane dysfunction.

RESULTS

Total yields of lipids and protein or pigments from microsomal and crude plastid membrane fractions are shown in Tables 1 and 2, respectively. Values represent the recovery from *ca* 80 g fr. wt of outer pericarp tissue. Yields of protein and PL from microsomes declined with storage of fruits at either 15° or 2°, whereas yields of CB and total steryl lipids declined relatively little. GL (MGDG + DGDG) levels, indicative of the extent of contamination of microsomes with plastid membranes, also declined with storage of fruits at either temperature. Yields of chlorophyll (Chl) and carotenes from the crude plastid fractions were fairly constant, except for plastids from fruits stored at 15° for 12 days, where partial ripening (to the turning stage) was associated with a decrease in Chl and an increase in carotenes. PL recovery from crude plastids increased with storage at 2° but not 15°. The yields of CB and steryl lipids from this fraction increased sharply with storage at either temperature, but particularly at 2°. Recovery of glycoglycerolipids (MGDG + DGDG + SQDG) from the crude plastid fraction varied with the temperature and duration of storage. Amounts of both MGDG and DGDG declined after four days at 15°, but were partially restored after 12 days. In contrast, with storage at 2° recovery of MGDG declined continuously while DGDG did not change. The yield of SQDG declined with storage at both 15° and 2°.

The PL:protein ratio in microsomes was unchanged after four days, but had declined after 12 days at either 15° or 2° (Table 3). The ratio of TMS to PL increased to the same extent with storage at 15° and 2°, but the FS:PL ratio increased much more at 2°. The microsomal CB:PL ratio also increased with storage, somewhat more at 2° than at 15°. In the crude plastid fraction, both TMS:PL and CB:PL increased to approximately the same extent at 15° and 2°, whereas an increase in FS:PL was greater at 2° after 12 days. An increase in the Chl a:b ratio

Table 1. Lipid and protein content of microsomal membranes from pericarp tissue of tomato fruit harvested when mature green and stored at chilling (2°) or nonchilling (15°) temperature for 0, four or 12 days

Storage temp.		15°		2°	
Days in storage	0	4	12	4	12
Protein (mg)	3.40±0.37	2.73±0.29	2.28±0.25	3.14±0.35	2.54±0.33
PL (μmol)	2.68±0.33	2.11±0.27	1.51±0.26	2.56±0.26	1.43±0.27
CB (nmol)	141±37	136±23	106±23	148±28	136±26
ASG (nmol)	357±26	368±31	335±33	396±31	260±18
SG (nmol)	112±17	129±20	113±19	140±18	110±15
FS (nmol)	40±8	34±6	34±6	61±7	77±11
SE (nmol)	11±3	12±3	17±4	18±4	25±6
MGDG (nmol)	127±30	79±18	54±15	78±19	40±9
DGDG (nmol)	86±22	58±9	42±13	62±14	29±7

Values represent the mean±s.d. (n=6 to 8).

Table 2. Lipid and pigment content of crude plastid fraction from pericarp tissue of tomato fruit harvested when mature green and stored at chilling (2°) or nonchilling (15°) temperature for 0, four or 12 days

Storage temp.		15°		2°	
Days in storage	0	4	12	4	12
Chl (μg)	373±42	353±44	288±38	391±48	356±45
Carotenes (μg)	15.5±2.8	16.4±2.8	33.8±5.9	15.6±2.7	13.4±2.6
PL (μmol)	1.38±0.22	1.41±0.24	1.37±0.20	1.60±0.24	1.82±0.28
CB (nmol)	75±19	95±18	121±17	110±18	168±29
ASG (nmol)	268±21	315±28	438±35	384±27	487±39
SG (nmol)	54±13	83±14	93±16	67±12	77±16
FS (nmol)	37±7	43±8	65±8	46±6	107±14
SE (nmol)	12±3	15±3	29±5	14±3	23±5
MGDG (nmol)	834±78	646±58	700±53	720±58	650±53
DGDG (nmol)	488±45	383±38	417±36	491±41	509±41
SQDG (nmol)	186±24	141±24	121±22	150±22	133±19

Table 3. Lipid and pigment ratios in microsomal membranes and crude plastids from pericarp tissue of tomato fruit harvested when mature green and stored at chilling (2°) or nonchilling (15°) temperature for 0, four or 12 days. All are mole ratios, with the exceptions of PL:Protein and FS:Protein (μmol mg^{-1}) and Chl:Carotenes (mass ratio)

Storage temp.		15°		2°	
Days in storage	0	4	12	4	12
Microsomes					
PL:Protein	0.79±0.06	0.77±0.05	0.66±0.06	0.82±0.07	0.56±0.07
FS:Protein	0.012±0.002	0.012±0.002	0.015±0.003	0.019±0.003	0.030±0.005
FS:PL	0.015±0.003	0.016±0.003	0.023±0.004	0.023±0.004	0.054±0.008
TMS:PL	0.19±0.02	0.25±0.03	0.32±0.04	0.23±0.03	0.31±0.04
CB:PL	0.053±0.011	0.064±0.012	0.070±0.014	0.058±0.011	0.095±0.017
Crude plastids					
Chl a:b	3.08±0.05	3.18±0.08	3.28±0.13	3.07±0.03	3.06±0.07
Chl:Carotenes	24.1±3.7	21.5±3.6	8.5±2.6	25.1±3.0	26.6±4.8
PL:Chl	3.70±0.44	3.99±0.45	4.76±0.49	4.09±0.35	5.12±0.63
GL:Chl	3.54±0.36	2.92±0.32	3.88±0.43	3.10±0.32	3.26±0.34
GL:PL	0.96±0.12	0.73±0.10	0.82±0.09	0.76±0.09	0.64±0.08
FS:PL	0.027±0.005	0.030±0.004	0.047±0.006	0.029±0.004	0.059±0.009
TMS:PL	0.26±0.03	0.30±0.03	0.43±0.05	0.31±0.03	0.37±0.05
CB:PL	0.054±0.009	0.067±0.013	0.088±0.017	0.069±0.014	0.092±0.019

Values represent the mean±s.d. (n=6 to 8).

occurred with storage at 15° but not 2°, and this was correlated with a large decline in the ratio of Chl to carotenes after 12 days at 15°. With storage at 15°, both GL:Chl and GL:PL in the crude plastid fraction had decreased after four days, but increased between four and 12 days. With storage at 2°, GL:Chl decreased slightly whereas GL:PL decreased sharply (largely because of an increase in PL; Table 2).

Changes in the sterol composition of steryl lipids in microsomes from cv 'Pik Red' tomato fruit after storage at 15° or 2° (Table 4) were similar to those previously observed for steryl lipids from pericarp tissue of cv 'Rutgers' fruit [17]. During storage the stigmasterol:sitosterol ratio increased in all four steryl lipids. The increase was greatest in FS, and it was consistently greater in microsomal steryl lipids from fruits stored at 15° vs 2°. There was also a dramatic decrease at both storage temperatures in the proportion of cholesterol in the SE and FS fractions, possibly via dilution. These same trends were noted for steryl lipids from the crude chloroplast fraction (data not shown).

Changes in the PL composition of microsomes and crude plastids were similar with storage at 15° and 2° (Table 5). A decrease in the PC:PE ratio occurred at both temperatures in both fractions, and this change was accompanied by a small increase in LPC. The proportion of PA was consistently greater in the crude plastid compared to the microsomal fraction; PA had increased in both fractions after 12 days at 15° but not 2°. There were several clear indications from the data in Table 5 that the crude plastid fractions were heavily contaminated with other cell membranes. The very large proportion of PE and a PC:PE ratio lower than that in the microsomes indicate the presence of much nonplastid membrane [18]. Also, the small amount of DPG (cardiolipin) shows that there was some mitochondrial contamination. Based on the percentage of PG, the major chloroplast PL [19], it is estimated that ≧80% of the total PL in this fraction was of extraplastidic origin.

On the day of harvest (day 0), the fatty acid compositions of PC and PE were quite similar in both membrane fractions, with 18:2 and 16:0 predominant (Table 6). By comparison, the fatty acids in ASG were highly saturated (>70% 16:0+18:0+20:0). PC, PE and ASG in microsomes and crude plastids differed by only slightly more fatty acid unsaturation in the latter fraction. The fatty acid compositions of MGDG, DGDG and SQDG from the crude plastid fraction were typical for these glycerolipids [20]. MGDG, the major GL, was highly unsaturated (*ca* 70% 18:3), and the order of unsaturation was MGDG > DGDG > SQDG. With storage of fruits at 15°, there were slight increases in the unsaturation index [U.I. = %18:1+2(%18:2)+3(%18:3)/100] of PC, PE and ASG in both membrane fractions (Table 7). For the glycerolipids MGDG, DGDG and SQDG in crude plastids, however, there was no change or a slight decline in U.I. at 15°. With storage at 2°, the increase in U.I. of PC and PE was smaller than that which occurred at 15° or nonexistent, whereas the U.I. of ASG increased as much or more than it did at 15°. In the crude plastid fraction, there was no change or a very slight increase in the U.I. of MGDG, DGDG and SQDG with storage at 2°.

Over the 12 days of storage at 15°, during which time fruits ripened from mature green to the turning stage, there were only modest changes in the distribution of total sterol among ASG, SG, FS and SE in both membrane fractions, namely, a small drop in ASG offset by slight increases in SG and SE (Table 8). In contrast, in

Table 4. Sterol composition of steryl lipids in microsomal membranes from pericarp tissue of tomato fruit harvested when mature green and stored at chilling (2°) or nonchilling (15°) temperature for 0, four or 12 days

Storage temp.		15°		2°	
Days in storage	0	4	12	4	12
ASG					
Cholesterol	4.3±0.3	4.0±0.2	4.0±0.3	4.0±0.3	4.2±0.3
Campesterol	7.3±0.5	7.9±0.3	8.1±0.2	7.8±0.5	7.9±0.6
Stigmasterol	16.6±1.6	20.1±1.4	21.8±1.8	17.9±1.6	18.7±1.8
Sitosterol	58.8±1.9	57.7±1.8	56.3±2.1	59.8±2.4	59.5±2.6
Isofucosterol	4.0±0.3	4.1±0.3	4.1±0.3	4.2±0.3	4.2±0.3
Others	9.0±1.2	6.2±0.7	5.7±0.5	6.3±1.1	5.4±1.0
SG					
Cholesterol	4.9±0.2	4.4±0.3	4.2±0.3	4.4±0.4	4.6±0.4
Campesterol	7.4±0.3	7.7±0.5	7.5±0.4	8.0±0.5	8.1±0.2
Stigmasterol	15.2±1.8	17.7±1.5	21.1±1.6	15.1±0.9	16.6±1.7
Sitosterol	61.3±1.0	58.9±1.7	57.0±2.0	60.9±1.8	60.3±1.0
Isofucosterol	5.1±0.4	5.1±0.4	4.6±0.3	4.9±0.5	3.6±0.4
Others	6.1±0.9	6.2±0.9	5.6±0.8	6.7±0.7	6.8±0.7
FS					
Cholesterol	17.4±1.4	12.7±1.2	11.8±1.3	7.4±0.8	6.1±0.7
Campesterol	7.1±0.5	6.9±0.4	7.0±0.6	6.0±0.3	5.7±0.3
Stigmasterol	19.3±1.0	26.3±2.1	30.1±2.4	22.8±1.5	28.2±1.9
Sitosterol	41.7±1.3	39.9±2.3	35.0±2.6	48.8±1.9	47.5±2.5
Isofucosterol	6.1±0.6	6.3±0.7	7.5±0.5	9.5±1.0	8.9±0.9
Others	8.4±1.1	7.9±1.0	8.6±0.9	5.5±0.7	3.6±0.6
SE					
Cholesterol	48.6±3.2	10.9±0.8	7.9±0.5	11.8±1.3	6.8±0.3
Campesterol	9.9±1.1	9.5±0.7	8.7±0.5	10.1±0.8	10.4±0.2
Stigmasterol	4.4±0.2	6.7±0.8	9.8±0.7	4.6±0.5	5.5±0.8
Sitosterol	23.5±2.3	56.3±2.2	55.6±1.8	57.4±2.3	60.5±2.2
Isofucosterol	6.2±0.7	7.4±0.5	8.0±0.4	6.6±0.4	6.9±0.5
Others	7.4±1.0	9.2±1.1	10.0±1.1	9.5±0.9	9.8±1.1

Values represent the mean±s.d. ($n=6$ to 8).

Table 5. Phospholipid composition of microsomal membrane and crude plastid fractions from pericarp tissue of tomato fruit harvested when mature green and stored at chilling (2°) or nonchilling (15°) temperature for 0, four or 12 days

Storage temp.		15°		2°	
Days in storage	0	4	12	4	12
Microsomal PL					
PC	53.7±2.1	49.8±1.8	46.8±2.4	49.9±2.4	48.8±2.3
PE	31.0±0.7	33.2±0.9	34.1±0.8	32.1±2.7	32.5±2.5
PI	6.8±0.8	6.6±0.6	6.7±1.0	7.6±1.1	7.5±1.5
PG	2.6±0.4	2.9±0.5	2.5±0.4	3.5±0.3	3.1±0.5
PA	1.8±0.4	2.8±0.7	4.3±1.2	2.0±0.5	1.9±0.4
LPE	2.4±0.2	2.5±0.4	2.6±0.3	2.2±0.4	2.6±0.3
LPC	1.7±0.4	2.2±0.3	3.0±0.4	2.7±0.5	3.3±0.7
Plastid PL					
PC	40.2±2.0	39.5±2.2	34.2±2.1	40.5±2.1	36.6±2.0
PE	27.1±1.9	28.2±1.9	31.0±1.8	27.2±1.6	30.3±1.7
PI	8.2±1.5	8.6±1.3	9.9±1.6	7.9±1.3	8.0±1.2
PG	12.8±2.2	10.9±2.0	10.2±2.5	11.9±1.8	10.3±2.1
PA	5.7±1.0	6.3±0.8	8.1±1.5	5.8±0.7	6.4±0.8
LPE	2.8±0.3	2.8±0.3	3.0±0.4	2.7±0.3	3.2±0.3
LPC	1.4±0.3	1.7±0.4	2.1±0.3	2.0±0.4	2.8±0.6
DPG	1.7±0.3	2.0±0.3	1.5±0.3	2.0±0.3	2.3±0.4

Values represent the mean±s.d. ($n=6$ to 8).

Table 6. Fatty acid composition of PC, PE and ASG in microsomal membranes and of PC, PE, ASG, MGDG, DGDG and SQDG in the crude plastid fraction from pericarp tissue of mature green tomato fruit on the day of harvest (Day 0)

	Microsomal membranes		
Fatty acid	PC	PE	ASG
16:0	32.7±1.9	29.9±2.2	69.9±1.4
18:0	4.0±0.3	3.8±0.3	8.1±0.4
18:1	4.2±0.5	2.0±0.3	1.8±0.3
18:2	49.2±1.6	49.9±2.3	13.9±1.1
18:3	8.7±0.6	7.3±0.5	2.8±0.3
20:0	1.1±0.2	1.7±0.3	3.3±40.5

	Crude plastid fraction		
Fatty acid	PC	PE	ASG
16:0	29.9±2.2	27.8±2.3	61.1±2.5
18:0	4.2±0.5	4.1±0.5	7.3±0.4
18:1	4.2±0.4	2.0±0.4	1.9±0.4
18:2	49.1±2.4	56.4±2.8	19.4±1.3
18:3	11.1±1.3	7.3±0.9	7.8±1.4
20:0	1.4±0.3	2.3±0.4	2.4±0.3

Fatty acid	MGDG	DGDG	SQDG
16:0	5.7±0.4	18.5±1.9	36.7±1.8
18:0	1.0±0.1	4.6±0.3	5.6±0.4
18:1	0.7±0.2	0.8±0.2	3.6±0.3
18:2	23.5±2.3	20.0±2.1	33.6±1.1
18:3	69.0±2.6	56.0±2.4	17.8±1.7
20:0	N.D.	N.D.	2.6±0.3

Values represent the mean±s.d. ($n=6$ to 8).

Table 7. Unsaturation Index (U.I.) of fatty acyl lipids in microsomal and crude plastid fractions from pericarp tissue of tomato fruit harvested when mature green and stored at chilling (2°) or nonchilling (15°) temperature for 0, four or 12 days

Storage temp.		15°		2°	
Days in storage	0	4	12	4	12
Microsomes					
PC	1.29	1.35	1.42	1.31	1.33
PE	1.24	1.35	1.40	1.28	1.31
ASG	0.38	0.39	0.41	0.46	0.48
Crude Plastids					
PC	1.36	1.40	1.41	1.39	1.37
PE	1.37	1.43	1.44	1.36	1.37
ASG	0.64	0.65	0.67	0.67	0.67
MGDG	2.55	2.63	2.56	2.64	2.63
DGDG	2.09	2.07	2.04	2.09	2.13
SQDG	1.24	1.24	1.15	1.25	1.26

U.I. equals the sum of the mol% of each unsaturated fatty acid multiplied by its number of double bonds and divided by 100.

Table 8. Distribution of steryl lipids (ASG, SG, FS and SE) in microsomes and of steryl and glycoglycerolipids (MGDG, DGDG and SQDG) in crude plastids from pericarp tissue of tomato fruit harvested when mature green and stored at chilling (2°) or nonchilling (15°) temperature for 0, four or 12 days

Storage temp.		15°		2°	
Days in storage	0	4	12	4	12
Microsomes					
Steryl lipid					
ASG	69	68	67	64	55
SG	22	24	23	23	24
FS	7	6	7	10	17
SE	2	2	3	3	4
Crude plastids					
Steryl lipid					
ASG	72	69	70	75	70
SG	15	18	15	13	11
FS	10	9	10	9	15
SE	3	4	5	3	3
Glycoglycerolipid					
MGDG	55	55	56	53	50
DGDG	32	33	34	36	39
SQDG	12	12	10	11	10

Values represent the mol% of total steryl or glycoglycerolipids in each membrane fraction.

microsomes from fruits stored at 2° for 12 days, there was a relatively large drop in the proportion of ASG which was offset principally by an increase in FS, and to a lesser extent by increases in SG and SE. The proportion of FS also increased in steryl lipids from the crude plastid fraction with storage at 2°. The proportions of the glycoglycerolipids MGDG, DGDG and SQDG in crude plastids changed very little with storage of fruits at 15°; there were slight increases in GL at the expense of SQDG (Table 8). With storage at 2°, decreases in the proportions of MGDG and SQDG were offset by an increase in DGDG. Over 12 days at 2° the MGDG:DGDG ratio in crude plastids dropped from 1.71 to 1.28, whereas little change occurred at 15°.

DISCUSSION

In previous studies of lipid changes in tomato fruit during ripening and chilling [16, 17, 21] analyses were performed on lipids extracted from sections of outer pericarp tissue. This study was undertaken to determine how the changes noted for the whole tissue are reflected in two subcellular membrane fractions. The data in Table 1 show a large reduction in the yield of PL and protein from microsomes after storage of fruits at either 15° or 2°. These data are subject to two interpretations: (i) there were in fact large losses of PL and protein from the membranes comprised in the microsomal fraction; or (ii) the yield/recovery of microsomes per unit fresh weight of tissue was relatively poor after storage of fruits (possibly

as a result of changes in membrane agglutination or sedimentation, or of less thorough disruption of the tissue with the mortar and pestle). In the case of fruits stored at 2°, the second interpretation is supported by the fact that PL loss in pericarp tissue of mature green fruits stored at 4° or 2° was found to be slight [16, 17], and by the fact that an increase in PL in the crude plastid fraction coincided with the decrease in microsomal PL (Tables 1 and 2).

The changes in PL composition in both membrane fractions with storage at either 15° or 2° may reflect the catabolism of PC. Specifically, the decrease in the PC:PE ratio coincident with the modest increase in LPC could result from the breakdown of PC by an A-type phospholipase. In addition, the increase in PA in both membrane fractions with storage of fruits at 15° indicates the probable involvement of phospholipase D in PC catabolism during ripening. This conclusion is supported by the observation that the level of PA invariably increases with ripening of tomato pericarp tissue (Whitaker, unpublished).

Changes in steryl lipid content and composition associated with ripening and chilling of tomato fruit were not as dramatic in microsomal membranes from pericarp of cv 'Pik-Red' as they were in total pericarp lipids from cv 'Rutgers' [17]. However, the same trends were noted; the proportion of FS relative to ASG, SG and SE increased much more at 2° than at 15°, whereas an increase in the ratio of stigmasterol:sitosterol, particularly in FS, was much more pronounced at 15° than at 2°. Metabolic studies have shown that ASG, SG, FS and SE can be rapidly interconverted in plant tissues [22, 23]. The increase in the proportion of FS with chilling of tomato fruits could result from decreased rates of glycosylation and esterification or from increased rates of deglycosylation and de-esterification. Concerning the changes in sterol composition during storage, it appears that with the advent of ripening at 15°, stigmasterol synthesis is greatly favoured and sitosterol synthesis all but ceases. In contrast, during storage at 2° and in the absence of ripening, synthesis of sitosterol continues, albeit at a reduced rate. Despite the minor structural difference between these two C_{29} phytosterols (a double bond in the alkyl side chain of stigmasterol) a change in the stigmasterol:sitosterol ratio may significantly alter the physical properties of plant cell membranes [24, 25].

The fatty acid composition of individual PL and glycolipids in microsomes and crude plastids changed very little during storage at either 15° or 2°, and the slight increase in the unsaturation index (Table 7) may represent a weak acclimation response [26]. However, total fatty acid data do not provide information about rearrangement or selective degradation of glycerolipid molecular species, which could play a role in altering membrane structure and function during ripening and chilling [26, 27]. The recent report of lipolytic acyl hydrolase and lipoxygenase activities associated with microsomal membranes from mature green tomato fruit [27] suggests that these enzymes could be involved in PL loss and fatty acid peroxidation during ripening and senescence.

Despite the obvious contamination of the crude chloroplast fractions with other cell membranes, data on the chloroplast-specific glycoglycerolipids (MGDG, DGDG and SQDG) give some insight into the changes in plastid membranes associated with ripening and chilling of tomato fruits. Changes in fatty acid composition and unsaturation at either storage temperature were surprisingly small. The apparent transient drop in the level of GL after four days at 15°, with recovery by 12 days, could reflect a reorganization of plastid lamellae with the onset of ripening and plastid transformation [28]. The most striking change in the chloroplast glycerolipids during postharvest storage was a sharp decline in the MGDG:DGDG ratio, which occurred at 2° but not at 15°. These data are in accord with a recent report that selective loss of MGDG, the major lipid in thylakoids, is specifically associated with chilling injury in pericarp tissue from mature green fruits of cv 'Capello' [16]. Loss of MGDG could be involved in the disorganization of chloroplast lamellae observed in ultrastructural studies of chill-injured tomato fruit [7, 14]. However, it should be noted that in pericarp tissue of cv. 'Rutgers' fruit, the decline in MGDG:DGDG after 12 days of chilling at 2° was relatively slight, whereas a significantly greater drop in MGDG:DGDG occurred during 12 days of ripening at 15° [21]. It would be of interest to compare both the effects of chilling on chloroplast ultrastructure and the symptoms of chilling injury in fruits of cvs 'Pik-Red' and 'Rutgers' grown under identical conditions.

EXPERIMENTAL

Plant material. Tomato plants (*L. esculentum* Mill.) cv 'Pik Red' 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. Fruits of uniform size (124 $\pm$ 15 g fr. wt) were hand-harvested at the mature green stage. Maturity was assessed by visual inspection of external colour and of the locular tissues, which generally showed some gel formation while the seeds remained firm and white. All harvests were made over a four-week period between mid-April and mid-May both in 1990 and 1991. After harvest, fruits were washed and blotted dry. One group was processed immediately, and additional groups were stored at 15° or 2° for either 4 or 12 days.

Isolation of plastids and microsomes. Outer pericarp tissue from two fruits was pooled for each isolation. The stem scar region and a 2 cm diam. disc at the blossom end of each fruit were excised, the fruits quartered and the locular tissue removed. Pericarp sections were blotted and 79 $\pm$ 5 g fr. wt were diced and immersed in 150 ml of cold homogenization medium (0.33 M D-sorbitol, 0.1 M tricine, 1 mM β-mercaptoethanol, 1 mM Na_4EDTA, pH 7.8 with KOH). After 15 min equilibration, the tissue was homogenized with a mortar and pestle, the homogenate filtered and crude plastid and microsomal membrane fractions isolated from the filtrate by differential centrifu-

gation as previously described for pericarp from bell pepper fruit [29]. The 2000 *g* pellet containing crude plastids was washed once in homogenization medium and once in 20 mM HEPES buffer, pH 8 prior to extraction. Microsomal membranes were recovered from the 13 000 *g* supernatant by centrifugation at 90 000 *g* for 30 min. The microsomes were resuspended in 8–10 ml of 20 mM HEPES buffer, pH 8, prior to extraction and two 50 μl aliquots were used for protein determination by the Bradford method [30]. A total of six to eight membrane isolations were performed with fruits from each of the five storage conditions (0 day, 4 days at 15° or 2°, and 12 days at 15° or 2°); three to four in 1990 and another three to four in 1991.

Lipid extraction, fractionation and analysis. Washed crude plastid pellets were extracted with boiling iso-PrOH then with $CHCl_3$–MeOH (2:1) and the microsomal membrane suspensions extracted with $CHCl_3$–MeOH (2:1), as previously described [29]. As an internal FS standard, 10 μg of lathosterol (cholest-7-en-3β-ol) was added to the total lipid extracts (TLE). After washing with 0.8% NaCl, then with MeOH–H_2O (1:1), $CHCl_3$ was evapd from the TLE under a stream of N_2 and lipids redissolved in 2 ml $CHCl_3$. A 25 μl aliquot of the plastid TLE was used for determination of chlorophyll content [31]; two 25 μl aliquots were withdrawn from both plastid and microsomal TLE for determination of total PL [32]. TLE were sepd into neutral lipid (NL), glycolipid (GlcL) and PL frs by CC; individual GlcL and PL were then isolated by TLC as described in ref. [29]. NL were dissolved in hexane, SE and FS sepd by sequential elution from silica Sep-Paks [33] and total carotenes in the SE fr. measured spectrophotometrically (A_{max} *ca* 449 nm) [34]. FS and FS derived from SE, ASG and SG by alkaline or acid hydrolysis were pptd with digitonin and analysed by FID GC using a 0.25 mm × 30 m SPB-1 fused silica capillary column [33]. FS and FS from SE were quantified by FID GC, whereas ASG, SG and CB were quantified using both the phenol–H_2SO_4 assay for sugars and HPLC with UV detection at 205 nm as described in ref. [35]. Individual PL were quantified by the method of Ames [32], and MGDG, DGDG SQDG were quantified as described in ref. [29]. Fatty acids of PL, MGDG, DGDG, SQDG and ASG were converted to their Me esters (FAME) with 14% (w/v) BF_3 in MeOH [33]. FAME were analysed by FID GC using a 0.25 mm × 15 m SP-2330 fused silica capillary column.

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