



INFLUENCE OF PRESTORAGE HEAT AND CALCIUM TREATMENTS ON LIPID METABOLISM IN 'GOLDEN DELICIOUS' APPLES

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(Received 15 October 1996)

Key Word Index—*Malus domestica*; Rosaceae; calcium; heat; lipids; membranes; postharvest; quality; storage.

Abstract—Heating 'Golden Delicious' apples for 4 days at 38° and/or pressure infiltrating the fruit with a CaCl₂ solution after harvest, maintains firmness and reduces decay during storage. The possibility that these beneficial effects involve changes in membrane lipid metabolism was investigated. Lipids of hypodermal cortical tissue were analyzed after 0, 1, 2 or 4 days at 38° and after storage (15 weeks at 0° plus 1 week at 20°) of fruit that were untreated (Ctl), heated 4 days at 38° (HT), infiltrated with 2% CaCl₂ (Ca) or heated then infiltrated (HT+Ca) before storage. Overall, effects of HT were much more pronounced than those of Ca and effects of HT+Ca were intermediate between those of HT or Ca alone. An initial phase of membrane damage induced by heating, indicated by glycerolipid loss over the first 1–2 days, could explain why HT for less than 3–4 days has an adverse effect on post-storage quality. HT effects on plastids, including accelerated chlorophyll and monogalactolipid loss, as well as carotenoid accumulation, are likely to cause the distinct yellowing of the fruit. HT-induced reductions in steryl glycosides and cerebroside prior to storage similar to those that occurred in Ctl and Ca fruit during storage, and the phospholipid (PL) content of HT fruit after storage was close to that of Ctl fruit at harvest. Also, the ratio of linoleate to oleate in PL was much higher in HT and HT+Ca than in Ctl fruit at the end of storage. One or more of these effects of HT on membrane lipids could be involved in the ultimate benefits to fruit quality. Published by Elsevier Science Ltd

INTRODUCTION

Both hot air heat treatment (HT) and pressure infiltration with solutions of calcium chloride (Ca) appear promising as a means of alleviating physiological disorders and reducing fungal decay during post-harvest storage of apple fruit [1–4]. Apples held at 38° for 4 days after harvest ripened more slowly and remained firmer than untreated fruit, both in storage and during subsequent shelf-life at 20° [5]. Heated fruit produced less ethylene and CO₂ during storage and were less susceptible to superficial scald [6] and fungal decay [7]. Similar benefits were noted when apples were infiltrated with 2–4% CaCl₂ prior to storage [8–10]. Heating, followed by calcium treatment, resulted in added retention of fruit firmness [1, 2, 11].

Effects of HT on the composition of apple cortical cell walls were investigated in an effort to establish a probable mode of action in the maintenance of firm-

ness [12–14]. Conversion of insoluble to soluble pectin was slowed and calcium pectate content was increased in heated compared with untreated fruit. However, softening-related phenomena, such as loss of neutral sugars, a rise in pectin methylesterase activity and de-esterification of pectin, were not changed by heating. It was concluded that the magnitude of firmness-retention did not correlate well with the kind and extent of changes in the cell wall.

Changes in membrane properties and composition as a consequence of heating have been less well characterized. Plasma membrane permeability and viscosity increased during heating, but declined to lower than control values during storage and subsequent shelf-life [15, 16]. The proportions of sterols and saturated fatty acids also increased during heating but by the end of 20° shelf-life after 0° storage, values were similar in plasma membrane from heated and unheated fruits [16]. As with the cell wall studies, the changes in membrane structure and function as a result of heating appeared to be insufficient for explaining the dramatic retention of firmness.

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Calcium treatments have long been known to maintain the firmness of apples in storage [17]. Hypothesized modes of action of calcium include rigidifying and retaining pectins in the cell wall and middle lamella, as well as stabilizing and maintaining the function of cell membranes. Recently, Picchioni *et al.* [18] reported the effects of Ca on lipid content and composition of outer cortical tissue in 'Golden Delicious' apples after 6 months at 0° and during a subsequent 2 weeks at 20°. The concentrations of all lipid classes were somewhat higher in Ca fruit at the end of storage but they generally differed little from those in untreated fruit after 1–2 weeks of shelf-life. The most dramatic difference in lipid metabolism was a transient increase in sterol conjugation that occurred in Ca but not in control fruit.

The present study was undertaken in order to characterize the influence of prestorage heat and calcium treatments, separately or combined, on lipid metabolism in the hypodermal cortex of 'Golden Delicious' apples. We were interested in determining: (1) why 1 or 2 days at 38° can be detrimental to quality, whereas 4 days are beneficial [5, 19]; and (2) how the treatments differ in their effects on membrane changes occurring during storage at 0° (15 weeks) and subsequent shelf-life at 20° (1 week).

RESULTS

Fruit firmness and colour

Pressure infiltration with 2% CaCl₂ was marginally more effective than a 4-day HT for maintaining firmness during storage; the combination of heat followed by Ca was slightly better than either treatment alone. Fruit-firmness at harvest (in N) was 89 ± 8 . After storage and shelf-life, firmness had declined to 50 ± 7 in untreated, 77 ± 9 in Ca, 73 ± 6 in HT, and 82 ± 7 in [HT+Ca] fruits ($n = 30$). The tissue calcium concentration after infiltration was $1554 \mu\text{g g}^{-1}$ dry wt, compared with $272 \mu\text{g g}^{-1}$ dry wt in untreated controls. HT prior to Ca reduced the efficiency of calcium infiltration, resulting in a tissue calcium concentration of $1119 \mu\text{g g}^{-1}$ dry wt. The effect of the treatments on peel colour was distinctly different. Ca largely prevented the partial yellowing noted during storage of untreated fruit, whereas HT, with or without Ca, caused a pronounced yellowing (subjective, visual assessment only).

Effects of heat treatment on lipid composition

To determine the rapid, prestorage effects of HT on lipid metabolism, lipids were extracted from hypodermal cortical tissue and analyzed after heating fruit for 0, 1, 2 or 4 days at 38°. The phospholipid (PL) content declined by *ca* 16% during the first day at 38°, then gradually rose to *ca* 89% of the initial amount

over the next 3 days [Fig. 1(a)]. In contrast, over the 4-day HT there was a continuous decline in the proportion of di-plus trienoic fatty acids and a concomitant steady rise in the proportion of saturated fatty acids in total PL [Fig. 1(b)]. As a result of these changes, the fatty acid unsaturation index of PL [UI = $\%18:1 + 2(\%18:2) + 3(\%18:3)/100$] fell from 1.44 to 1.26.

Among the sterol lipids, free sterols (FSs) were the major class, sterol glycosides (SGs) were second in abundance and acylated sterol glycosides (ASGs) comprised only a very minor fraction. FS content was unaffected by heating, whereas SGs declined by *ca* 21%, with most of the change occurring over the first 2 days [Fig. 2]. ASGs had increased after 1 day at 38° but had returned to the initial concentration after 4 days.

The galactolipids, mono- and digalactosyl-diacylglycerol (GLs; MGDG and DGDG), were affected very differently by heating [Fig. 3(a)]. MGDG dropped sharply (67%) during the first day at 38°, then reached a plateau at *ca* 25% of the initial amount after 2 days of heating. In contrast, DGDG showed only a slight decline (*ca* 7%) over the entire 4-day HT. Although somewhat erratic from sample to sample, the ratio of the absorbance of lipid extracts at 455 vs 668 nm showed a *ca* two-fold increase over the 4 days of heating [Fig. 3(b)].

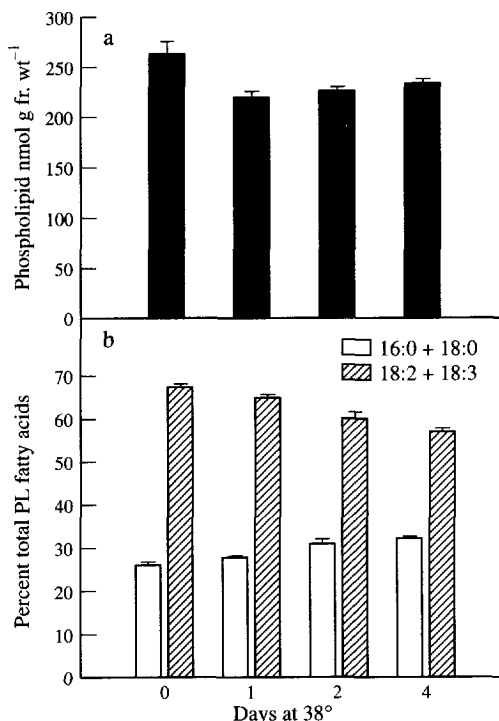


Fig. 1. Phospholipid (PL) content (a) and proportions of saturated (16:0+18:0) and polyunsaturated (18:2+18:3) PL fatty acids; (b) in hypodermal cortical tissue of 'Golden Delicious' apples after 0, 1, 2 or 4 days at 38°. Palmitic, stearic, linoleic, and linolenic acids are denoted by 16:0, 18:0, 18:2, and 18:3, respectively. Errors bars indicate s.d. ($n = 5$).

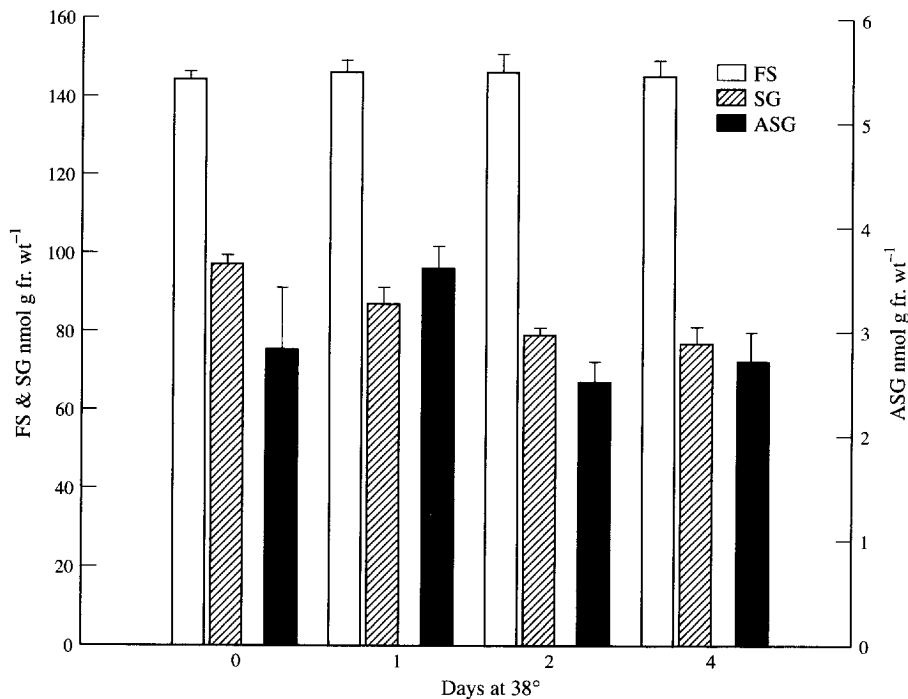


Fig. 2. Free sterol (FS), steryl glycoside (SG) and acylated steryl glycoside (ASG) content in hypodermal cortical tissue of 'Golden Delicious' apples after 0, 1, 2 or 4 days at 38°. Error bars indicate s.d. ($n = 5$).

Effects of HT and/or Ca on lipid changes during storage

After storage for 15 weeks at 0° plus 1 week at 20°, the PL content of hypodermal cortical tissue in

untreated fruit had increased by *ca* 21% [Fig. 4(a)]. This PL increase was slightly reduced in Ca fruit, whereas in HT fruit the PL content was about the same as that in the prestorage controls and had increased only by *ca* 10% compared with prestorage heated fruit. PL content in fruit given the combined heat and calcium treatment was intermediate between that of fruit treated with calcium or heat alone. Along with the increase in PL during storage of untreated fruit, there were changes in PL composition (Table 1), most notably an increase in the ratio of phosphatidylcholine (PC) to phosphatidylethanolamine (PE). This trend was noted regardless of the prestorage treatment (data not shown), although the PC:PE ratio increased a bit more in HT and [HT + Ca] fruit (to 1.84 compared with 1.74 in controls). Prior to storage, the ratio of linoleic to oleic acid (18:2/18:1) in total PL was > five-fold higher in untreated compared with HT fruit [Fig. 4(b)]. After storage, however, this ratio had decreased > nine-fold in untreated fruit and had increased 1.5-fold in HT fruit. Relative to the 18:2/18:1 ratio in PL of stored, untreated fruit, values were *ca* 3.3-, 2.5- and 1.7-fold higher in PL of [HT + Ca], HT and Ca fruit, respectively. Regardless of which treatment fruit received, 18:2/18:1 was much higher in PE than in PC.

Both the FS:PL and TSL:PL (TSL = total steryl lipid = FS + SG + ASG) mole ratios declined substantially during storage of untreated fruit, mostly as a consequence of the increase in PL content (Fig. 5). In contrast, at the end of storage the FS:PL and TSL:PL ratios in HT fruit were only slightly lower than those in the prestorage controls. These steryl

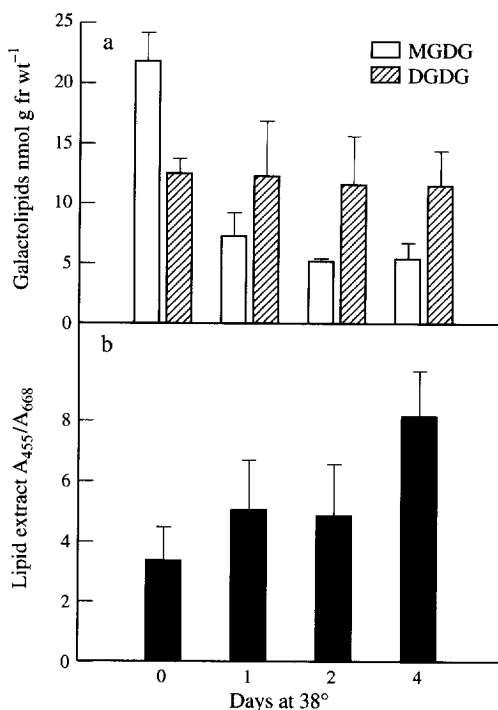


Fig. 3. Galactolipid (mono- and digalactosyldiacylglycerol; MGDG and DGDG) content in (a) and ratio of absorbance at 455 vs 668 nm in lipid extracts from (b) hypodermal cortical tissue of 'Golden Delicious' apples after 0, 1, 2 or 4 days at 38°. Error bars indicate s.d. ($n = 5$).

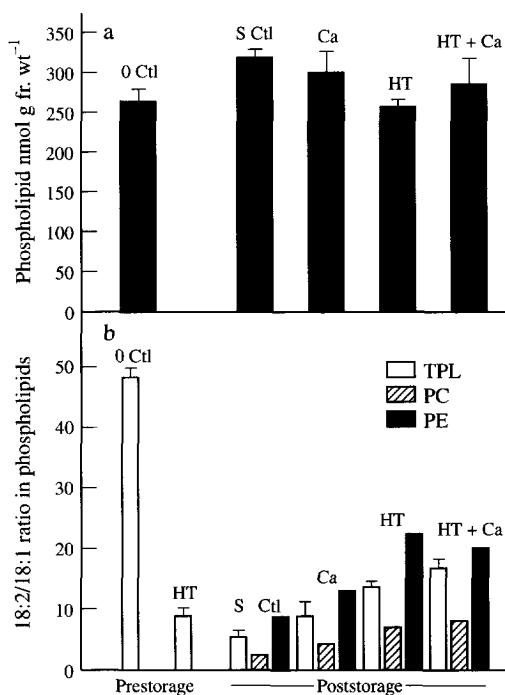


Fig. 4. Phospholipid (PL) content (a) and linoleic/oleic acid (18:2/18:1) ratio of total PL (TPL), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (b), in hypodermal cortical tissue from 'Golden Delicious' apples. Untreated fruit were sampled at harvest (0 Ctl) and after storage for 15 weeks at 0° plus 1 week at 20° (S Ctl). Fruit heated 4 days at 38° (HT) were sampled both after heating and post-storage. Fruit infiltrated with 2% CaCl₂ (Ca) or heated then infiltrated (HT + Ca), were sampled post-storage. Error bars indicate s.d. ($n = 5$). Values for 18:2/18:1 in PC and PE are from one determination using pooled PL from five fruits.

lipid to PL ratios were marginally higher in stored calcium-treated compared with untreated fruit; for [HT + Ca] fruit, values were intermediate between those of fruit given a prestorage treatment with calcium or heat alone.

Only minor changes in FS composition occurred during storage of untreated fruit (Table 1). This was also true for HT fruit; at the end of storage, there was little variation in FS composition among fruit given

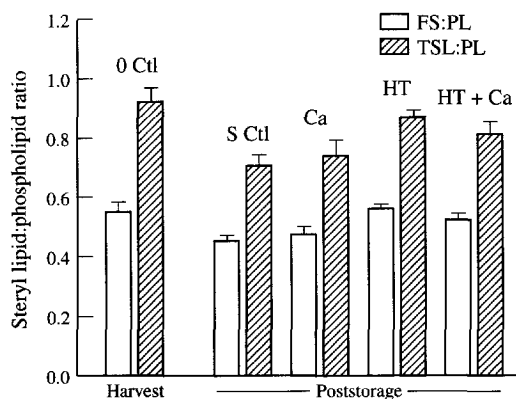


Fig. 5. Free sterol to phospholipid (FS:PL) and total steryl lipid to phospholipid (TSL:PL) mole ratios in hypodermal cortical tissue of 'Golden Delicious' apples that were untreated, heated 4 days at 38° (HT), infiltrated with 2% CaCl₂ (Ca) or heated then infiltrated (HT + Ca), prior to storage for 15 weeks at 0° plus 1 week at 20°. Untreated fruit were sampled both at harvest (0 Ctl) and post-storage (S Ctl). TSL included free sterol, steryl glycoside and acylated steryl glycoside. Error bars indicate s.d. ($n = 5$).

the four different treatments (data not shown). Sitosterol was the predominant FS and was always $\geq 90\%$ of the total FS. Also, sitosteryl glucoside was always $> 90\%$ of the total SG, both before and after storage, regardless of treatment (data not shown).

The steryl lipid distribution (FS:SG:ASG mole ratios) in hypodermal cortical tissue of untreated fruit changed substantially during storage (Table 2). The proportion of FSs increased, while that of SGs decreased, largely as a result of the 25% decline in SGs. A 4-day heat treatment prior to storage caused a remarkably similar change in the relative proportions of FSs and SGs. In addition, at the end of storage, the steryl lipid distribution in all fruit was essentially the same, regardless of prestorage treatment. Thus, FS:SG:ASG changed little with storage of HT fruit, apart from a small increase in the proportion of ASG. A decline in cerebroside (CB) content (*ca* 22%), comparable with that in SG content (*ca* 25%), also occurred during storage of untreated fruit

Table 1. Phospholipid (PL) and free sterol (FS) compositions of hypodermal cortical tissue from untreated 'Golden Delicious' apples at harvest and after storage for 15 weeks at 0° plus 1 week at 20°. Values represent the wt % of total PL or FS and indicate the mean $\pm$ s.d. ($n = 5$)

PL	Harvest	Poststorage	Free sterol	Harvest	Poststorage
PC	42 $\pm$ 1	51 $\pm$ 1	Campesterol	3 $\pm$ 1	2 $\pm$ < 1
PE	37 $\pm$ 1	30 $\pm$ 1	Stigmasterol	1 $\pm$ < 1	1 $\pm$ < 1
PA	8 $\pm$ 1	8 $\pm$ 1	5,25-Stigma.	1 $\pm$ < 1	1 $\pm$ < 1
PI	7 $\pm$ 1	7 $\pm$ 1	Sitosterol	90 $\pm$ 1	92 $\pm$ 1
PS	3 $\pm$ < 1	2 $\pm$ < 1	5,7-Stigma.	3 $\pm$ 1	2 $\pm$ 1
PG	3 $\pm$ < 1	2 $\pm$ < 1	Others	2 $\pm$ 1	2 $\pm$ 1
PC:PE ratio	1.13	1.74			

Abbreviations: PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, Phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; Stigma., stigmastadienol.

Table 2. Effect of heat and/or calcium treatment on steryl lipid ratios and on steryl glycoside (SG) and cerebroside (CB) content in the hypodermal cortex of 'Golden Delicious' apples before or after storage for 15 weeks at 0° plus 1 week at 20°. Values for SG and CB indicate the mean $\pm$ s.d. ($n = 5$)

Treatment		FS:SG:ASG (mole ratios)	SG (nmol g fr. wt ⁻¹)	CB (nmol g fr. wt ⁻¹)
Pre-storage	At harvest	59:40:1	97 $\pm$ 2	32 $\pm$ 2
	4 days 38°	65:34:1	77 $\pm$ 4	27 $\pm$ 3
Post-storage	None (control)	65:33:2	73 $\pm$ 3	25 $\pm$ 1
	2% CaCl ₂ inflt.	64:34:2	74 $\pm$ 1	26 $\pm$ 1
	4 days 38°	64:34:2	76 $\pm$ 3	25 $\pm$ 1
	4 days 38° + 2% CaCl ₂	64:34:2	77 $\pm$ 5	25 $\pm$ 1

Abbreviations: FS, free sterol; ASG, acylated steryl glycoside.

(Table 2). As observed for FS:SG:ASG, heat, by itself, caused a similar reduction in CB and SG content; after storage CB and SG contents were similar in all fruit regardless of treatment.

The GL (MGDG + DGDG) content of hypodermal cortical tissue from untreated fruit decreased by *ca* 24% during storage [Fig. 6(a)]. In contrast, a small increase in total GL occurred during storage of HT fruit, as well as a pronounced increase in the ratio of MGDG to DGDG from 0.5 to 1.6 [Figs 3(a) and 6(a)]. It should be noted, however, that prior to storage, the GL content in HT fruit was only 50% of that in

untreated controls [Fig. 3(a)]. Thus, at the end of storage, total GL content was 30% lower in HT compared with untreated fruit. Poststorage GL concentrations in Ca and [HT + Ca] fruit were 16 and 34% lower, respectively, than those in the controls. Despite the variation in GL content among fruit given the different treatments, the MGDG:DGDG ratio was similar in all fruit after storage, ranging from *ca* 1.6 to 1.7 [Fig. 6(a)].

The ratio of the absorbance of lipid extracts at 455 vs 668 nm was increased > two-fold after storage of untreated fruit, whereas for Ca fruit there was little change relative to prestorage controls [Fig. 6(b)]. Extracts from poststorage HT fruit had by far the highest A_{455}/A_{668} ratio; five-fold higher than that of prestorage control and two-fold higher than that of prestorage HT fruit extracts. The A_{455}/A_{668} ratio of extracts from [HT + Ca] fruit was intermediate between that of extracts from HT and untreated fruit after storage.

DISCUSSION

The types and amounts of lipids in cortical tissue of preclimacteric apples determined in the present work were, generally, in close agreement with the findings of Bartley [20] and Galliard [21]. Also, the increase in total PL, the increase in PC:PE and the decrease in GLs that we found during storage (15 weeks at 0° plus 1 week at 20°) were previously reported to occur with ripening at 12° from the pre- through post-climacteric stages. Concerning the steryl lipid changes associated with ripening-storage, our results matched those of Bartley [20], who found that there was no change in FS and a decrease in SG in post- compared with pre-climacteric fruit. Galliard [21], however, reported a substantial increase in FS (*ca* 35%) and a resultant increase in FS:PL from 0.6 to 0.9, after ripening at 12°.

Heat treatment had a number of pronounced effects on lipid metabolism in 'Golden Delicious' fruit; the pattern of changes over the 4 days at 38° may explain why heating for only 1 or 2 days can be detrimental to post-storage quality [19]. The sharp decline in PL

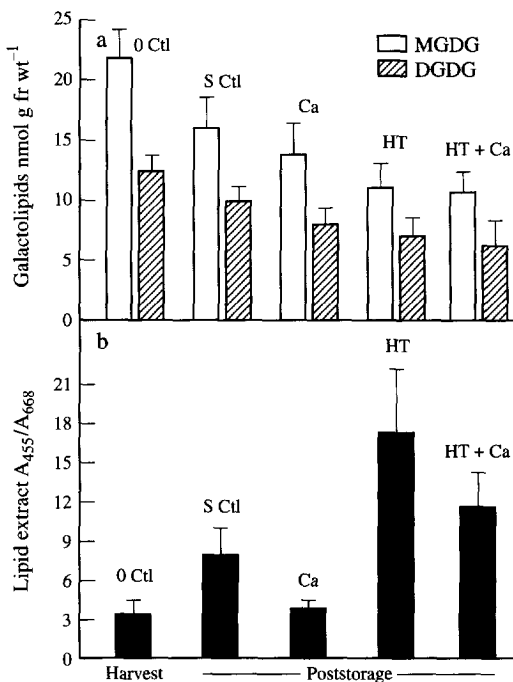


Fig. 6. Galactolipid (mono- and di-galactosyldiacylglycerol; MGDG and DGDG) content in (a) and ratio of absorbance at 455 vs 668 nm in lipid extracts from (b) hypodermal cortical tissue of 'Golden Delicious' apples that were untreated, heated 4 days at 38° (HT), infiltrated with 2% CaCl₂ (Ca) or heated then infiltrated (HT + Ca), prior to storage for 15 weeks at 0° plus 1 week at 20°. Untreated fruit were sampled both at harvest (0 Ctl) and post-storage (S Ctl). Error bars indicate s.d. ($n = 5$).

and MGDG during the first day at 38°, indicates extensive lipid catabolism and, probably, membrane damage. Transfer of fruit to 0° at this point, may cause further perturbation of metabolism and propagation of degradative reactions. After 4 days of heating, it seems that acclimatization to the high temperature has occurred; PL have been partially resorted and the contents of SG, CB and MGDG have stabilized. In accord with this assessment, Lurie *et al.* [16] found that electrolyte leakage and plasma membrane microviscosity of cortical tissue from 'Golden Delicious' apples increased over the first 3 days at 38° but then decreased during day 4. The decline in unsaturation of PL fatty acids with heating, also noted in [16], is a typical response of plant tissues to heat-stress temperatures, that appears to involve new synthesis of less-unsaturated PL [22].

Although HT and Ca have both been shown to maintain firmness and reduce decay severity during storage of apples, it is apparent that the mechanisms by which the two treatments exert these effects are different. Generally, calcium treatment appears to maintain juvenility by reducing the rate of metabolism and delaying senescence [23]. It has been proposed that HT, perhaps through synthesis of heat-shock proteins and coincident interruption of normal protein synthesis, inhibits physiological processes which require that new proteins be made while stimulating others which do not [15]. One clear indication of the differential effects of heat and calcium treatments is the peel colour following storage. The pronounced yellowing of HT fruit reflects accelerated chlorophyll loss and carotenoid synthesis, whereas in Ca fruit these processes are retarded and the fruit remain green. Our work has shown that these pigment differences were discernable in the cortical tissue layer just beneath the peel and that heating caused rapid loss of MGDG, the major lipid of plastids and particularly of thylakoids [24]. In the future, a time-course study of changes in galactolipid contents and plastid ultrastructure in apple peel during heat treatment would be of interest.

Picchioni *et al.* [18] examined the influence of Ca on lipid metabolism in 'Golden Delicious' apples during post-storage shelf-life. Amounts of all the cortical tissue lipids (PL, FS, SG, ASG, MGDG and DGDG) were shown to be higher in Ca fruit after 6 months at 0°. However, 1 week after transfer to 20°, lipid differences between treated and control fruit were much less evident, despite the clear improvement in retention of firmness conferred by calcium. There is unfortunately little basis for comparison between these results [18] and those from the present study, because in that investigation, the duration of storage of 0° was longer, data for prestorage fruit were lacking and control fruit were pressure-infiltrated with water.

Lurie *et al.* [16] determined the effects of HT on the composition and function of plasma membrane from cortical tissue of 'Golden Delicious' fruit. With respect to the temperature and duration of heating, storage

and poststorage shelf-life, this study was similar to ours. It is questionable to compare results of lipid analysis for an isolated membrane fraction with those obtained for an entire tissue but a few apparent discrepancies should be addressed. During HT for 4 days at 38°, it was noted that plasma membrane PL content did not change, whereas FSs increased by ca 40%. In contrast, PL in hypodermal cortical tissue rapidly declined then partly recovered during HT, while FSs remained constant. During storage at 0°, an increase in plasma membrane PL was three-fold greater in heated than in unheated fruit, but 1 week after transfer to 20° there was only 12% more PL in the plasma membranes of HT fruit. PL content in hypodermal cortical tissue increased by ca, 21% in control and 10% in HT fruit during storage and shelf-life; the PL content was 19% lower in HT than in unheated fruit after storage.

Heat-shock is known to inhibit protein synthesis [15] and plasma membrane lipid contents were expressed on a per mg protein basis in ref. [16]. Thus, a decline in plasma membrane protein coincident with PL loss could, in part, explain the trends observed for PL and FS content. It is also possible that the PL increase during storage of HT fruit was greater in the plasma membrane than in other cell membranes. The FS:PL ratios reported for apple plasma membrane [16] were more than one order of magnitude lower than those determined for cortical tissue in this study and previously [20, 21]. This is very surprising, in view of the fact that the plasma membrane is generally enriched in sterols [25]. Possibly, cholesterol oxidase, used in the assay of FSs in ref. [16], is inhibited by some lipid component, such as SG, resulting in low estimates of FS content.

Overall, the results of the present work indicate that HT has a much greater effect than Ca on lipid metabolism in apple fruit and that combination of the two treatments generally has a smaller effect than HT alone. After storage and shelf-life, lipid differences in tissue of heated compared with untreated fruit included lower PL and GL content, higher steryl lipid to PL ratios and a higher 18:2/18:1 ratio in PL. It is interesting that HT *per se* induced reductions in SG and CB similar to those that occurred in untreated fruit during storage; at the end of storage, the PL content in heated fruit was close to that in controls at harvest. Lurie *et al.* [16] concluded that heated apples recovered rapidly from stress and acclimated more successfully to 0° than did control apples. This beneficial effect of HT can, perhaps, at least, in part, be ascribed to one or more of the observed 'adjustments' in membrane lipids.

EXPERIMENTAL

Plant material. 'Golden Delicious' apple fruit (*Malus domestica*, Borkh.) were harvested from a commercial orchard in southern Pennsylvania in the preclimacteric stage (ethylene production was <0.1

$\mu\text{l g}^{-1}$ and the climacteric rise in respiration had not yet begun). After randomization, groups of 20–40 fruit were treated as follows (1) no treatment (control); (2) 1, 2 or 4 days of heating at 38° ; (3) pressure infiltration for 3 min at 103 kPa with a 2% (w/v) soln of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; and (4) 4 days of heating at 38° followed by pressure infiltration with 2% CaCl_2 . Prior to HT, apples were placed in tray-packed boxes with perforated polyethylene bags as liners. One lot of 10 control fruit and lots of 10 fruit heated 1, 2 or 4 days at 38° were processed immediately after treatment. In addition, lots of control, 4-day heat-treated, calcium-infiltrated and 4-day heat-treated plus calcium-infiltrated fruit were stored for 15 weeks 0° in air followed by 1 week at 20° prior to processing. Sampling of apple tissue for lipid analysis was as follows. A 2-mm layer of peel and outer flesh was removed from each fruit with a mechanical peeler and discarded. The next 2–3 mm of outer cortical tissue were then cut with the peeler from the equatorial region and 2.5-g portions from two fruits were combined to make up one 5-g sample. Each sample was inserted immediately into a 50-ml Pyrex culture tube and frozen in liquid N_2 . Sets of five tubes (five samples per treatment) were placed in 900-ml lyophilizer jars and freeze-dried for 72 hr. Sample tubes were then sealed with a cork and wax film, and stored at -80° until used. Measurement of fruit firmness and determination of total Ca content in the cortical tissue were performed as reported in ref. [1].

Lipid extraction, fractionation and analysis. Culture tubes containing the freeze-dried apple tissue were chilled on ice followed by the addition of 15 ml of $\text{CHCl}_3\text{--MeOH}$ (2:1) and 10 μg of cholestanol (Sigma) as an int. FS standard. Samples were homogenized for 30 sec with a Polytron tissue homogenizer and this procedure was repeated after 1 min of cooling on ice. Homogenates were vacuum-filtered through 5.5-cm glass fibre discs pretreated with 3 ml of $\text{CHCl}_3\text{--MeOH}$ (2:1) and supported by a small Büchner funnel. Tissue cakes were rinsed with an additional 9 ml of the solvent mix. Combined filtrates were washed with 0.85% NaCl soln, followed by $\text{MeOH--H}_2\text{O}$ (1:1). The lower CHCl_3 phase containing total lipids was evapd under a stream of N_2 . Total lipid extracts (TLE) were redissolved in 2 ml of CHCl_3 and *A* readings were made at 455 and 668 nm (as indices of carotenoid and chlorophyll content, respectively). After removal of two 20- μl aliquotes for PL phosphate assay [26], TLE were stored sealed under N_2 at -80° until sepd.

Total lipids were sepd into neutral lipid, glycolipid and PL frs by silicic acid CC and individual lipid classes were isolated using TLC on silica gel 60 plates [27]. Fatty acid Me esters (FAMES) were produced by incubating acyl lipids with 14% BF_3 or 0.6 M KOH in MeOH. FSs and sterols derived from SGs by acid hydrolysis were isolated by digitonin pptn [28]. FAMES and sterols were analyzed and quantified by FID-GC [29, 30]. SGs, SGs derived from ASGs, and CBs, were quantified by C_6 -HPLC with UV moni-

toring at 205 nm [31, 32]. Individual PL classes (e.g. PC) and GLs (MGDG and DGDG) were quantified by normal-phase silica HPLC with evaporative light scattering detection [18].

Acknowledgements—We wish to thank Arthur Reyes and George Brown for their valuable technical assistance. Reference to a brand or firm name does not constitute endorsement by the U.S. Dept of Agriculture over others of a similar nature not mentioned.

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