Phytochemistry, Vol 31, No. 8, pp 2627–2630, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00
Pergamon Press Ltd

CHANGES IN GALACTOLIPID AND PHOSPHOLIPID LEVELS OF TOMATO FRUITS STORED AT CHILLING AND NONCHILLING TEMPERATURES

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(*Received* 19 *November* 1991)

Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato fruit; chilling; galactolipids; phospholipids; plastid membranes; postharvest; ripening.

Abstract—Mature-green stage two (MG2) and mature-green four to breaker-stage (MG4/BK) tomato (*Lycopersicon esculentum*) fruits (harvested 30 and 36 days postanthesis, respectively) were stored at chilling (2°) or nonchilling (15°) temperature for zero, four or 12 days. Lipids extracted from pericarp tissue were analysed for mono- and digalactosyldiacylglycerols (MGDG and DGDG) and total phospholipids (PL). Results were similar for MG2 and MG4/BK fruits. At 2°, PL increased ≈ 7–11% during the first four days in storage, declining slightly by day 12. In contrast, PL declined ≈ 10–15% during storage at 15°, mostly over the first four days. Total galactolipids (GL) declined at both 2° and 15°, but losses were greater (≈ 25–35%) at 15°. Loss of GL at 15° was more rapid for MG4/BK compared with MG2 fruits, reflecting the more advanced stages of ripening after four and 12 days of storage. The ratio of MGDG to DGDG had dropped after 12 days at either 2° or 15°. The decline was greatest (from ≈ 1.8:1 to 1.2:1) in MG4/BK fruit stored at 15°, which had ripened to the pink stage. These results conflict with a recent report that loss of MGDG is associated with chilling, but not with ripening, of tomato fruits.

INTRODUCTION

Tomato fruits are prone to the physiological disorder known as chilling injury when exposed to low but non-freezing temperatures in the range of 0 to 10° for a week or more [1–3]. The two main consequences of chilling injury are the failure to ripen properly and increased susceptibility to postharvest decay [4–6]. Dysfunction of one or more cell membranes (loss of semipermeability or enzymatic activity) at chilling temperature is thought to be the primary event which ultimately leads to injury [7–9]. Tomato fruits are particularly susceptible to chilling injury at the mature-green stage of development [9, 10], and partial ripening of tomatoes as well as other fruits of tropical or subtropical origin reduces their sensitivity to chilling temperatures [3, 4, 11]. Ultrastructural studies of pericarp tissue from mature-green tomatoes have shown that disorganization of chloroplast internal lamellae occurs after seven to 10 days of chilling [8, 12]. This finding supports the conclusion that disruption of chloroplast membranes during chilling interferes with the transformation to chromoplasts after rewarming of the fruits [12]. In accord with this hypothesis, a recent report indicated that selective loss of monogalactosyldiacylglycerol (MGDG), the major glycerolipid in thylakoid membranes, is associated with chilling injury of mature-green and breaker-stage tomato fruits [13]. In our previous study of lipid changes during chilling of 'Rutgers' tomato fruits, steryl lipids and phospholipids were analysed but galactolipids were not [14]. This work was undertaken primarily to determine whether loss of MGDG is specifically induced by chilling in fruits of the 'Rutgers' cultivar.

RESULTS

The levels of total phospholipids (PL) and galactolipids (GL) and of MGDG and digalactosyldiacylglycerols (DGDG) in pericarp tissue of mature-green stage two (MG2) and mature-green four to breaker-stage (MG4/BK) fruits on the day of harvest are shown in Table 1. Recovery of these glycerolipids was reproducible; the s.d. for sets of six to eight samples did not exceed 10%. The PL content, and to a lesser extent the GL content, was lower in MG4/BK compared with that of MG2 pericarp. This decline in glycerolipid content with increasing maturity of the fruit was slightly greater on a dry wt than on a fr. wt basis.

On both a dry and fr. wt basis, the PL content of pericarp tissue from both MG2 and MG4/BK fruits had increased by 7–11% after four days at 2° (Table 2). After 12 days at 2°, the pericarp PL content was still 4–7% greater than that in control fruit at harvest. In contrast, after four days at 15° the PL content of pericarp from MG2 and MG4/BK fruit had decreased by 7–8% and 13–17%, respectively (Table 2). Storage at 15° for 12 days resulted in a slight additional decline (minus 2–3%) in the PL content of MG2 fruit, whereas in MG4/BK fruit there was a partial restoration of the PL content (plus 7–10%) between the fourth and 12th day of storage.

After four days of storage, the GL level in pericarp tissue of MG2 fruit had declined by 10–13% at 2° and by 4–6% at 15° (Table 2). However, while there was a partial recovery in the GL content of MG2 fruit between the fourth and 12th day at 2°, there was an additional 19–21% drop in GL content during this storage interval

at 15°. The GL content in pericarp of MG4/BK fruit had declined by 13–14% at 2° and by 18–21% at 15° after four days of storage (Table 2). Between the fourth and 12th day of storage, the GL content in MG4/BK fruit dropped by only another 1–4% at 2° compared with an additional 14–16% at 15°.

For both MG2 and MG4/BK fruits, storage at 2° resulted in a sharp increase in the PL:GL ratio over the first four days, with little or no change over the next eight days (Table 3). In contrast, during storage at 15° there was little change in the PL:GL ratio after four days, but after 12 days it was higher than that in comparable fruit stored at 2°. There was little or no change in the MGDG:DGDG ratio in pericarp of MG2 and MG4/BK fruits after four days at 2°, but after 12 days a moderate decline was evident (Table 3). In MG2 fruits stored at 15°, the MGDG:DGDG ratio increased slightly over the first four days then declined over the next eight days to about the same extent as that in fruits at 2°. The MGDG:DGDG ratio in pericarp of MG4/BK fruits at 15° had decreased slightly after four days but dramatically after 12 days of storage.

Table 1. Total PL, total GL, MGDG and DGDG content of pericarp tissue from 'Rutgers' tomato fruit on the day of harvest (day 0)

Maturity at harvest	Lipid	Lipid content (g^{-1} dry wt)	Lipid content ($10\ g^{-1}$ fr. wt)
MG2	PL	9.11±0.35	4.12±0.23
	GL	2.36±0.17	1.07±0.10
	MGDG	1.53±0.12	0.69±0.07
	DGDG	0.83±0.07	0.38±0.04
MG4/BK	PL	7.46±0.33	3.74±0.08
	GL	2.08±0.08	1.04±0.07
	MGDG	1.33±0.06	0.67±0.04
	DGDG	0.75±0.03	0.37±0.02

Fruits were harvested at the MG2 or MG4/BK stage of maturity (30 or 36 days postanthesis, respectively). Values indicate lipid content in μmol±s.d. ($n=6$ to 8) on either a dry or fr. wt basis.

DISCUSSION

A recent report showed a close correlation between the duration of chilling (at 4°) and loss of the chloroplast lipid MGDG in pericarp tissue of MG and BK 'Capello' tomato fruit [13]. The results of the present study with fruit of cv 'Rutgers' differ from those of this cultivar, and do not support the conclusion that loss of MGDG is a specific consequence of chilling in tomato fruits. Although there were differences in methodology between the two studies (notably the temperatures of storage and duration of chilling), there is an adequate basis for comparison of major similarities and differences in the results obtained. When the effects of chilling on pericarp GL are considered alone, the data for MG4/BK 'Rutgers' fruit and MG 'Capello' fruit are quite similar; over 12 days total GL declined ≈17%, with a corresponding moderate drop in the MGDG:DGDG ratio. However, the effects of ripening on GL content were completely opposite in the two studies. Over 12 days at 12 or 20°, total GL and the MGDG:DGDG ratio increased with ripening of 'Capello' fruit, whereas after 12 days of ripening at 15°, total GL in 'Rutgers' fruit had declined by 25–35%, with a large decline in the MGDG:DGDG ratio. In both MG 'Capello' and 'Rutgers' fruit, total PL had increased 5–10% after 12 days of chilling, suggesting an attempted acclimation to low temperature. A decrease in PL with ripening was also noted for both cultivars, but the loss of PL was far more rapid and extensive in 'Capello' fruit.

Chilling sensitivity and postharvest ripening in tomatoes are dependent upon fruit maturity at harvest as well as various preharvest factors [9, 10, 15, 16]. The different results obtained in the present study may be due in part to these factors, although greenhouse-grown fruit of roughly comparable ripening stages were used. Chilling sensitivity and ripening characteristics also vary considerably among different tomato cultivars [17–19]. Fruit of cv 'Rutgers' ripen rapidly with extensive softening [18] and show

Table 2. Percentage changes in PL and GL contents of pericarp tissues from 'Rutgers' tomato fruits during storage for four or 12 days at chilling (2°) or nonchilling (15°) temperature

Maturity at harvest	Storage		Lipid content: PL		Lipid content: GL	
	Temp.	Time	1 g dry wt	10 g fr. wt	1 g dry wt	10 g fr. wt
MG2	2°	4 days	108±3	111±4	87±2	90±3
		12 days	107±4	106±9	99±11	96±8
	15°	4 days	93±4	92±8	96±4	94±7
		12 days	91±8	89±6	75±8	73±7
MG4/BK	2°	4 days	109±6	107±6	87±5	86±5
		12 days	104±5	106±6	83±7	85±7
	15°	4 days	87±3	83±3	82±4	79±8
		12 days	94±5	93±9	66±8	65±7

Fruits were harvested at the MG2 or MG4/BK stage of maturity (30 or 36 days postanthesis, respectively). Values indicate percentages of PL and GL contents in 0 day controls (see Table 1). ±s.d. ($n=6$ to 8) on a dry or fr. wt basis.

Table 3. PL:GL and MGDG:DGDG ratios in pericarp tissues from 'Rutgers' tomato fruits stored at chilling (2°) or nonchilling (15°) temperature for 0, 4 or 12 days

Maturity at harvest	Storage Temp.	Time	Lipid ratio PL:GL	MGDG:DGDG
MG2	—	0 days	3.88±0.36	1.84±0.10
	2°	4 days	4.76±0.21	1.95±0.05
		12 days	4.22±0.36	1.60±0.06
	15°	4 days	3.74±0.13	2.09±0.06
		12 days	4.78±0.58	1.55±0.07
MG4/BK	—	0 days	3.60±0.15	1.79±0.07
	2°	4 days	4.53±0.48	1.79±0.06
		12 days	4.53±0.32	1.55±0.08
	15°	4 days	3.81±0.27	1.65±0.12
		12 days	5.13±0.69	1.18±0.10

Fruits were harvested at the MG2 or MG4/BK stage of maturity (30 or 36 days postanthesis, respectively). Values represent the PL:GL or MGDG:DGDG mole ratio±s.d. ($n=6$ to 8).

intermediate tolerance to chilling [19]. It would be of interest to know how 'Capello' compares with 'Rutgers' with respect to these physiological traits. Preliminary results of work with plastids from chilled and nonchilled fruits of 'Pik-Red' indicate that this cultivar responds similarly to 'Capello', i.e. the MGDG:DGDG ratio declines with chilling but increases slightly with ripening.

Two recent reports support the conclusion that separate effects of low temperature on chloroplasts and on one or more cytoplasmic membranes are involved in chilling injury in tomato fruit [8, 20]. Disruption of interior chloroplast membranes at chilling temperature may cause inhibition of lycopene synthesis [12, 21], while dysfunction of the tonoplast and/or plasma membrane leads to surface pitting and susceptibility to invasion by pathogens [5, 20]. Differences in membrane lipid composition and metabolism among tomato cultivars may account for variation in chilling sensitivity and in the expression of chilling injury symptoms.

If loss of MGDG during chilling of tomato fruits results in disruption of thylakoids and subsequent inhibition of carotenogenesis, what mechanism is involved? A report on degradation of lipids in chilled cucumber fruit showed that breakdown of GL in peel tissue preceeded that of PL by several days [22]. Degradation of these glycerolipids was via peroxidation, as evidenced by a decrease in fatty acid unsaturation and an increase in ethane evolution, and it did not occur until after rewarming. The reported chilling-induced loss of MGDG in 'Capello' tomato fruits occurred during chilling without rewarming [13]. No data on the fatty acid composition of GL after chilling were provided, but preliminary results with 'Pik-Red' fruit showed a slight increase in unsaturation of MGDG species after 12 days at 2°. Thus, MGDG loss may occur via hydrolysis, transacylation, or additional glycosylations. Further studies are required, preferably with several tomato cultivars, to assess the importance of MGDG loss during chilling in the subsequent inhibition of lycopene synthesis and plastid transformation.

EXPERIMENTAL

Plant material. Tomato plants (*Lycopersicon esculentum* Mill. cv Rutgers) were grown in a greenhouse with supplemental lighting as previously described [14]. Flowers were tagged at anthesis and hand-pollinated. In two separate expts, fruits were harvested at 30 and 36 days postanthesis. Based on visual inspection of pigmentation, locular gel formation and seed development, fruits from the 2 harvests were at the MG2 and MG4/BK stages of development, respectively [23]. Fruits were washed, air-dried and stored in darkness at 15° or 2° for 0, 4 or 12 days. MG2 fruits at 15° had ripened to the breaker stage after 4 days and to the turning stage after 12 days. MG4/BK fruits at 15° had ripened to the turning stage after 4 days and to the pink stage after 12 days. Fruits at 2° did not ripen during storage. Pericarp sections from individual fruits were frozen in liquid N_2 and stored at −80° prior to lipid analysis [14]. At least 6 tissue samples were analysed for each storage condition (temp. × duration).

Lipid extraction, fractionation and quantification. Samples of frozen pericarp tissue (≈10 g) were lyophilized overnight. After dry wt determination, samples were crushed with a spatula and homogenized in 30 ml of $CHCl_3$–MeOH (2:1) with ×3 15 sec bursts of a Polytron tissue homogenizer. The homogenate was filtered through a sintered glass funnel and the tissue residue re-extracted with an additional 10 ml of $CHCl_3$–MeOH. The comb extracts were washed with 0.8% NaCl, then MeOH–H_2O (1:1). After centrifugation, the $CHCl_3$ phase containing total lipids was evapd under a stream of N_2. The dried lipids were dissolved in 2 ml of $CHCl_3$ prior to silicic acid CC. Neutral lipids, glycolipids and PL were eluted as described in ref. [14]. PLs were quantified by the method of ref. [24]. MGDG and DGDG were isolated from the glycolipid fr. by TLC and quantified as previously described [25].

Acknowledgements—The author wishes to thank J. N. Livsey for supplying the plant material used in this study and S. K. Jones for her invaluable technical assistance.

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