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Calcium delays senescence-related membrane lipid changes and increases net synthesis of membrane lipid components in shredded carrots

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Abstract

The influence of Ca²⁺ on firmness and membrane lipid structural components was evaluated during storage of shredded (wound-stressed) carrot tissues (*Daucus carota* L. 'Caropak'). During 10 days of storage at 10°C and 95% RH, Ca²⁺ treatment (applied as 1% w/v CaCl₂ upon shredding) provided 6–16% greater firmness retention than did a water control treatment. Calcium accentuated net increases in total phospholipid and monogalactosyldiacylglycerol concentrations after 4 and 10 days of storage, and in acylated steryl glycoside concentrations after 4 days of storage. Calcium partially counteracted a net reduction in steryl glycoside concentration which occurred following 4 days of storage, and prevented an increase in the free sterol: phospholipid ratio following 10 days of storage. Calcium preserved membrane integrity of carrot shreds not only by delaying senescence-related membrane lipid changes, but also by apparently augmenting membrane restructuring processes.

Keywords: *Daucus carota* L.; Fresh-cut vegetable; Lipid catabolism; Membrane repair; Wounding

1. Introduction

During the past decade, there has been increasing demand for fresh-cut vegetables and fruits, but maintaining their quality has been a significant problem (Huxsoll et al., 1988). Fresh-cut tissues are subjected to severe injury during preparation, and this initiates degradative reactions which shorten storage life (Bolin and Huxsoll, 1991). Controlling these reactions could thus provide significant benefits to the fresh-cut products industry.

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The essential role of Ca^{2+} in delaying plant senescence is largely associated with its stabilizing influence on cell membranes (Ferguson, 1984), and the induction of membrane lipid catabolism is a characteristic feature of wounded, quiescent plant storage organs (Kahl, 1982) and of fresh-cut products (Brecht, 1995). Accordingly, the use of Ca^{2+} has been suggested as a way to delay senescence in fresh-cut tissues (Rolle and Chism, 1987).

Carrot shreds are a popular fresh-cut commodity, and Ca^{2+} treatments have the potential to maintain their textural qualities for up to 10 days of storage (Izumi and Watada, 1994). In a study of membrane lipid and ultrastructural changes in shredded carrots, Ca^{2+} pretreatment was reported to prevent an increase in cell permeability that began 3 days after wounding (Picchioni et al., 1994). This finding presumably reflects a protective influence of Ca^{2+} on cell membrane systems (Leopold and Willing, 1984).

A more striking and previously undocumented characteristic of stored carrot shreds is the apparent intensive membrane 'repair' process, which may persist for as long as 10 days at 10°C. This phenomenon was revealed by both membrane lipid analysis and transmission electron microscopy (Picchioni et al., 1994). Results indicated that shredded carrot tissues undergo a prolonged period of active lipid synthesis necessary for the biogenesis of new membranes.

In view of these new findings, the question arose as to whether Ca^{2+} treatment could intensify the repair processes, and through this action, maintain membrane integrity and keeping quality. Thus, in the present study, our objective was to investigate the effect of Ca^{2+} treatment on tissue firmness and on the relative changes in concentrations of membrane structural lipid components during storage of shredded carrot tissue.

2. Materials and methods

2.1. Plant material

'Caropak' carrots (an Emperor-type cultivar) grown by Brandt Farms, Inc. (Imaly City, MI) were obtained from a local retail market. After peeling, 5-cm long root sections were shredded using a food processor (Model DLC-10, Cuisinarts Corp.) to provide the test material. Shreds were approximately 4 mm wide, 50 mm long, and 2 mm thick.

2.2. Firmness measurements

Freshly prepared shreds (500-g lots) were immersed for 2 min in 2.5-l of either distilled water (0% CaCl_2) or 1% w/v CaCl_2 (99% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, Sigma Chemical Co.). The pH of the solutions ranged from 6.6 to 7.0.

Following the immersion treatments, shreds were placed in a salad spinner for 30 s at 200 rpm to remove residual treatment solution adhering to the tissue surfaces. Triplicate 50-g subsamples of shredded tissue were evaluated for firmness (0 days storage) using the methods described by Izumi and Watada (1994). The force required to shear 40 g of the shredded tissue (expressed in N) was used to define firmness. Additional triplicate 50-g subsamples were weighed into plastic dishes and stored in an environmentally

controlled room at 10°C and 95% RH for 4 or 10 days. Samples were placed inside of separate 10-l plastic containers (one each for 4 and 10 days storage). The containers were covered with a polyethylene bag and aerated with humidified air at the rate of 24 ml min⁻¹ (450 g total tissue weight per storage container). Firmness measurements were then recorded (as described above) at both 4 and 10 days following storage.

2.3. Lipid class analysis

Freshly prepared shreds (250-g lots) were immersed in 1.5-l of the treatment solutions as described above. Triplicate 20-g subsamples were frozen in liquid N₂, placed in sealed bags under N₂ gas, and then stored at -70°C (prestorage 0-day samples) to await lipid analyses. The remaining shredded samples were stored immediately after treatment at 10°C and 95% RH for 4 or 10 days. Throughout storage, the 10-l storage containers were aerated with humidified air at the rate of 16 ml min⁻¹ (255 g total tissue weight per storage container). At the end of 4 and 10 days storage, samples were frozen in liquid N₂ and stored under N₂ gas at -70°C to await lipid analyses.

Frozen tissues were lyophilized, ground into a fine powder, and 200-mg subsamples were homogenized in 2:1 (v/v) CHCl₃:MeOH with three, 15-s bursts of a Polytron homogenizer. The homogenates were filtered, then the tissue residue was resuspended in 2:1 (v/v) CHCl₃:MeOH and filtered again. The combined filtrates were washed with 0.85 (w/v) NaCl and then 1:1 (v/v) MeOH:H₂O. The CHCl₃ phase containing the total lipids was evaporated under N₂ and the lipid residue was taken up in 1 ml CHCl₃. Lipid components (neutral lipids, glycolipids, and phospholipids) were separated by passing the total lipid extract through a silica Sep Pak cartridge (Waters, Milford, MA) using the elution procedure outlined previously (Picchioni et al., 1994).

Free sterols in the neutral lipid fraction were precipitated with digitonin and analyzed by capillary GLC-FID (Whitaker and Lusby, 1989) using lathosterol (cholest-7-en-3 β -ol; Sigma Chemical Co.) as an internal standard. The carrier gas (He) flow rate was maintained at 1 ml min⁻¹ and the oven temperature was isothermal at 260°C. The injector temperature was 300°C and the detector temperature 350°C.

Glycolipid and phospholipid fractions were prepared for HPLC injections and analyzed by HPLC as described earlier (Picchioni et al., 1994, 1996). Detection was achieved using a Varex IIA evaporative light scattering detector (Varex Corp., Burtonsville, MD). The detector N₂ flow rate was maintained at 2.5 l min⁻¹ and the drift tube temperature at 90°C throughout all analyses. For calibration, authentic glycolipid standards were obtained from Matreya, Inc. (Pleasant Gap, PA) and phospholipid standards from Sigma Chemical Co. Lipids were eluted using gradient systems of 2-PrOH:hexane:H₂O (phospholipids) or 2-PrOH:hexane without water (glycolipids) as described previously (Picchioni et al., 1994).

2.4. Statistical analyses

The experiment was completely randomized as a 3 \times 2 factorial (3 storage periods and 2 immersion treatments), and analysis of variance was performed using MSTAT-C version 2.11 (Michigan State University, East Lansing). Significant *F*-tests for

main effects of the immersion treatment and storage period, and the interaction between treatment and storage period are noted in presentation of the data. Lipid class concentration data are presented as a percentage of the day 0 value (2 storage periods only for storage main effects). Unless noted, each observation is reported as the mean \pm SE of 3 sample replicates.

3. Results and discussion

3.1. Firmness changes

Firmness (resistance to shear) averaged 6–16% greater in Ca^{2+} -treated shreds compared with water control (0% CaCl_2) shreds, both immediately after treatment and throughout the 10-day storage period at 10°C (Table 1). The immediate increase in firmness of Ca^{2+} -treated shreds (Table 1) was also observed in Ca^{2+} -treated apples (Abbott et al., 1989).

Following 4 days of storage, firmness had increased by 25% in control shreds and by 17% in Ca^{2+} -treated shreds. During days 4–10 of storage, firmness continued to increase in Ca^{2+} -treated shreds, but not in control shreds.

These results are similar to those from a recent study involving Ca^{2+} -treated carrot shreds (Izumi and Watada, 1994), which concluded that the effects of Ca^{2+} in maintaining textural quality during storage were due to the stabilizing influence of Ca^{2+} on cell membranes and cell walls. The previous study also showed that treatment of carrot shreds with 1% CaCl_2 increased tissue Ca^{2+} concentration over 3-fold relative to controls.

3.2. Lipid class evaluation

The average lipid concentrations or ratios (dry matter basis) on day 0 (water control and 1% CaCl_2 treatments combined) were as follows: total phospholipid, 727 ± 19 mg 100 g⁻¹; total free sterol:total phospholipid molar ratio, 0.209 ± 0.006 ; acylated steryl glycoside, 88 ± 4 mg 100 g⁻¹; steryl glycoside, 30 ± 2 mg 100 g⁻¹; total free sterol, 83 ± 2 mg 100 g⁻¹; monogalactosyldiacylglycerol, 78 ± 5 mg 100 g⁻¹; and digalactosyldiacylglycerol, 239 ± 17 mg 100 g⁻¹.

Table 1

Texture (*N*) of 'Caropak' carrot shreds at three storage times following treatment with distilled water (0% CaCl_2) or with 1% CaCl_2 . Shreds were immersed in treatment solutions for 2 min and subsequently stored at 10°C and 95% RH for 0, 4, or 10 days. Each value represents the mean of three sample replicates. Mean separation within columns by Duncan's multiple range test ($P < 0.05$). Main effects of treatment and storage, and treatment \times storage (*F*-tests) significant at $P < 0.001$, 0.001, and 0.01, respectively

| Time in storage (days) | CaCl_2 in treatment solution (w/v) | |
|------------------------|---|----------|
| | 0% | 1% |
| 0 | 3508 (b) | 3944 (c) |
| 4 | 4382 (a) | 4626 (b) |
| 10 | 4230 (a) | 4912 (a) |

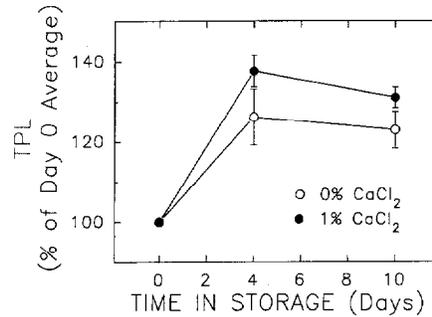


Fig. 1. Changes in total phospholipid (TPL) concentrations during storage of shredded 'Caropak' carrots pretreated with water (0% CaCl₂) or 1% CaCl₂ (treatment and handling described in Table 1). Main effects of treatment (*F*-test) were significant at $P < 0.05$. Each value represents the mean \pm SE of three sample replicates.

Total phospholipid (TPL) concentrations increased sharply during the first 4 days of storage, and the extent of the increase was greater in Ca²⁺-treated shreds (Fig. 1). The Ca²⁺ effect persisted throughout 10 days of storage, although no significant change in TPL concentration occurred between days 4 and 10 in either treatment.

The absence of a continuous increase in TPL concentration throughout 10 days of storage contrasts earlier findings with non-Ca²⁺-treated shreds of 'Apache' carrot (also an Imperator-type cultivar) under similar experimental conditions (Picchioni et al., 1994). Apparently, carrot cultivars may differ in their capacity to synthesize membrane phospholipids following wounding and long-term storage.

The percentage mass distribution among the phospholipid classes – phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), and lysophosphatidylcholine (LPC) – was unaffected by treatment during storage (individual treatment data not presented). Regardless of the treatment, however, the proportion of the two dominant phospholipids (PC and PE) changed inversely between 0 and 4 days of storage, such that the PC:PE mass ratio decreased (Table 2).

Table 2

Changes in the distribution of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidic acid (PA) (expressed as the weight percent of the total phospholipids) and in the PC:PE mass ratio in shredded 'Caropak' carrots (treatment and handling described in Table 1). Storage-related changes were not influenced by CaCl₂ treatment. Each value represents the pooled mean of water (0% CaCl₂) and 1% CaCl₂ treatments ($n = 6$). Mean separation within columns by Duncan's multiple range test ($P < 0.05$) following a one-way analysis of variance. Main effects of storage (*F*-test) were significant at $P < 0.001$

| Time in storage (days) | Phospholipid class | | | PC:PE ratio |
|------------------------|--------------------|----------|---------|-------------|
| | PC | PE | PA | |
| 0 | 55.9 (a) | 26.7 (b) | 6.2 (b) | 2.09 (a) |
| 4 | 52.1 (b) | 29.5 (a) | 6.3 (b) | 1.78 (b) |
| 10 | 48.8 (c) | 29.6 (a) | 8.6 (a) | 1.65 (b) |

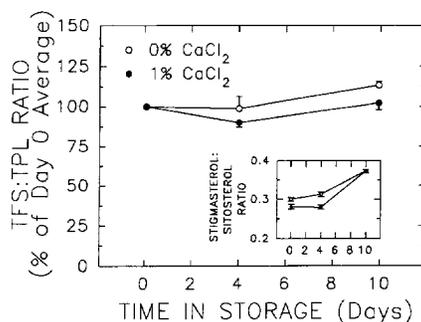


Fig. 2. Changes in the total free sterol:total phospholipid molar ratio (TFS:TPL) and in the stigmasterol:sitosterol mass ratio during storage of shredded 'Caropak' carrots pretreated with water (0% CaCl₂) or 1% CaCl₂ (treatment and handling described in Table 1). Significant main effects (*F*-tests) were as follows: TFS:TPL (treatment and storage, $P < 0.05$); stigmasterol:sitosterol (treatment, storage, and treatment \times storage, $P < 0.01$). Values represent the mean \pm SE of three sample replicates.

An increase in the proportion of PE relative to PC is known to increase membrane rigidity (Leshem et al., 1992), and increased membrane rigidity (decreased lipid fluidity) is associated with senescence of plant tissues and loss of membrane protein function (Duxbury et al., 1991). Thus, the decrease in the PC:PE ratio in both treatments may reflect senescence of the carrot tissue.

Between 4 and 10 days of storage, PE remained constant, but a further reduction in the proportion of PC was closely matched by an increase in the proportion of PA (Table 2). Phosphatidic acid is known to be a membrane phospholipid hydrolytic product resulting from the action of phospholipase-D (Larsson et al., 1990). Thus, the increase in PA at the expense of PC between 4 and 10 days of storage may have partly arisen from membrane degradative processes. Calcium treatment did not curb the rate of PA accumulation (data not presented).

Immediately after treatment, the ratio of free stigmasterol:free sitosterol was greater in control shreds than in Ca²⁺-treated shreds (see inset of Fig. 2). Following 4 days of storage, the stigmasterol:sitosterol ratio increased in controls, but remained unchanged in the Ca²⁺ treatment. After 10 days of storage, the stigmasterol:sitosterol ratio had increased sharply in both treatments.

In model plant phospholipid bilayer systems, sitosterol has been shown to possess greater efficiency in ordering phospholipid fatty acid chains than stigmasterol, and this characteristic is believed to be important in reducing water permeability and in stabilizing membranes (Schuler et al., 1991). In our study, the effect of Ca²⁺ treatment on delaying the increase in the stigmasterol:sitosterol ratio had occurred only minutes between the time shreds were prepared and stored at -70°C . This finding may indicate that Ca²⁺ treatment partially reversed cellular disorganization resulting from wounding.

Although relatively small changes in the total free sterol:total phospholipid molar ratio (TFS:TPL) occurred during storage (Fig. 2), the TFS:TPL ratio in control shreds had increased following 10 days storage, whereas the TFS:TPL ratio of Ca²⁺-treated shreds never increased above the day 0 value. Increase in the sterol:phospholipid ratio is one of the most conspicuous changes which occurs in senescing plant organs

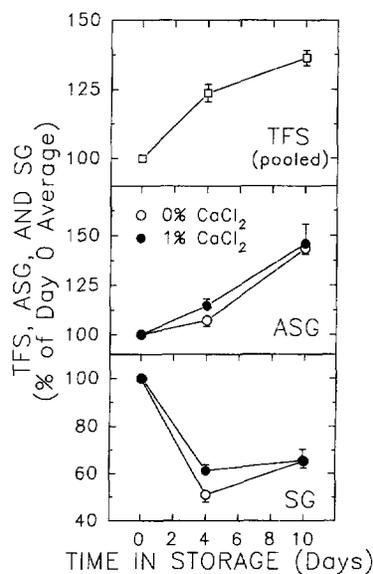


Fig. 3. Changes in total free sterol (TFS), acylated steryl glycoside (ASG), and steryl glycoside (SG) concentrations during storage of shredded 'Caropak' carrots pretreated with water (0% CaCl₂) or 1% CaCl₂ (treatment and handling described in Table 1). Significant main effects (*F*-tests): TFS (storage, *P* < 0.05); ASG and SG (storage, *P* < 0.01). Each value represents the pooled mean of six sample replicates for TFS (water and 1% CaCl₂ combined) or the mean ± SE of three sample replicates (ASG and SG).

(Thompson, 1988). The maintenance of lower TFS:TPL ratios in Ca²⁺-treated shreds was due to the greater net accumulation (or less catabolism) of phospholipids.

Calcium had no effect on total free sterol (TFS) concentrations during storage, although TFS concentrations had increased by an average of 23 and 37% following 4 and 10 days of storage, respectively (see pooled Ca²⁺-treated and control shred results in Fig. 3). Sitosterol represented the majority of the TFS mass, followed by stigmasterol and campesterol (averages of 56.2 ± 1.2, 16.3 ± 0.4, and 10.2 ± 0.2 mg 100 g⁻¹ dry wt, respectively, at 0 days).

Following 4 days storage, concentration changes of acylated steryl glycosides (ASG) differed greatly from steryl glycoside (SG) concentration changes (Fig. 3). After 4 days, ASG concentrations averaged 11% greater than the day 0 value, whereas SG concentrations decreased by an average of 44%. As a molar percentage of the total steryl lipids, SG decreased from an average of 15 to 7%, which was accompanied by the increase in TFS from 56 to 63% during this period. There were no other time-dependent or Ca²⁺-dependent changes in the molar distribution of steryl lipid classes (data not shown).

The 4-day changes in ASG and SG concentrations in Ca²⁺-treated shreds (as a percentage of day 0 values) were significantly different than those in control shreds (*t*-tests significant at *P* < 0.05 and 0.01 for ASG and SG, respectively). The physiological basis of the Ca²⁺-dependent changes in ASG and SG concentrations following 4 days storage is not clear. There is growing appreciation for the importance of sterol

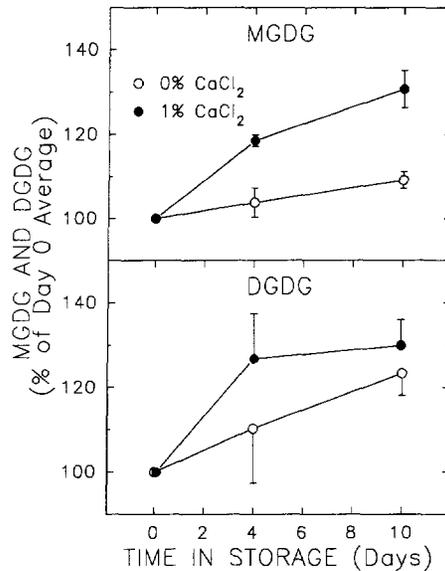


Fig. 4. Changes in monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) concentrations during storage of shredded 'Caropak' carrots pretreated with water (0% CaCl₂) or 1% CaCl₂ (treatment and handling described in Table 1). Significant main effects (*F*-tests) occurred only for MGDG (treatment and storage, *P* < 0.001 and 0.05, respectively). Each value represents the mean \pm SE of three sample replicates.

conjugation processes in plant membrane function, particularly the plasma membrane (Hartmann and Benveniste, 1987; Larsson et al., 1990; Moreau and Preisig, 1993). In light of this importance, Ca²⁺ treatment may have contributed to membrane stabilization by partially (but temporarily) counteracting the net reduction in SG concentration and elevating the net accumulation of ASG.

Following 10 days storage, both control and Ca²⁺-treated shreds had experienced nearly identical changes in ASG and SG concentrations as compared to the day 0 value.

Only a marginal (9%) increase in monogalactosyldiacylglycerol (MGDG) concentration occurred in control shreds during 10 days of storage (Fig. 4). However, Ca²⁺ treatment significantly increased the net accumulation of MGDG after both 4 and 10 days of storage (19 and 31% above the day 0 value, respectively). A similar trend occurred with digalactosyldiacylglycerol (DGDG), but the averages were more variable and the *F*-test for treatment was not significant (Fig. 4).

The greater net accumulation of galactolipids, particularly MGDG, suggests that Ca²⁺ treatment may have an important influence on maintaining the structure of plastid membranes during storage through increased availability of galactolipid pools (Mudd, 1967; Miernyk, 1985). Carrot chromoplasts are heavily enriched with carotene (Frey-Wyssling and Schwegler, 1965), and presumably, stabilization of chromoplast membranes would delay the onset of carotene degradation associated with senescence. However, total carotenoid concentrations during storage were unaffected by the treatment in our study (data not shown).

Extensive documentation has summarized metabolic and ultrastructural changes in wound-stressed plant storage organs (for example, see Kahl, 1974; Barckhausen, 1978; Mazliak and Kader, 1978; Theologis and Laties, 1980). This large body of data is dominated by findings from the so-called 'aging' systems of 24 h or less. However, such results may be irrelevant when fresh-cut tissues are stored for periods considerably longer than 1 day. To our knowledge, there are no previous quantitative accounts of membrane structural lipid changes in fresh-cut (wound-stressed) vegetable tissues over a 10-day storage period. The lack of this information may be because interest in fresh-cut vegetables has developed only recently.

Despite the absence of membrane lipid metabolic studies on fresh-cut vegetables, some long-established traits of carrot roots may be of relevance to our findings. First, non-growing carrot cells derived from secondary vascular tissues of intact roots can be induced (by wounding and slicing) to undergo a period of active metabolism (biosynthesis) for 24 h (ap Rees and Royston, 1971). Second, cells of Umbelliferous species possess a high capacity for somatic embryogenesis (Noggle and Fritz, 1976). Clearly, complete embryogenesis/regeneration requires more than 24 h, but it, too, is highly dependent on anabolic pathways (e.g., membrane lipid synthesis and membrane restructuring).

Visual evidence for membrane lipid synthesis and restructuring in shredded carrot tissue (e.g., accumulation of endoplasmic reticulum 10 days after wounding) has been obtained (Picchioni et al., 1994), and we confirmed this phenomenon in the present study (data not shown). However, it was not possible to detect a quantitative difference in the biogenesis of endoplasmic reticulum between Ca^{2+} -treated and control tissues during the storage period.

Somatic embryogenic capacity varies among carrot genotypes (Steward et al., 1964). With the two cultivars used in our work, we have observed differing amounts of increase in membrane lipid concentrations in wounded and stored tissues. This raises the interesting possibilities of genotypic variation in the capacity for membrane restructuring in response to wounding, in cell regenerating capacity, and in storage longevity. More detailed study would be needed to verify these possibilities.

4. Conclusion

The above results provide evidence indicating that Ca^{2+} treatment preserves membrane integrity of wound-stressed carrot tissues (stored shreds). Not surprisingly, the effect of Ca^{2+} involves deferral of senescence-related membrane lipid changes. However, as shown in the present study, it may also include regulation of membrane system repair processes, and appropriately, it has recently been suggested that Ca^{2+} serves an essential biological function in the healing of membrane injuries (McNeil, 1991). Because of the fundamental importance of membrane function in senescing tissues and the growing interest in fresh-cut and table-ready fruits and vegetables, this area of postharvest biology is in need of greater attention.

References

- Abbott, J.A., Conway, W.S. and Sams, C.E., 1989. Postharvest calcium chloride infiltration affects textural attributes of apples. *J. Am. Soc. Hort. Sci.*, 114: 932–936.
- ap Rees, T. and Royston, B.J., 1971. Control of respiration in disks of carrot storage tissue. *Phytochemistry*, 10: 1199–1206
- Barckhausen, R., 1978. Ultrastructural changes in wounded plant storage tissue cells. In: G. Kahl (Ed.), *Biochemistry of Wounded Plant Tissues*. Walter de Gruyter, New York, pp. 1–42.
- Bolin, H.R. and Huxsoll, C.C., 1991. Effect of preparation procedures and storage parameters on quality retention of salad-cut lettuce. *J. Food Sci.*, 56: 60–67.
- Brecht, J.K., 1995. Physiology of lightly processed fruits and vegetables. *HortScience*, 30: 18–22.
- Duxbury, C.L., Legge, R.L., Paliyath, G., Barber, R.F. and Thompson, J.E., 1991. Alterations in membrane protein conformation in response to senescence-related changes. *Phytochemistry*, 30: 63–68.
- Ferguson, I.B., 1984. Calcium in plant senescence and fruit ripening. *Plant Cell Environ.*, 7: 477–489.
- Frey-Wyssling, A. and Schwegler, F., 1965. Ultrastructure of the chromoplasts in the carrot root. *J. Ultrastructure Res.*, 13: 543–559.
- Hartmann, M.A. and Benveniste, P., 1987. Plant membrane sterols: isolation, identification, and biosynthesis. *Methods Enzymol.*, 148: 632–650.
- Huxsoll, C.C., Bolin, H.R. and King, A.D., Jr., 1988. Physicochemical changes and treatment for lightly processed fruits and vegetables. In: J.J. Jen (Ed.), *Quality Factors of Fruits and Vegetables*. Chemistry and Technology. American Chemical Society, Washington, DC, pp. 203–215.
- Izumi, H. and Watada, A.E., 1994. Calcium treatments affect storage quality of shredded carrots. *J. Food Sci.*, 59: 106–109.
- Kahl, G., 1974. Metabolism in plant storage tissue slices. *Bot. Rev.*, 40: 263–314.
- Kahl, G., 1982. Molecular biology of wound healing: the conditioning phenomenon. In: G. Kahl and J.S. Schell (Eds.), *Molecular Biology of Plant Tumors*. Academic, New York, pp. 211–267.
- Larsson, C., Møller, I.M. and Widell, S., 1990. An introduction to the plant plasma membrane – its molecular composition and organization. In: C. Larsson and I.M. Møller (Eds.), *The Plant Plasma Membrane*. Springer-Verlag, New York, pp. 1–15.
- Leopold, A.C. and Willing, R.P., 1984. Evidence for toxicity effects of salt on membranes. In: R.C. Staples and G.H. Toenniessen (Eds.), *Salinity Tolerance in Plants*. Wiley, New York, pp. 67–76.
- Leshem, Y., Shewfelt, R.L., Willmer, C.M. and Pantoja, O., 1992. *Plant Membranes – A Biophysical Approach to Structure, Development, and Senescence*. Kluwer Academic, Boston, p. 29.
- Mazliak, P. and Kader, J.C., 1978. Lipid metabolism in aging plant storage tissues. In: G. Kahl (Ed.), *Biochemistry of Wounded Plant Tissues*. Walter de Gruyter, New York, pp. 123–154.
- McNeil, P.L., 1991. Cell wounding and healing. *Am. Scientist*, 79: 222–235.
- Miernyk, J.A., 1985. The isolation and characterization of nongreen plastids. In: H.F. Linskens and J.F. Jackson (Eds.), *Modern Methods of Plant Analysis*, Vol. 1. Springer-Verlag, New York, pp. 259–295.
- Moreau, R.A. and Preisig, C.L., 1993. Lipid changes in tobacco cell suspensions following treatment with cellulase elicitor. *Physiol. Plantarum*, 87: 7–13.
- Mudd, J.B., 1967. Fat metabolism in plants. *Annu. Rev. Plant Physiol.*, 18: 229–252.
- Noggle, G.R. and Fritz, G.J., 1976. *Introductory Plant Physiology*. Prentice-Hall, Englewood Cliffs, NJ, p. 522.
- Picchioni, G.A., Watada, A.E., Roy, S., Whitaker, B.D. and Wergin, W.P., 1994. Membrane lipid metabolism, cell permeability, and ultrastructural changes in lightly processed carrots. *J. Food Sci.*, 59: 597–605.
- Picchioni, G.A., Watada, A.E. and Whitaker, B.D., 1996. Quantitative high-performance liquid chromatography analysis of plant phospholipids and glycolipids using light-scattering detection. *Lipids*, 31: 217–221.
- Rolle, R.S. and Chism, G.W. III., 1987. Physiological consequences of minimally processed fruits and vegetables. *J. Food Qual.*, 10: 157–177.
- Schuler, I., Milon, A., Nakatani, Y., Ourisson, G., Albrecht, A.M., Benveniste, P. and Hartmann, M.A., 1991. Differential effects of plant sterols on water permeability and acyl chain ordering of soybean phosphatidylcholine bilayers. *Proc. Natl. Acad. Sci.*, 88: 6926–6930.

- Steward, F.C., Mapes, M.O., Kent, A.E. and Holsten, R.D., 1964. Growth and development of cultured plant cells. *Science*, 143: 20–27.
- Theologis, A. and Laties, G.G., 1980. Membrane lipid breakdown in relation to the wound-induced and cyanide-resistant respiration in tissue slices. *Plant Physiol.*, 66: 890–896.
- Thompson, J.E., 1988. The molecular basis for membrane deterioration during senescence. In: L.D. Noodén and A.C. Leopold (Eds.), *Senescence and Aging in Plants*. Academic, New York, pp. 51–83.
- Whitaker, B.D. and Lusby, W.R., 1989. Steryl lipid content and composition in bell pepper fruit at three stages of ripening. *J. Am. Soc. Hort. Sci.*, 114: 648–651.