THE EFFECTS OF TEMPERATURE DIFFERENTIAL AND SURFACTANT ON THE POSTHARVEST INFILTRATION OF CALCIUM SOLUTION INTO JONATHAN APPLE FRUIT

By

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ABSTRACT

THE EFFECT OF TEMPERATURE DIFFERENTIAL AND SURFACTANT ON THE POSTHARVEST INFILTRATION OF CALCIUM SOLUTION INTO JONATHAN APPLE FRUIT

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Deterioration of apple fruit quality during storage has been shown to be related to low levels of calcium (Ca) in the fruit. Current orchard and postharvest practices to increase fruit Ca are not completely satisfactory. The effects of temperature differentials and surfactants on solution infiltration were examined to possibly provide a direct and efficient means to enrich fruit with Ca.

Calcium infiltration into mature 'Jonathan' apple fruit was achieved by submersion of warm fruits in cold solutions of 2 and 4% $CaCl_2$. Temperature reduction of the submerged fruit decreased the pressure of gases within the intercellular spaces (ICS). The cooling solution was forced into the ICS of the cortex via open lenticels as a result of the difference between the ambient pressure and the pressure of the cooled internal gases. Solution infiltration was markedly enhanced by the addition of surfactant, L-77, in the cooling solution.

Since fruit Ca increase was proportional to the quantity of infiltrated $CaCl_2$ solution as measured by fruit weight gain, the increase in fruit Ca could be accurately estimated by the weight gain from a known $CaCl_2$ concn in the cooling solution. Increases as great as 16 mg

Ca/100 g fresh weight were readily achieved, whereas increase of only 1 or 2 mg Ca/100 g fresh weight have been reported for the conventional dip or drench methods.

The fruit Ca increase was increased by increasing $CaCl_2$ concn in the cooling solution, increasing initial fruit temperature, decreasing cooling solution temperature, increasing submersion duration and decreasing surface tension of the cooling solution. Morphological characteristics of the fruit, such as the quantity of open lenticels, are proposed as additional factors affecting infiltration of the cooling solution.

These results suggest that hydrocooling with a refrigerated $CaCl_2$ solution would offer a practical means of Ca enrichment. An adequate increase of fruit Ca to maximize the storage and market life of the fruit could be achieved after harvest, but prior to fruit storage, in this manner.

Dedicated to my parents, father-in-law and my dear wife.

Without any of them, this work would never have started.

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INTRODUCTION

The useful life of a mature but unripe apple fruit under optimal storage conditions is limited by how slow it progresses through naturally occurring senescence processes, decay caused by pathological agents and its susceptibility to physiological disorders.

Low levels of calcium (Ca) in the fruit are implicated in a number of physiological disorders during storage such as bitter pit, cork spot and Jonathan spot. In addition, water core, internal breakdown, low temperature breakdown, lenticel spot, scald and rot may be intensified by sub-optimal levels of Ca (3). A more fundamental role of Ca in delaying the senescence of apple fruit is evident by the faster post-climacteric rate of respiration of fruit with low Ca content (1), the more rapid loss of membrane integrity of pit-prone fruits (2, 22), and the lower permeability of fruit tissue low in Ca (39). The obvious solution to the problem of Ca deficiency is to increase the Ca content of the fruit to the extent that it will eliminate or considerably minimize the occurrence of these Ca-deficiency related disorders.

Considerably effort has been devoted to the development of methods to increase the Ca content of the fruit, yet none of the current methods appears adequate. There is great need for the development of an economical and reliable means to increase the Ca content of the fruit sufficiently to retard the development of disorders.

LITERATURE REVIEW

Most Ca is deposited in apple fruit during the first 4 to 6 weeks of growth and development following anthesis. Subsequently, little Ca moves from the vegetative part of the tree to the fruit, but some Ca may move from the fruit to the leaves and shoots under certain conditions (48). Young fruits, because of a relatively large surface area and a highly permeable cuticle, have a high rate of transpiration. Ca absorbed by the roots is transported to the fruit via xylem and is relatively mobile. With increasing fruit size, transpiration diminishes. Assimilates are transported via the phloem, in which Ca is not mobile (35). Two possible means of increasing the Ca content of the fruit are to decrease the leaf/fruit ratio subsequent to the cessation of the influx of Ca to the young fruit and to supply Ca ion directly to the fruit.

It has been known for many years that severe pruning in the dormant period increases the likelihood of bitter pit in the subsequent apple crop (9). It was concluded that the increase in tree vigor due to severe pruning resulted in a high leaf/fruit ratio and, hence, more competition from the leaves for water and nutrients. Summer pruning reduces the leaf/fruit ratio and minimizes the problem associated with severe pruning in the dormant period (32). The fruit Ca is reported to be increased by approximately 1 mg/100 g fresh weight when summer pruning is employed (27). This increase from summer pruning, however, does not provide an adequate increase in Ca content of the fruit to

overcome the storage disorder problems. Furthermore, the labor cost prohibits its adoption by growers in the U.S.A.

Attempts have been made to increase the Ca content of the fruit by spraying the trees with Ca salt solutions during the growing season. Prebloom calcium chloride or lime sprays on 'Spartan' apple trees did not increase fruit Ca level or decrease breakdown incidence (20), whereas, postbloom Ca salt sprays have been helpful. In general, their effectiveness when applied after fruit set increases with increasing concentration of the Ca salt and frequency of spraying. The highest usable concentration is limited by the level which damages the leaves or fruits (44). Four or more sprays of 0.6% (W/V) $CaCl_2$ to the tree or a single spray of 2 or 4% (W/V) $CaCl_2$ just before harvest seems to be similarly effective for increasing the Ca content of the fruit by about 40 ppm dry weight or more (31). Ca chloride and Ca nitrate have proven to be more effective than Ca lactate or Ca acetate (46). Ca phosphate has proven ineffective as a tree spray (23).

A postharvest dip or drench of apple fruit with 4% (W/V) $CaCl_2$ solution, the most widely used and most effective method to remedy the Ca-deficiency related disorders, generally increases the Ca content by at least 80 ppm (31). In general, Ca uptake by the fruit increases with increasing concentration of the Ca salt, with the highest concentration being limited by the level at which an unacceptable amount of damage occurs to the treated fruits. $CaCl_2$ is the most effective among the Ca salts. The addition of a wetting agent alone to the dip solution may reduce the amount of $CaCl_2$ retained at the surface of the fruit (31), and hence, reduce the amount of Ca uptake (24). Modifying the viscosity of the dip solution by thickener, Kelzan (Keltrol) or arrowroot,

greatly increased the Ca uptake by the fruit, with up to 825 ppm dry weight increase being reported (24, 8). The thickeners apparently caused the adherence of a greater volume of the dip solution to the fruit surface, favoring Ca penetration into the fruit (19). Combining a thickener and a wetting agent resulted in greater uptake of Ca by the fruit (24) with a higher rate of uptake (8) than with either alone. Unfortunately, the solution is difficult to prepare in large quantity since vigorous stirring is required to make the gel-like solution. Spillage of this slippery solution can be a safety hazard to the workers.

Lecithin, a phospholipid used as a general food additive, added in either a $Ca(NO_3)_2$ or a $CaCl_2$ dip solution reduced the incidence of bitter pit and enhanced the Ca uptake by the fruit (8, 37). It is postulated that lecithin, a highly polar compound with a lipophilic fatty acid "backbone" and hydrophilic choline "head", might assist the movement of Ca ion through the waxy cuticle of apple fruit (37). Accordingly, three Ca ion-containing lipophilic compounds which may penetrate through the hydrophobic cuticle were synthesized and applied to 'Golden Delicious' apples (38). In the short run, the compounds enchanged the rate of Ca uptake, but after prolonged storage, none of these compounds was more effective than $CaCl_2$ in reducing fruit softening. The residue of lecithin on the surface of the fruit at the end of the storage period must be removed, and this washing is an additional cost. Furthermore, lecithin is a rather expensive chemical.

It was observed that the dip solution was absorbed by 'Jonathan' apple fruit grown in Australia, when the temperature of the dip solution was lower than that of the fruit (42). The amount of dip solution absorbed increased with decreasing temperature of the dip solution and

increasing duration of dipping. The solution entered the fruit through the open calyx, typical of the cultivar employed, and moved into the core cavity. There was no advantage in respect to controlling breakdown by using a 5°C dip solution instead of a 20°C dip solution for 20°C fruits. A similar treatment applied to 'Spartan' apples (19) revealed that 38°C fruit dipped in 0°C solution resulted in an increase of 53 ppm Ca in the fruit flesh.

Applying a vacuum to the surface of the $CaCl_2$ solution in which apples are immersed results in air being forced out of the fruits, so that upon returning to normal pressure, a small amount of solution enters into the fruits. Vacuum infiltration (225 mm Hg for 2 min) with solution up to 4% (W/V) $CaCl_2$ has increased the mean Ca content in 'James Grieve' apple from 3.3 to 15 mg/100 g fresh weight. Fruits thus treated were found (43) to be free of bitter pit and breakdown, more firm, and greener in color after 12 weeks of storage in air at 3.5°C than untreated fruits. New Zealand 'Cox's Orange Pippin' and Australian 'Granny Smith' apples responded extremely well to the vacuum infiltration methods (43).

Positive pressure has proven to be effective in forcing a Ca salt solution into the fruit. The amount of Ca uptake can be controlled by the Ca concentration in the solution, the amount of pressure and the duration of treatment (30). A 518.2 to 1036.4 mm Hg positive pressure has increased the fresh weight of the fruit by 1 to 4%. Pressure infiltrated 'Golden Delicious' apples had no loss in firmness after prolonged storage at 0°C. This effect of Ca on firmness was obtained regardless of whether the fruits were treated soon after harvest or after three months of storage (29). Both the vacuum and pressure infiltration

methods, if performed on an commercial scale, would require large, strongly constructed metal chambers that would be costly to build and time consuming to operate.

OBJECTIVES OF THE STUDY

Results of the pressure and vacuum infiltration methods suggest that the migration of solution in the the intercellular spaces (ICS) of a submerged apple fruit can be induced, provided a positive pressure is established between the ambient atmosphere and the internal atmosphere of the ICS of the fruit cortex.

Another possible method of establishing a favorable pressure relationship is by temperature differential. A warm fruit submerged in a cold solution used as a cooling medium could provide a pressure differential and may cause solution infiltration. Previous studies (19, 42) indicated that the submersion of warm fruit in a cold $CaCl_2$ solution did not yield more effective control of fruit breakdown than the conventional dip or drench method. The ineffectiveness was attributed to either the uptake of solution into seed cavities through open calyx or to the insufficient increase in fruit Ca content, which could be the result of insufficient amount of solution infiltration into the fruit cortex.

Assuming that the solution infiltrated into the fruit via some of the open lenticels at the surface of the fruit, the amount of solution infiltrated could be increased by either increasing the temperature differential or reducing the threshold pressure required to initiate solution infiltration. There are limitations on the magnitude of the temperature differential that can be safely induced by hydrocooling the

fruit. The threshold pressure required for solution infiltration through small apertures depends on many factors, one of which is the surface tension of the solution (41). The lower the surface tension, which can be reduced by the addition of a surfactant, the lower the threshold pressure.

It was the purpose of this study to investigate the effects of surfactants and temperature differentials on solution infiltration in 'Jonathan' apple fruit, and to assess these treatments in increasing the Ca content of the fruit for prolonging their storage life.

GENERAL MATERIALS AND METHODS

The 'Jonathan' apple fruit used came from three lots obtained from two sources. Lot A was harvested Sept. 23rd, 1977 at the Horticulture Research Center, East Lansing. Lot B and C were purchased on Dec. 16th, 1977 and Feb. 3rd, 1978, respectively, from the Rasch Brothers Orchards near Sparta, Michigan. The latter were harvested on Sept. 23rd and 24th, 1977 and stored CA without postharvest calcium treatment.

The fruit was stored at -0.5°C in one bushel wooden crates overwrapped with 1.5 mil polyethylene bag to maintain high relative humidity. The fruits were sorted and randomized within the lot before storage so that subsequent handling would be minimized.

The fruits for experiments were sorted to remove those with visible surface defects of a physiological, pathological or mechanical nature. Each fruit was selected for weight (85 - 145 g) and diameter (64 - 70 mm). The pedicle, if present, of the fruit was trimmed to a length of approximately 6 mm to minimize weight gain caused by hydration of dry pedicle. All loose particles and debris on the surface of the fruit were removed with puffs of air.

The selected and prepared fruits were held in a room or growth chamber at the desired experimental temperature for 24 to 36 hours prior to the treatment. It was found that 24 hours was necessary for the temperature at the center of the fruit to equilibrate to the temperature of the room or growth chamber. The temperatures of the solution were conditioned the same manner as that of the fruits.

All experimental solutions were prepared with distilled water containing either 2 or 4% (W/V) $CaCl_2$[1/]. Blue food color[2/], when used was added at a rate of 0.1% (V/V). The surfactants, X-77[3/] and L-77[4/], employed in some experiments, were added at a rate of 0.1%, which is tenfold the critical micelle concn of both surfactants (6, 11).

The submersion treatments utilized 5 liters of treatment solution contained in an 8 liter bucket kept in a room at the temperature of the solution. Ten fruits were placed into the bucket and kept submerged just below the surface of the solution with a piece of perforated plastic disc for a selected period of time. This simple arrangement ensured that fruits of different replicates within a treatment received the same amount of cooling. Upon removal from the solution, the fruits were rinsed in tap water and blotted with paper towels, with particular attention to drying of the stem and calyx ends of the fruits. The fruits were then placed on their sides in a 21 ± 1°C room for 20 min, then then turned over and held for another 20 min, to ensure complete evaporation of water adsorbed on the surface of the fruit before weight measurements were taken.

The weight of each apple was measured before and after submersion treatment with a Mettler 160 top loading balance to the nearest mg. The weight change was expressed as either mg/fruit or mg/100 g fresh weight.

1/ $CaCl_2$ anhydrous with purity of 96.3%. J. T. Baker Chemical Co. Phillipsburt, N.J.

2/ 4% dye content, Seeley-Morris Extract Co. Detroit, Mich.

3/ Chevron Chem. Co. Ortho Division.

4/ Union Carbide Corp.

Tissue blocks, 2 x 2 x 2 mm^3 in size, containing lenticels were excised from apple fruits and fixed in FFA solution (15). Dehydrated in serial tertiary butyl alcohol - ethyl alcohol solutions and embedded in paraffin (15). Serial sections of the embedded tissue were cut with a rotatory microtome at 12 μm thickness. Sections were mounted, stained with fast green and counter-stained with Sudan VI.

Freshly excised tissue blocks containing lenticels were mounted and frozen in Optimum-Temperature Compound[1/] on metal stubs (33). Sections 32 μm in thickness were cut in a cryostat at -22°C and placed on glass slides. A drop of OTC was placed over the section on the glass slides and covered with cover-slip. The edges of the cover-slip were sealed with melton wax. Cryostat sections of apple fruit thus mounted could be preserved for two weeks.

The X-ray intensities of Ca and Cl in apple fruit tissue were measured with an electron microprobe[2/] operated at 15 KeV accelerating voltage, 0.02 μA sample current at 500X magnification. For semi-quantification of the elements (34), the electron beam was set to scan horizontally at 1 msec/200 μm and vertically at 80 msec/160 μm over an area 160 x 200 μm^2 on the tissue surface approximately 2 mm beneath the cuticle. The X-ray intensities of Ca (K_α, 3.359 Å) and Cl (K_α, 4.728 Å) were counted for 10 sec and repeated 10 times on the same area.

The calcium content was determined by atomic absorption spectrophotometry for whole fruit of known weight with seeds removed. The tissue was macerated in 100 ml of deionized distilled water in a Wareing

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1/ OTC, -15 to -30°C, Fisher Scientific Co.

2/ ARL, Model EMX-SM

blender for 3 min. The addition of water gave the macerated tissue a uniform consistency. An aliquot of approximately 10 g was weighted to the nearest mg, and transferred to a crucible and air-dryed at 50°C for 12 hr prior to being ashed at 550°C for 10 hr. The ash was dissolved in 5 ml of 0.5 N HCL with 1% $LaCl_3$ (49). The ash solution was analyzed with an atomic absorption spectrophotometer[1/]. The concn of Ca in ppm in the ash solution was computed from a standard curve constructed with solutions of known concn of Ca. The Ca content of the fruit was then calculated accordingly and expressed as mg Ca/100 g fresh weight.

The temperature at the center of the apple fruit was measured through a hole, 6.4 mm in diameter, bored perpendicularly to the stem-calyx axis from the equator to the center of the fruit. A thermometer, with resolution to 0.1°F, was inserted and the opening was sealed with non-phytotoxic molding rubber to prevent the seepage of cooling solution into the fruit. The fruit containing the thermometer was lowered into the cooling solution so that the whole fruit was submerged with the fruit-thermometer junction at the surface of the solution. The temperature readings were taken at two-minute intervals for forty minutes, starting immediately after the fruit was completely submerged.

1/ Beckman, Model DB-G grading spectrophotometer equipped with Model 1501 atomic absorption accessary with laminar flow burner assembly.

EXPERIMENT I. FRUIT WEIGHT GAIN DURING HYDROCOOLING

Introduction: The intercellular spaces (ICS) of apple fruit flesh occupy up to 25% of the total volume of the fruit at maturity (36). The major portion of the ICS is filled with gas (14). In a study of apple fruit porosity, nitrogen gas under 40 cm water column pressure introduced to the center of the fruit flowed through the fruit with ease via the interconnecting portion of the ICS and exhausted to the external atmosphere, presumably, via open lenticels (14). A temperature reduction of the fruit lowers the pressure in the ICS. When totally submerged in an aqueous solution, the ambient pressure could force the external solution into the ICS, and if so, it could be measured as a weight gain of the fruit. The purpose of this experiment was to study the possible solution infiltration into the totally submerged fruit during hydrocooling by means of fruit weight gain and occurrence of blue coloration in the fruit flesh.

Materials amd Methods: Variables considered were solutions either with or without blue food dye, fruits at either 22.5 or -0.5°C, and solutions at either 22.5 or -0.5°C, for a total of eight treatment combinations. Thirty fruits were subjected to each treatment for a period of one hour. All solutions contained 4% (W/V) $CaCl_2$. Weight gain was measured for individual fruit as mg/fruit. Since the standard deviations of the treatments were nearly proportional to their means, data were transformed by $\log_{10}$ and subjected to one-way analysis of variance (21).

Results: The presence or absence of food dye in the cooling solution was not significantly related to weight gain of the fruit, whereas the solution temperature and fruit temperature contributed significantly to weight gain. The highly significant interaction of solution temperature and fruit temperature (Table 1) indicated their effect on weight

Table 1. Statistical evaluation of the effects of food dye (FD), solution temperature (ST) and fruit temperature (FT) on weight gain of 'Jonathan' apple fruits.

Source of variation	d.f.	F
total	239	
treatment	7	59.6**
FD	1	0.01
ST	1	93.2**
FT	1	210.5**
FD X ST	1	0.1
FD X FT	1	5.6
ST X FT	1	107.7**
FD X ST X FT	1	0.2
error	232	

** significant at $\alpha = 0.01$

gain to be interdependent. The submersion of warm fruit (22.5°C) in cold solution (-0.5°C) yielded a substantial increase in weight of the fruits over the other treatments (Table 2). The presence of blue coloration in the cortex, which could be observed through the cuticle (Figure 1), occurred only to fruits with substantial weight gain. It was indicative that the weight gain was a result of the infiltration of the cooling solution into the fruit.

Based on the assumption that weight gain is due, at least partially, to the infiltration of solution, any dissolved chemicals in the

Figure 1. The surface of a 'Jonathan' apple fruit hydrocooled in 4% $CaCl_2$ solution tinted with blue food dye. Note the blue color around the lenticel. 20X.

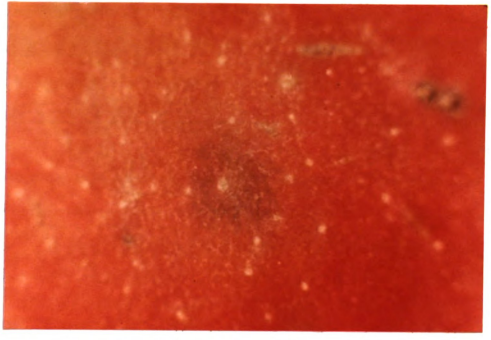


Table 2. The effect of solution temperature (ST) and fruit temperature (FT) on mean weight gain of 'Jonathan' apple fruit submerged for one hour.

Treatment ST (°C)	FT (°C)	Weight gain (mg/fruit)	
-0.5	-0.5	18.1	2.5[1/]
-0.5	22.5	328.3	27.1
22.5	-0.5	17.7	1.6
22.5	22.5	27.2	2.6

[1/] S. E. based on 60 replications

solution should enter the fruit with the solution unless there is some mechanism to exclude the entry of the chemicals, but not water and dye. If weight gain results entirely from solution infiltration, 328 mg/fruit weight gain from 4% $CaCl_2$ aqueous solution would be equivalent to an increase of 3.69 mg Ca/100 g fresh weight for a fruit weighing 120 g (Appendix I). When this amount is added to the average native Ca content, 1.49 mg Ca/100 g fresh weight (Exp. VI), the total Ca content of the fruit would approach the 5.5 mg Ca/100 g fresh weight that is considered adequate for Ca to produce a beneficial effect on apples during long-term storage (28). Since a one-hour submersion period is impractical for commercial use, a quicker means of Ca infiltration is needed.

EXPERIMENT II. THE DISTRIBUTION OF DYE SOLUTION IN APPLE CORTEX AND THE ANATOMICAL DIFFERENCES BETWEEN OPEN AND CLOSED LENTICELS

Introduction: Areas of blue coloration were observed beneath the cuticle of warm fruit (22.5°C) which exhibited a substantial amount of weight gain following submersion in cold (-0.5°C) dye solution (Exp. I). An examination of the distribution of dye solution in the fruit cortex was made to provide clues for the cause of fruit weight gain during hydrocooling. Since the lenticel offers a possible portal of entry for the solution, its structure was carefully observed.

Materials and Methods: The flesh of apple fruits showing blue coloration were examined visually and with the aid of a dissecting microscope. Tissue blocks containing a lenticel surrounded by a small amount of dye, shown as a dyed lenticel in Figure 1, and containing a lenticel without dye (non-dye lenticel) excised from the same fruit which had been submerged briefly in 4% $CaCl_2$ solution with 0.1% blue food color were processed by the paraffin method for anatomical examination. Tissue blocks with dyed and non-dyed lenticels were also sectioned with a cryostat and examined.

Results: The blue coloration always appeared in the tissue adjacent to some of the lenticels on the fruits submerged in cold dye solution. In cross section, this coloration was located at the periphery beneath the cuticle in isolated areas (Figure 2). The mean depth of penetration was 3 mm, with a maximum depth of 7 mm. No coloration was observed in

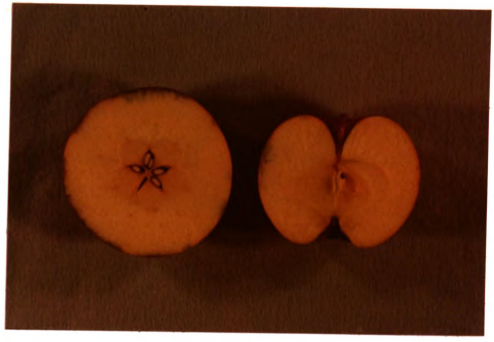
the cortex near the pedicle or calyx, nor in the seed cavity. Employing the dissecting microscope, the blue color was seen clearly within the intercellular spaces (Figure 3). Sections of dyed lenticels prepared by the cryostat showed that the dye was distributed as discrete blue dots beneath the lenticels (Figure 4).

Serial sections of the non-dyed lenticels revealed that some had no aperture at the cuticle surface (Figure 5), others had aperture, but the surrounding cells were tightly packed without an open space between them (Figure 6). Serial sections of the dyed lenticels show that all had apertures at the cuticle and the surrounding cells were loosely arranged with open spaces between them (Figure 7). Some of these open spaces connected the cuticle aperture and the intercellular spaces (Figure 7).

The presence of coloration immediately adjacent to lenticels suggested that these lenticels were the portals of entry for the solution. The anatomical examinations of the open lenticels with dye penetration confirmed that their structure would permit the entry of solution under pressure into the cortex of the fruit. The discrete blue dots in the cryostat section of open lenticels strongly suggested that there were specific routes through which the solution migrated to the cortical tissue. Since the cortex is composed of thin-walled parenchyma cells and randomly distributed intercellular spaces, the logical route is the intercellular spaces. This is substantiated by the presence of blue color in the intercellular spaces at the edge of the dye-colored area where individual sections of the intercellular spaces containing blue color were discerned.

Figure 2. Cross section of a 'Jonathan' apple fruit hydrocooled in 4% $CaCl_2$ solution tinted with blue food dye. Note the blue coloration at the periphery of the fruit immediately beneath the cuticle.

Figure 3. Cross section of cortical tissue of 'Jonathan' apple fruit hydrocooled in 4% $CaCl_2$ solution tinted with blue food dye. Note the irregular-shaped blue coloration between the parenchuma cells, where the intercellular spaces are located. 120X.



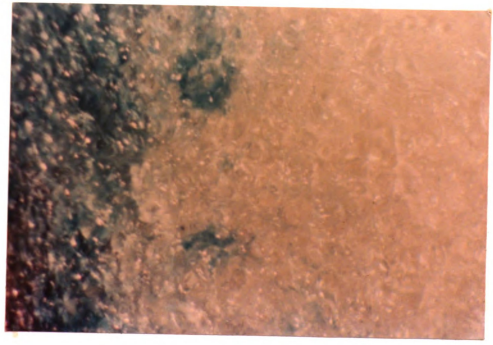
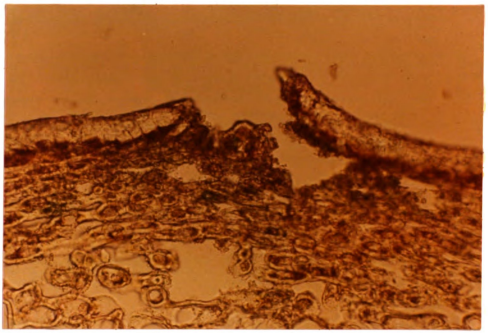


Figure 4. Cross section of a dyed apple lenticel, or an open lenticel, prepared by the cryostat. Note the dye is distributed as discrete blue dots as indicated by arrows. 2,444X.

Figure 5. Structure of a non-dyed apple lenticel, or a closed lenticel (C.S.). Note that there is no aperture at the cuticle. 2,444X.



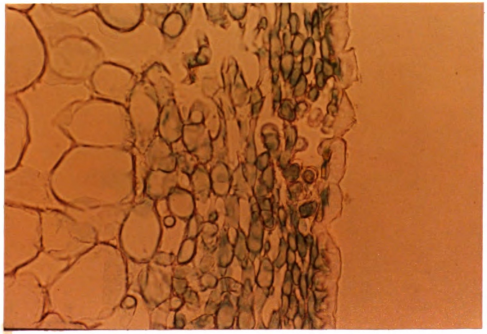
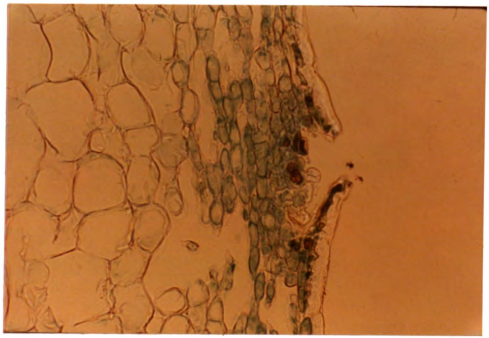
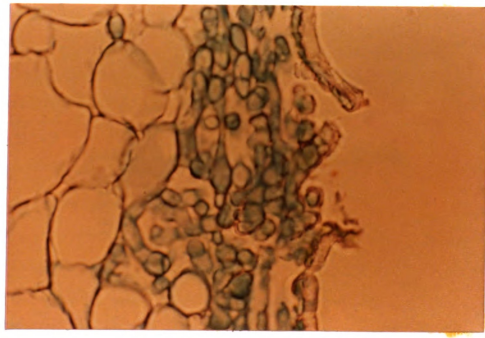


Figure 6. Structure of a non-dyed apple lenticel, or a closed lenticel (C.S.). Note the tightly packed cells around the aperture at the cuticle. 1,000X.

Figure 7. Structure of a dyed apple lenticel, or an open lenticel (C.S.). Note the loosely arranged cells around the aperture at the cuticle. 2,444X.





EXPERIMENT III. DYE AS A TRACER FOR $CaCl_2$ IN APPLE FRUIT TISSUE

Introduction: It was found that warm intact apple fruit submerged in a cold 4% $CaCl_2$ solution tinted with blue food dye gained weight substantially (Table 2) and had blue coloration in the cortical tissue. The dye in the solution was shown to be incidental and not causal to fruit weight gain (Table 1). The presence of this blue coloration in the cortical tissue in fruits having weight gain suggests solution infiltration into the cortical tissue. The $CaCl_2$, as a solute, likely entered into the cortical tissue as well. An experiment was conducted to determine whether $CaCl_2$ entered the cortical tissue and, if so, to relate its presence to the distribution of the dye in the fruit cortical tissue.

Materials and Methods: Three warm (21°C) fruit were submerged in cold (-1°C) 0.1% blue food dye solution with or without 4% $CaCl_2$ for a period of one hour. Tissue blocks, 3 x 3 x 3 mm^3 in size, with cuticle were excised from both colored and non-colored regions of the same fruit. After freeze drying (40), they were mounted on carbon discs with Television Tube-Koat[1/] and coated with a thin layer of carbon in a vacuum evaporator[2/], a method of tissue preparation that prevents redistribution of water soluble compounds in the tissue. Both calcium and chlorine were semi-quantified with a microprobe.

1/ G. E. Electrons.

2/ Varian, Model VE 10.

Results: The X-ray intensities of the elements obtained from plant tissue prepared as outlined above can be positively correlated but cannot be transformed to determine the concentration of the elements in the tissue. Comparisons of X-ray intensities, therefore, were limited to the same element in tissue of different treatments. The presence of blue dye in the tissue did not affect the Ca and Cl content in the tissue. The presence of $CaCl_2$ in the treatment solution yielded a tremendous increase of Ca and Cl in the tissue; furthermore, the increase occurred only in tissue with blue coloration (Table 3).

Table 3. The mean X-ray intensity of calcium and chlorine in cortical tissue of 'Jonathan' apple fruit hydrocooled in dye solution either with or without $CaCl_2$.

Coloration of cortical tissue	$CaCl_2$ in solution	X-ray intensity (counts per 10 sec.) Calcium		Chlorine	
+	+	443.2	56.3[1/]	344.7	58.6
-	+	19.7	0.6	1.5	0.4
+	-	31.2	3.4	1.8	0.7
-	-	33.7	7.9	2.1	0.5

[1/] S.E. of 3 replications.

There is no doubt but that Ca had entered the cortical tissue of the fruit. Its presence was indicative that hydrocooling of apple fruit with solution containing $CaCl_2$ could increase the Ca content of the fruit. The dye proven to be a good tracer of $CaCl_2$ at the termination of the submersion treatment. The distribution of blue coloration in apple flesh described in Exp. II was similar for calcium. Since Ca applied to the fruit surface was found (47) to penetrate the cuticle

and migrate into the core of the fruit, it is likely that Ca once in the intercellular spaces would move throughout the fruit.

EXPERIMENT IV. THE EFFECT OF SURFACTANTS ON FRUIT WEIGHT GAIN

Introduction: A possible means for increasing the rate of solution infiltration and thereby shortening the duration of submersion is to lower the surface tension of the solution by adding a surfactant. It has been demonstrated that mass solution infiltration through open stomata of leaf can be induced by adding a small amount of surfactant to the solution (11). The opening of the ICS at the open lenticels, which may serve as portals of entry for solution infiltration, have similar dimensions to stomata. Since X-77 and L-77 can lower the surface tension of water to 33 and 24 Newton respectively (6, 11). These two surfactants were tested to study their effect on fruit weight gain which could be resulted from solution infiltration induced by hydrocooling.

Materials and Methods: The weight gains of 50 fruits with a 21°C initial temperature submerged in a 4% $CaCl_2$ plus 0.1% X-77 aqueous solution at -1°C for 10, 20, 30, 45 and 60 min were measured. Similarly, weight gains of 50 fruits at 21°C were obtained in 4% $CaCl_2$ plus 0.1% L-77 aqueous solution at -1°C for 5, 10, 15 and 20 min. Weight gains of 40 fruit at 21°C were obtained in 4% $CaCl_2$ aqueous solution at -1°C for 15, 30, 45 and 60 min as controls.

Results: A 20-min treatment with X-77 gave a weight gain equivalent to 60-min submersion in solution without the surfactant. L-77 was more effective than X-77, producing in 20 min more than triple the weight

gain in 60 min for the control (Table 4). It is evident that the surfactants markedly enhanced fruit weight gain. Since one of the effects

Table 4. The effect of surfactants on mean weight gain of 21°C 'Jonathan' apple fruit during submersion in 4% $CaCl_2$ solution at -1°C.

Submersion duration (min)	Mean weight gain (mg/fruit) control		X-77 (0.1%)		L-77 (0.1%)	
5	-		-		164.8	13.6[1/]
10	-		79.6	1.4	423.5	32.5
15	57.1	6.7	-		672.9	38.7
20	-		271.4	26.7	906.1	56.0
30	119.6	20.2	518.6	35.8	-	
45	202.9	31.5	718.2	43.8	-	
60	283.9	29.8	1010.7	79.5	-	

[1/] S.E. of 50 replications.

of surfactant is to enhance solution infiltration through small appertures, this result supported the hypothesis that fruit weight gain was resulted from solution infiltration. With the aid of L-77, an adequate amount of Ca, 5.71 mg/fruit, could be infiltrated into the fruit in less than 10 min.

EXPERIMENT V. THE EFFECT OF SUBMERSION DURATION AND INITIAL FRUIT TEMPERATURE ON FRUIT WEIGHT GAIN

Introduction: The weight gain of warm fruit submerged in cold solution was shown to be, most likely, due to the infiltration of solution into the intercellular spaces. As the fruit is cooled, the volume of gas within the intercellular spaces is reduced, which results in a lower pressure in the intercellular spaces as compared to the ambient atmosphere pressure. It is assumed that this pressure differential is the driving force for solution infiltration. According to the gas laws this pressure differential is proportional to the temperature drop (ΔT), which is the difference in temperature of the fruit before and after cooling. With ΔT being a function of both the initial fruit temperature (T_o) and the cooling, or submersion duration (t), an experiment was designed to study the effect of these two factors on fruit weight gain at a constant cooling medium temperature (T_1).

The hydrocooling machinery employed in the cooling of fresh produce is designed to maintain the temperature of cooling water at approximately 5°C, the temperature used in this experiment. The range of initial temperature of the fruit chosen for this experiment was 10 to 20°C, as the approximate range of the temperature for 'Jonathan' apples harvested in Michigan.

Materials and Methods: The weight gain of fruits initially at 10, 15 and 20°C when submerged in 2% $CaCl_2$ plus 0.1% L-77 solution that was

refrigerated to maintain 5 ± 0.5°C for 10, 20 and 30 min were measured. Fifty fruits were used in each treatment.

Results: The fruit weight gains increased significantly with both increasing submersion duration and with higher initial fruit temperature (Table 5). Since it was proposed that the amount of solution infil-

Table 5. The mean weight gain of 'Jonathan' apple fruit under simulated hydrocooling conditions (solution temperature: 5 ± 0.5°C, 2% $CaCl_2$ plus 0.1% L-77).

initial fruit temperature	submersion period					
	10 min		20 min		30 min	
	mean weight gain (mg/100 g fr. wt.)					
10°C	53.6	4.8[1/]	147.2	10.9	282.0	18.5
15°C	230.0	15.9	452.6	27.4	595.4	30.7
20°C	367.1	29.3	808.2	52.7	938.4	56.8

[1/] S.E. of 50 replications.

trated could be affected by both T_o and t at a constant T_1, the results suggested that fruit weight gain was a reflection of solution infiltration. Furthermore, these data could serve as a guide for determining the required submersion duration for fruits of various initials temperatures in order to achieve a desired amount of weight gain, and hence, increase in Ca content if the total amount of solution infiltrated could be measured as fruit weight gain.

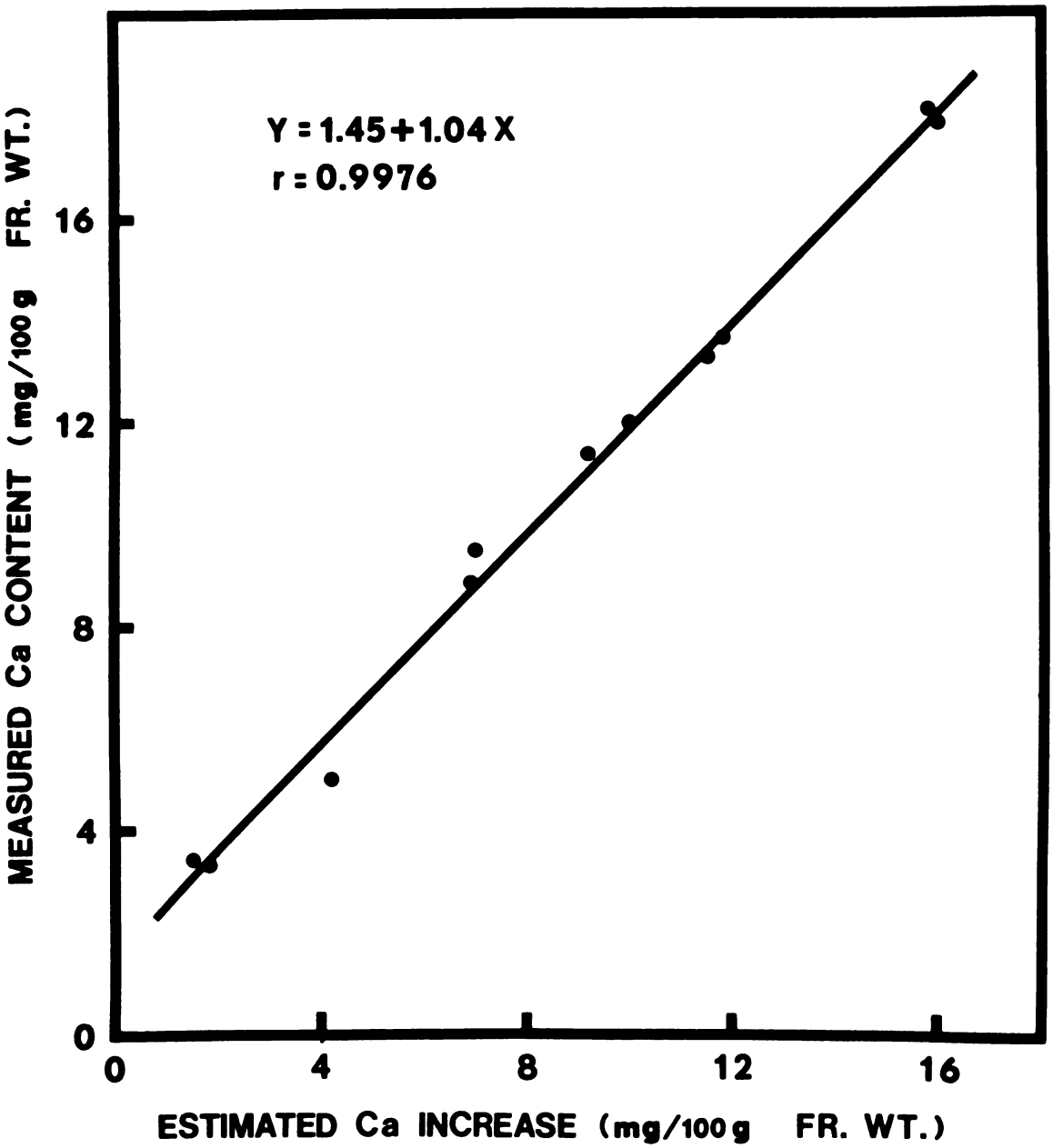
EXPERIMENT VI. THE RELATIONSHIP BETWEEN FRUIT WEIGHT GAIN AND Ca CONTENT IN THE FRUIT

Introduction: It was established in Exp. III. that Ca entered the fruit cortex with the solution, yet the amount of Ca entering the fruit relative to the weight gain was not determined. The relationship is herein studied together with an investigation of the validity of the estimation for the increase in Ca content of apple fruit based on weight gain, which is assumed to be the result of solution infiltration, in known concn of $CaCl_2$, as estimated in Appendix I.

Materials and Methods: Weight gains were measured for 30 fruits at a temperature of 21°C submerged in a solution of 2% $CaCl_2$ plus 0.1% L-77 at -1°C for 10, 20 and 30 min. Fruits with weight gains ranging from approximately 220 to 2300 mg/100 g fresh weight were chosen at an increment of approximately 400 mg/100 g fresh weight, each in duplicate. The Ca content of these fruits was determined by atomic absorption spectrophotometry, and linearly correlated with the estimated increases in Ca content based on weight gains. The Ca contents of 10 untreated fruits were also measured.

Results: The linear correlation of measured and estimated increases in Ca content for fruits with various amounts of weight gains was nearly perfect, $r = 0.9976$ (Figure 8). The slope of the regression equation was 1.04 with a 99% confidence interval of 1.10 to 0.98, which included the value of 1.00. It is concluded that for each unit of increase in

Figure 8. The relationship of measured total Ca content to the estimated increase in Ca content determined on the basis of weight gain for 'Jonathan' apple fruit hydrocooled in a solution containing 2% $CaCl_2$ plus 0.1% L-77, a surfactant.


Y = 1.45+1.04 X
r = 0.9976
MEASURED Ca CONTENT (mg/100g FR. WT.)
ESTIMATED Ca INCREASE (mg/100g FR. WT.)
0
4
8
12
16

estimated Ca, there is the same unit of increase in the measured Ca in the fruit. The intercept of the regression equation was 1.45 with an estimated S.E. of 0.014, which was not significantly different from the Ca content, 1.49 ± 0.042 mg Ca/100 g fresh weight, of the untreated fruits.

The total Ca content of the fruit, according to the regression equation, is the sum of the native Ca content and the estimated infiltrated Ca derived from the weight gain. It is obvious that the weight gain is a true reflection of the weight of the solution infiltrated into the fruit. Accordingly, the final Ca content of fruit hydrocooled in a solution containing a known concentration of $CaCl_2$ can be estimated with a reasonable degree of accuracy from the weight gain and the native Ca content of the fruit.

EXPERIMENT VII. SOLUTION INFILTRATION INTO APPLE FRUIT INDUCED BY PARTIAL VACUUM

Introduction: The fruits of Exp. I. had been warmed from 0 to 21°C before they were subjected to the submersion treatments in -0.5°C solution. There was the possibility that the warming had inflicted certain changes on the fruit which favored solution infiltration during hydrocooling. For example, it has been shown (7) that the number of open lenticels, which are the portal of entry for solution infiltration, may increase when fruits are exposed to an environment of low humidity. The relative humidity in the room in which the fruits of Exp. I. were warmed was 30 to 35% and 90 to 95% in the storage room.

Infiltration of solution into apple fruit can also be achieved by partial vacuum (43). From the gas laws it can be calculated that a 55 torr pressure drop has the same effect as a 21°C temperature drop on the volume change of an ideal gas (Appendix II). This moderate pressure drop should have little, of any, greater effect on the integrity of anatomical structure of the fruit than has 21°C temperature drop.

It was the purpose of this experiment to investigate the amount of solution infiltrated into the fruit before and after being warmed from 0 to 21°C. Since three different lots of apple fruit were used in this study, it was important also to determine if the apples had the same property regarding solution infiltration.

Materials and methods: A 55-torr pressure drop was applied to fruits submerged in an aqueous solution of 4% $CaCl_2$ containing 0.1% X-77, a

wetting agent, for 45 min. The partial vacuum was released slowly over a period of 1 min followed by 14 min of soaking before the termination of the treatment. The X-77 facilitated the escape of air from the open lenticels that would ensure subsequent solution infiltration. Thirty fruits from lot A at 0°C were treated in 0°C solution. Thirty other fruits of the same lot were warmed to 21°C, then treated in 21°C solution. Another 30 fruits from each of the three lots were warmed to 21°C and treated in 21°C solution. Weight gains were measured for individual fruits of all treatments.

Results: The mean weight gain of apple fruits at 0 and 21°C of 262 ± 28.2 and 328 ± 36.7 mg/fruit, respectively, were not significantly different. It has been shown that the only portal of solution entry for these apples is an open lenticel. Since it has been shown (Exp. VI) that the weight gain is the sole result of solution infiltration, it is evident that the status of open lenticels, both the total number of open lenticels of a fruit and the degree of opening of individual lenticel, was not affected by the temperature change.

The mean weight gain of fruits from the three lots were 328.6 ± 36.7, 307.8 ± 32.2 and 282.9 ± 35.7 mg/fruit. Since they were not significantly different from each other, it is indicative that they were of similar property pertaining to solution infiltration.

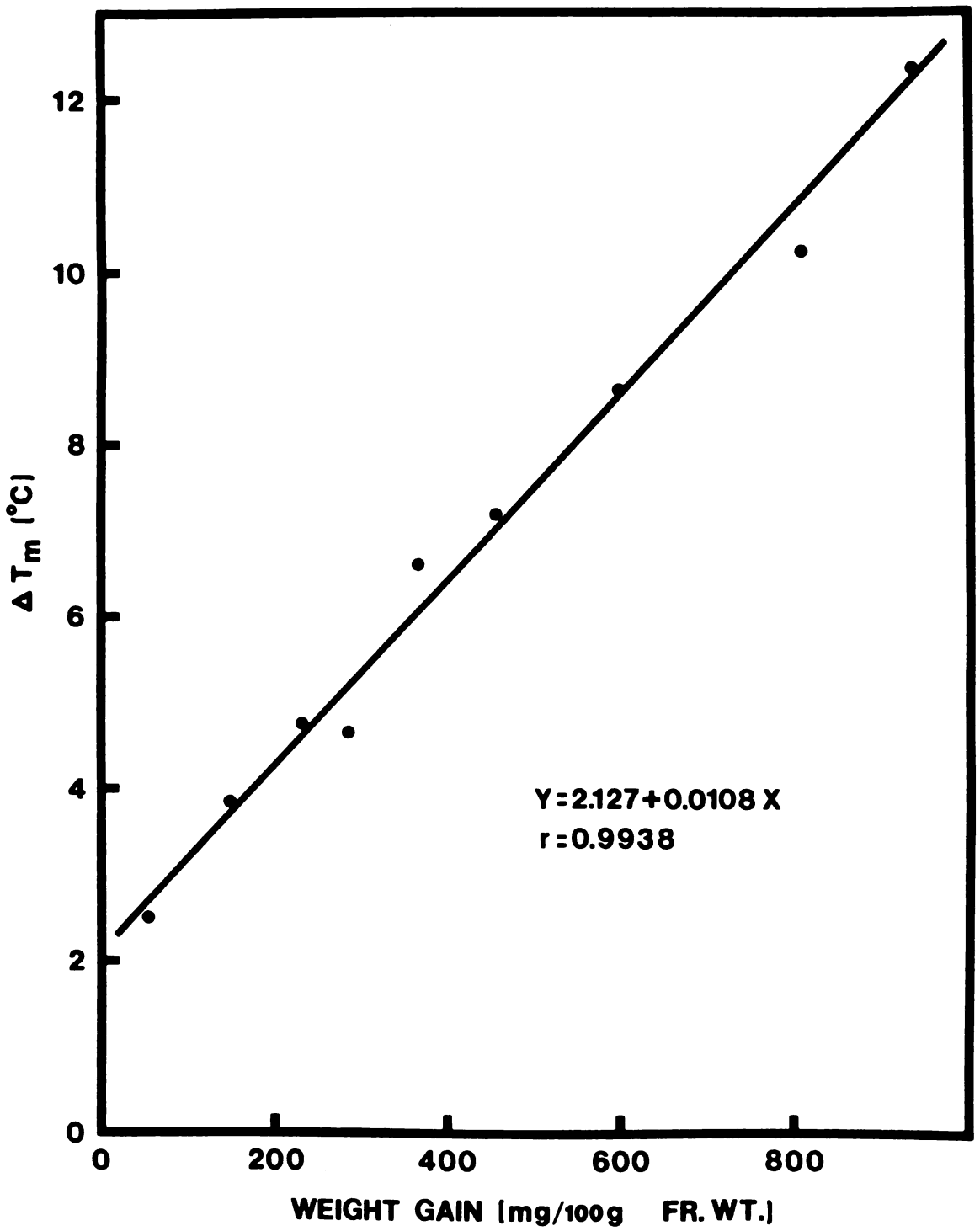
EXPERIMENT VIII. THE EFFECT OF FRUIT TEMPERATURE REDUCTION ON SOLUTION INFILTRATION

Introduction: In previous experiments an increase in weight gain of the fruit was affected by the initial temperature of the fruit and the length of the submersion duration, both of these factors affect the extent of fruit cooling. Weight gain induced by hydrocooling has been shown to be a result of the amount of solution infiltrated into the fruit cortex (Exp. VI). The volume occupied by the infiltrated solution, ΔV, is the difference between the structural volume of the ICS and the volume occupied by the cooled internal atmosphere. The maximum available ΔV can be estimated (Appendix V), and is shown (Appendix IV) to be a linear function of ΔT, the temperature reduction of the fruit. This experiment was designed to study the quantitative relationship between ΔT and fruit weight gain.

Materials and Methods: The change in temperature during cooling at the center of the fruit was measured by a thermometer sealed into the fruit under the same treatment condition as weight gain was measured in Exp. V. Duplicate fruits of 6.4, 6.7 and 7.0 cm in diameter were used in each cooling treatment. The mass average temperature, T_m, was then calculated using the information derived from the change of temperature at the center of the fruit during cooling (Appendix IV). The mean ΔT_m's of fruits of each cooling treatment were then correlated with the corresponding mean weight gains (Table 5).

Results: The ΔT_m's and weight gains obtained under similar cooling conditions are almost perfectly correlated in a positive linear fashion, r = 0.9938 (Figure 9). This provides further support that solution infiltration is induced by cooling of the fruit. Furthermore, the amount of solution infiltrated can be predicted from ΔT_m using the regression equation. Unfortunately, as shown by Kopelman et al. (18), the cooling of individual apple fruit, thus ΔT_m, cannot be predicted with reasonable accuracy from the initial temperature of the fruit, cooling medium temperature, cooling period and the size of the fruit. Otherwise, solution infiltration of 'Jonathan' apple fruit under various cooling conditions could be estimated by calculation. Nevertheless, the data of Table 5 should serve as a useful guide for the practical application of hydrocooling to enrich fruit with Ca.

Figure 9. The relationship between ΔT_m and solution infiltration measured as weight gain of 'Jonathan' apple fruit resulted from hydrocooling. $\Delta T_m = T_{mo} - T_{mt}$; whereby, T_{mo} = initial mass average temperature of the fruit and T_{mt} = mass average temperature at the end of submersion period t.


12
10
8
6
4
2
0
ΔTm [°C]
0
200
400
600
800
WEIGHT GAIN [mg/100g FR. WT.]
Y=2.127+0.0108 X
r=0.9938

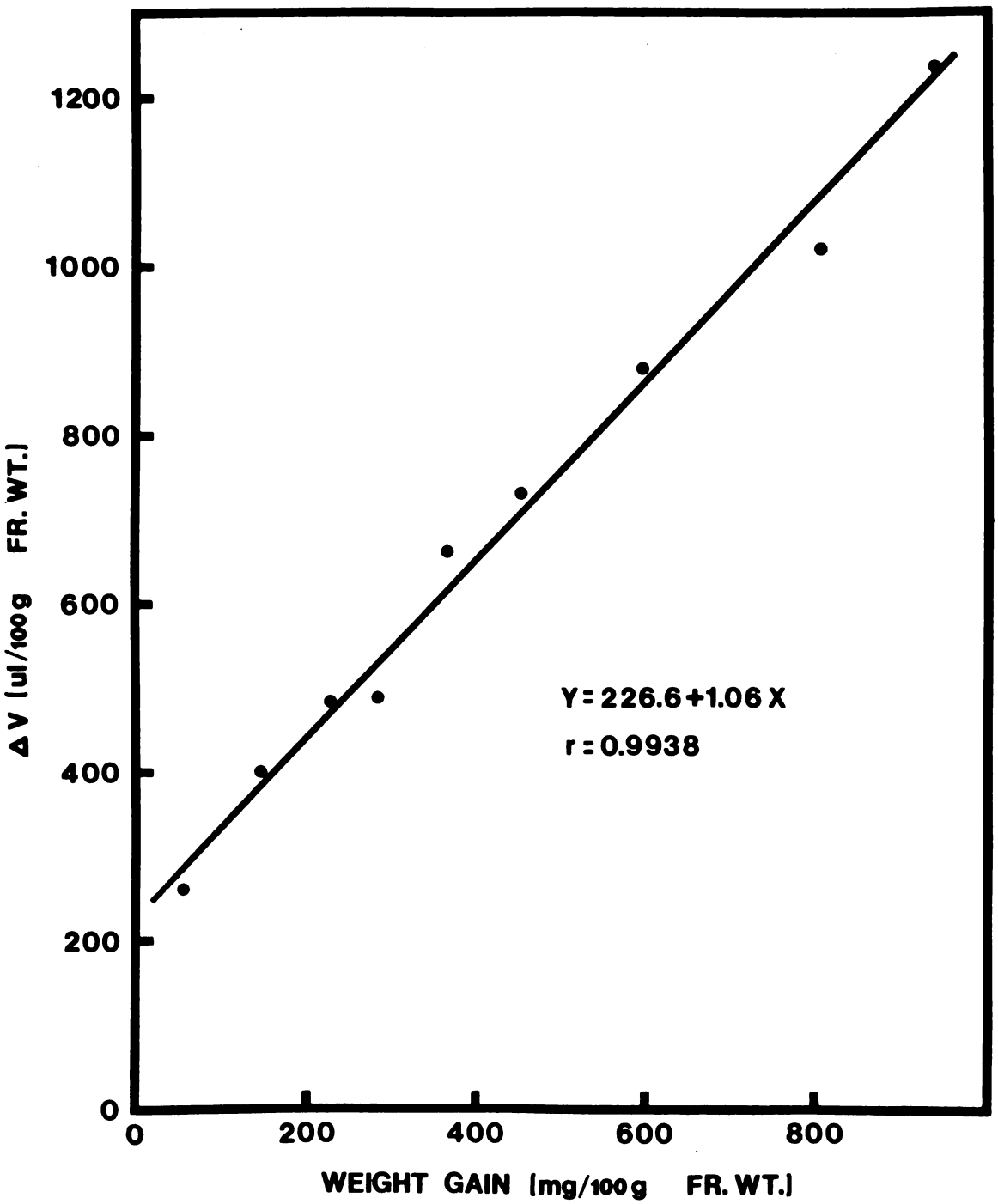
EXPERIMENT IX. THE RELATIONSHIP BETWEEN SOLUTION INFILTRATION AND THE VOLUME CHANGE OF THE GAS WITHIN THE INTERCELLULAR SPACES OF 'JONATHAN' APPLE FRUIT

Introduction: Anatomical examination (Exp. II) indicated that solution infiltrated into the cortex of the fruit was located in the intercellular spaces. Since it was shown that the amount of solution infiltrated was a linear function of ΔT_m (Figure 9) and that the maximum available ΔV was proportional to ΔT_m (Appendix III), weight gain, therefore, is also a linear function of the maximum available ΔV. A remaining question, however, is whether or not the maximum available ΔV induced by cooling is large enough to accommodate the volume of the observed amount of solution infiltrated.

Materials and Methods: The mean ΔT_m's and their corresponding mean weight gains in previous experiment were employed. The maximum available ΔV's were calculated from the ΔT_m's with certain assumptions, as detailed in Appendix V, and correlated with the mean weight gains in Table 5.

Results: The linear correlation between the maximum available ΔV and weight gain was nearly perfect ($r = 0.9938$), as shown in Figure 10. Since the density of 2% $CaCl_2$ aqueous solution is 1.01 (13), each mg of the solution should occupy 0.99 µl of volume. The regression coefficient of the regression equation is 1.06 with a 99% confidence interval of 1.15 to 0.97, which obviously includes 0.99. In other words, disregarding the value of the intercept, for each µl of the maximum

Figure 10. The relationship between the estimated maximum ΔV and solution infiltration measured as weight gain of 'Jonathan' apple fruit resulted from hydrocooling. See Appendix III and V for definition and determination of ΔV.


1200
1000
800
600
400
200
0
0
200
400
600
800
ΔV [ul/100g FR. WT.]
WEIGHT GAIN [mg/100g FR. WT.]
Y = 226.6 + 1.06 X
r = 0.9938

available induced by cooling, there is a corresponding mg of solution infiltration. The numerical value of the ΔV's in all cases were larger than that of the weight gains. It is seemingly evident that the maximum available ΔV induced by cooling can accommodate the volume of the observed amount of solution infiltrated into the fruit.

DISCUSSION

The intercellular spaces (ICS) within the fruit flesh is bounded by a continuous network of cell walls which can be considered as a semi-rigid matrix. The available air spaces (AAS) is defined as the combined volume occupied by the seed cavity and portions of the ICS that communicate to the ambient atmosphere through open lenticels. The gases in the AAS are cooled, when a warm apple fruit is submerged in a cold solution, the pressure within the AAS decreases. The cold solution is introduced into the readily accessible AAS when the pressure difference between the solution and the fruit AAS exceeds the frictional resistance of the open lenticels. This threshold pressure is related to the surface tension of the cooling solution and decreases as the surface tension is reduced. Further reduction in fruit temperature is associated with more infiltrated solution which occupies the volume, ΔV, the difference between structural volume of the AAS and the volume occupied by the cooled internal atmosphere. The fruit temperature reduction was measured as ΔT, the difference between the initial temperature (T_o) and the final temperature (T_t) of the fruit during the cooling period (t). The amount of solution infiltrated into the fruit was measured as weight gain, the difference in weight before and after the fruit was hydrocooled.

Studies (7, 16) of the ontogeny and anatomical structure of apple lenticels have shown the presence of open and closed lenticels which

are indistinguishable to the naked eye. Microscopic examinations (7) revealed that open lenticel had an aperture at the cuticle as compared to no aperture for the closed lenticels. There were connecting channels between open lenticels and the ICS of the cortical tissue for some of the open lenticels, which would permit the atmosphere within the ICS to be in continuum with the ambient atmosphere. This concept of continuum of atmosphere within and without the fruit is supported by evidence that a gas introduced under slight pressure into the seed cavity of an apple fruit was exhausted easily to the external atmosphere by Hoff and Dostal (14). Additionally, this provided evidence for the assumption (14) that open lenticels were the port of gas exhaustion, and to the conclusion by Burg and Burg (5) that gas exchange in apple fruit occurred through open lenticels.

It was found in Exp. I. that in the absence of ΔT, and thereby no ΔP, there was no substantial weight gain, even though the fruit was submerged in solution for a considerable period. The very small weight gains recorded for fruits in these treatments could have resulted from hydration of the fruit tissue itself. The existence of ΔT when warm fruit is submerged in cold solution caused the solution to infiltrate the cortical tissue which resulted in a substantial weight gain. Similar observations were made for 'Jonathan' apple fruit grown in Australia by Scott and Wills (42); however, contrary to our Michigan fruit, theirs had open calyx canals so that the infiltrated solution moved mostly into the seed cavity.

Exp. II. demonstrated that as the ΔT was gradually developed, solution infiltration was initiated at the open lenticels where the AAS is most accessible to the solution. Solution tinted with food dye

caused blue coloration around some lenticels, the same phenomenon as observed by previous researchers (7). Since the ΔP is developed in a direction perpendicular to the fruit surface and toward the center of the fruit, the solution is forced into the ICS of the cortical tissue immediately beneath the cuticle.

$CaCl_2$ as a solute in the cooling solution entered the fruit in an amount proportional to the quantity of the infiltrated solution. Consequently, the amount of Ca increase in the fruit could be accurately estimated based on the weight gain obtained in a solution of known concentration of $CaCl_2$. It is likely that other solutes and many suspended particles small enough to be accommodated by the passageways of the open lenticels and the ICS would enter the fruit with the solution. This direct infiltration of solutes into the fruit cortex would possibly permit the use of lower concentration of chemical in solution employed for other postharvest treatment of the apple fruit. For example, diphenylamin (DPA) is employed for the control of superficial scald, a common storage disorder of apple fruit throughout the world. Presumably, the uptake of DPA into the flesh of the fruit is accomplished by diffusion of DPA residue on the surface of the fruit across the cuticle. It was found that the incidence of scald was inversely correlated to the concentration of DPA in the fruit flesh (12). In order to achieve an adequate concentration of DPA in the fruit flesh, believed to be 8 ppm (12), 2000 ppm or higher concentration of DPA is used in the drench or dipping solution. With direct infiltration of DPA solution into the fruit, the concentration of DPA in the treatment solution could be dramatically reduced.

Fungicides are sometimes used in the drench or dip solution for postharvest treatment of apple fruits to prevent decay caused by certain fungi (11). If used in the cooling solution, it is expected that the fungicides be infiltrated into the fruit flesh. Attention should be paid to the fact that the fungicide residue in the fruit flesh does not exceed the maximum allowable concentration.

The uptake of Ca, supplied to the fruit as postharvest dip or drench, into the apple flesh has been proven (4, 47) to be the result of diffusion of Ca salt residue on the surface of the fruit across the cuticle. The superior effectiveness of $CaCl_2$ in controlling the disorders over other Ca salts is attributed by many researchers to its hydroscopicity. This physical property allows $CaCl_2$ to absorb sufficient amount of moisture from the humid storage room and, thus, exist as ions in solution for easier diffusion. The solution infiltration method possibly provides a means for reexamining the effect of other Ca salts in prolonging the storage life of apple fruit.

It was shown (Figure 9) that the amount of solution infiltrated into the fruit is a linear function of ΔT. The intercept of the linear equation, or the lag of the weight gain behind ΔT could be attributed to a threshold pressure, occurring as a result of certain characteristics of the open lenticels in conjunction with the surface tension of the solution, below which no solution infiltration was possible. Disregarding the intercept, it is apparent that increasing ΔT is accompanied by proportional increases in the amount of solution infiltrated. When the cooling medium temperature (T_1) is maintained constant, increases in initial fruit temperature, T_o, and submersion duration, t, cause and increase in ΔT, and hence increase the weight gain of the fruit.

Surfactants were employed to reduce the surface tension of the solution and thereby decrease the threshold pressure to be overcome for initiating solution infiltration through small apertures, as shown by Schönherr (41). This served as a means for shortening the submersion period (t) required to achieve a given amount of solution infiltration. The use of L-77, a surfactant that reduces the surface tension of pure water to 24 Newton (6), in the cooling solution resulted in a dramatic enhancement in weight gain (Table 4). The increase in solution infiltration achieved in this manner makes the hydrocooling method of postharvest Ca treatment for apple fruits a highly feasible possibility for practical use.

There are several factors that would affect the potential increase in Ca for apple fruit by the hydrocooling method. One is the concentration of Ca salt in the cooling solution. For a given weight gain, the Ca content of the fruit may be increased by increasing the concentration of Ca salt in the solution. The actual increase in fruit Ca was calculated (Appendix I) and verified in Exp. VI. for fruit hydrocooled in 2% $CaCl_2$ solution. It is anticipated that the increase in Ca content for fruit treated in solution with other concentrations of $CaCl_2$ or other Ca salts could be estimated equally well by modification of the formula used in Appendix I.

It was shown that the increase in Ca content of the fruit was a function of the amount of solution infiltrated into the fruit, therefore, factors affecting solution infiltration would also influence the increase in Ca content of the fruit.

It is likely that the ICS of an apple fruit consists of some isolated compartments without opportunity for direct gas exchange with

other part of the ICS or open lenticels (14). Most ICS, however, consists of interconnecting compartments linked to at least one open lenticel. The former type of ICS probably does not participate in the creation of ΔP, the driving force for solution infiltration, whereas, the latter type of ICS together with the seed cavity is greatly important in the creation of ΔP. It was derived from the gas laws (Appendix III) that the maximum ΔV was proportional to V_1, the assumed volume of AAS, as long as P and $\Delta T/T_1$ remain constant. Since it was shown (Figure 10) that the amount of infiltrated solution was a linear function of maximum ΔV, the size of V_1 obviously affects the amount of solution infiltrated into the fruit.

It is postulated that the combined effect of V_1 and the lenticel status, the number of open lenticels per fruit and the extent of individual lenticel opening, on solution infiltration could be measured by the relationship between weight gain and ΔT in a solution of given surface tension. This relationship deserves further investigation because it is likely to vary by variety, cultural conditions and other factors affecting the growth and development of the fruit.

The results of Exp. VI. in which surfactants were added to the cooling solution indicate that the weight gain is inversely proportional to the surface tension of the solution. This conforms with the knowledge (41) that lowering of the surface tension reduced the pressure required for initiating solution infiltration through small apertures.

The extent of fruit cooling is important in that weight gain is a linear function of ΔT (Figure 9), and furthermore, ΔT is a function of T_o, T_1 and t. Weight gain increases with increasing T_o and t, and with

decreasing T_1. Although the ΔT of any object with regular geometric shape and known thermal properties can be predicted accurately for any combination of T_o, T_1 and t, the cooling of 'Jonathan' apple fruit cannot be predicted with reasonable accuracy (18). If accurate cooling could be predicted, the weight gain of apple fruit induced by hydrocooling could be predicted for any combination of T_o, T_1 and t in conjunction with the characterized relationship between weight gain and ΔT of a given variety. Nevertheless, various amount of weight gain, and thus, the amounts of increase in Ca content, can be obtained experimentally for the combinations of T_o, T_1 and t, that are of practical usage. From this information, the desired amount of Ca and, possibly, any other water soluble chemicals could be infiltrated into the fruit by utilizing the appropriate combination of T_o, T_1 and t. Further studies in this area are needed before recommendations to growers can be made.

CONCLUSION

Solution infiltration into the cortex of 'Jonathan' apple fruit was induced by cooling the fruit submerged in a solution at lower temperature than the fruit. The solution entered the fruit via open lenticels and moved to the intercellular spaces in the cortex at the periphery of the fruit beneath the cuticle. The amount of solution infiltrated was measured as a weight gain of the fruit.

$CaCl_2$ and, presumably, all other water soluble chemicals, entered into the fruit with the solution. The amount of increase in fruit Ca was proportional to fruit weight gain.

Addition of a surfactant, such as L-77, greatly enhanced the rate of solution infiltration. Increase of fruit Ca up to 16 mg Ca/100 g fresh weight was readily achieved with the aid of L-77.

The amount of Ca increase of the fruit was affected by initial fruit temperature, temperature of the cooling solution, submersion duration, surface tension of the cooling solution, concentration of Ca salt in the solution and certain morphological characteristics, such as number of open lenticels per fruit.

A predetermined amount of increase in fruit Ca content could be achieved by subjecting the fruit to a specific hydrocooling condition. This hydrocooling method of postharvest Ca treatment for apples is a highly feasible possibility for practical use.

APPENDIX

Appendix I. The estimation of the increase in fruit Ca based on weight gain for apple fruit submerged in a solution of known $CaCl_2$ content.

P: purity of $CaCl_2$ in %.

C: concentration of $CaCl_2$ solution in %.

Δwt: weight gain of the fruit in mg.

wt: weight of the fruit before treatment in mg.

The proportion of Ca in $CaCl_2$ on a weight basis: 40/111 = 0.3604

The increase in Ca content in mg Ca/100 g fr. wt.:

$$(0.3604 \cdot \Delta wt/wt) \cdot 100 \cdot P \cdot 10^{-2} \cdot C \cdot 10^{-2}$$

$$= 3.604 \cdot 10^{-3} \cdot P \cdot C \cdot \Delta wt/wt$$

Example:

P = 93.6%. the purity of chemical grade anhydrous $CaCl_2$ used in the experiments.

C = 4%

Δwt = 328 mg

wt = 120 g

mg Ca/100 g fr. wt. = $3.604 \cdot 10^{-3} \cdot 93.6 \cdot 4 \cdot 328/120 = 3.69$

Appendix II. The effect of 55 mm Hg partial vacuum and 21°C temperature drop on the ΔV.

Under constant temperature,

$$P_1V_1 = P_2V_2 \quad (1)$$

When $P_1 = 760$ mm Hg, $P_2 = 760 - 55 = 705$ mm Hg

substituting these values into Eq. (1),

$$760\ V_1 = 705\ V_2, \text{ or } V_1 = 705/760\ V_2 \quad (2)$$

$$\text{Let } \Delta V = V_2 - V_1 \quad (3)$$

substituting Eq. (2) into Eq. (3),

$$\Delta V = V_2 - 705/760\ V_2$$
$$= (1 - 705/760)\ V_2$$
$$= 0.07\ V_2 \text{ or } \Delta V/V_2 = 7\%$$

The volume of gases within the fruit increases as the pressure is lowered. As gas expands part of it escapes from the fruit through open lenticels as air bubbles, which comprises 7% of V_2. Upon returning to 760 mm Hg, the ΔV can be replaced with the solution, and the proportionality is retained, i.e., 7% of V_1 can be replaced by the solution.

Under constant pressure,

$$V_1/V_2 = T_1/T_2 \quad (4)$$

When $T_1 = 273 + 21 = 294°K$, $T_2 = 273 + 0 = 273°K$

substituting these values into Eq. (4),

$$V_1/V_2 = 294/273, \text{ or } V_2 = 273V_1/294 \quad (5)$$

$$\text{Let } \Delta V = V_1 - V_2 \quad (6)$$

substituting Eq. (5) into Eq. (6),

$$\Delta V = V_1 - 273\ V_1/294$$
$$= (1 - 273/294)\ V_1$$
$$= 0.07 \text{ or } \Delta V/V_1 = 7\%$$

As the temperature of the fruit is lowered, the volume that the gas will occupy decreases if the pressure remains constant. This reduces the volume by 7% of V_1, and represents the maximum amount of volume the solution may occupy. Accordingly, a 55 mm Hg pressure drop and a 21°C temperature drop would cause approximately 7% of the volume of the intercellular spaces of the fruit to be the maximum available space that may be occupied by the infiltrated solution.

Appendix III. The relationship between ΔT_m and ΔV of 'Jonathan' apple fruit during cooling.

The maximum amount of volume (ΔV) available for solution infiltration induced by hydrocooling can be calculated as the following:

Under the condition of constant pressure, the gas law states that:

$$V_2/V_1 = T_2/T_1 \qquad (1)$$

where,

T_1 = initial temperature, T_2 = final temperature

V_1 = volume of gas at T_1, V_2 = volume of gas at T_2

Rearranging Eq. (1),

$$1 - V_2/V_1 = 1 - T_2/T_1, \text{ or}$$

$$(V_1 - V_2)/V_1 = (T_1 - T_2)/T_1 \qquad (2)$$

Let $\Delta V = V_1 - V_2$ and $\Delta T = T_1 - T_2$, where $T_1 > T_2$

Substituting ΔV and ΔT into Eq. (2)

$$\Delta V/V_1 = \Delta T/T_1, \text{ or}$$

$$\Delta V = (V_1/T_1) \cdot \Delta T, \text{ or } \Delta V = V_1 (\Delta T/T_1)$$

The equation states that ΔV is a linear function of ΔT.

In the case of these experiments, V_1 is the volume of space in the ICS, which is constant regardless of the temperature of the fruit.

Appendix IV. The derivation of mass average temperature from the temperature at the center of apple fruit.

The relationship between mass average temperature, T_m, and the temperature at the center of apple fruit, T_c, is given (17):

$$T_m = T_1 - K(T_1 - T_c) \quad \text{(a)}$$

Where, T_1 = The temperature of the cooling medium.

$$K = J_m/J_c = 3/\beta_1^3(\sin \beta_1 - \beta_1 \cos \beta_1) \quad \text{(b)}$$

Where, J_m = The lag factor for T_m.

J_c = The lag factor for T_c.

β_1 = The first root of the transcendental function,

$N_{\beta_1} = 1 - \beta_1 \cot \beta_1$, in radian.

and $$J_c = T_a/(T_o - T_1) \quad \text{(c)}$$

Where, T_a = The intercept of the regression equation of log $(T_{ct} - T_1)$ vs time t.

T_o = The initial temperature of the fruit.

$T_{ct} = T_c$ at time t.

T_a is obtained by taking the value of the intercept of the linear regression equation of log $(T_{ct} - T_1)$ vs t. J_c is calculated by substituting T_a into Eq. (c).

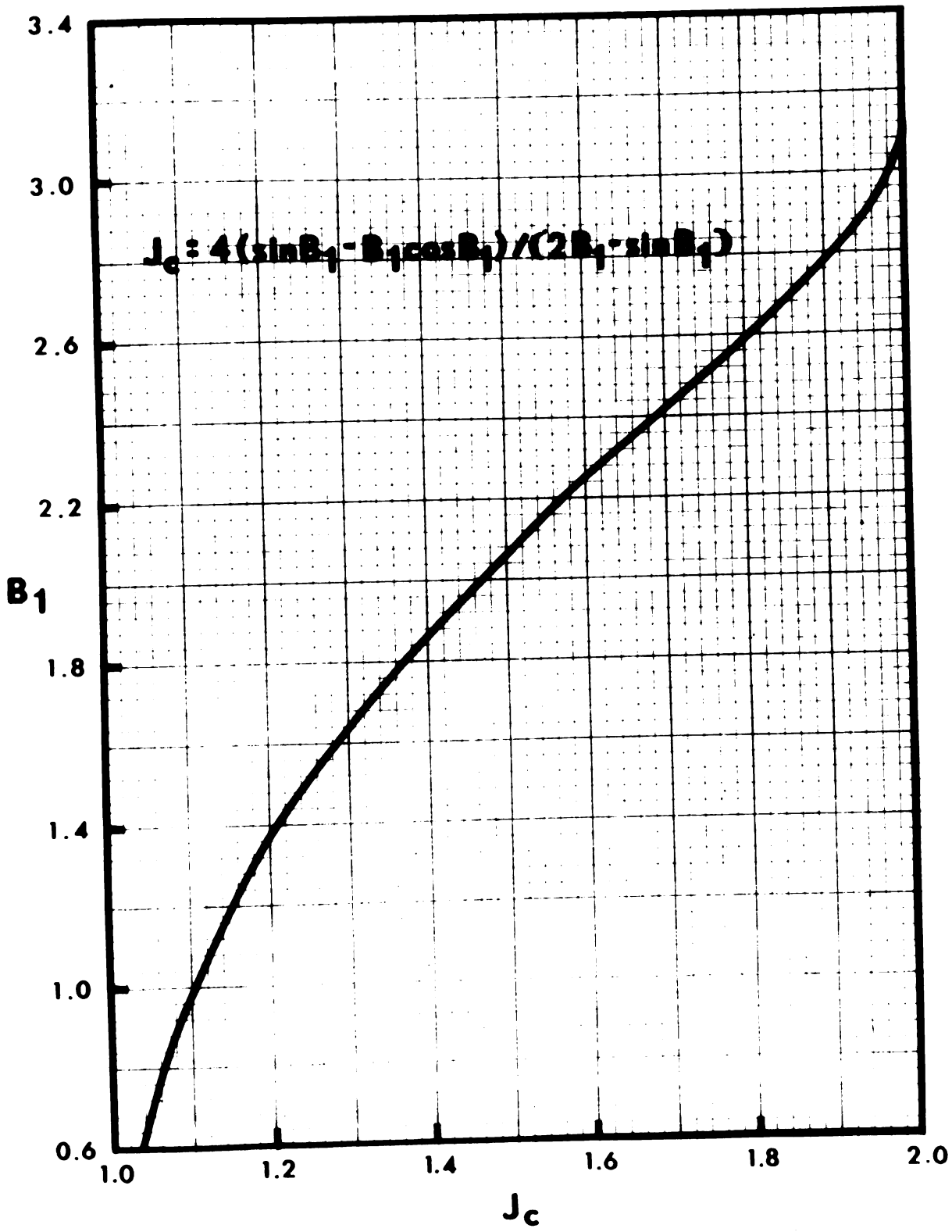
Given (17):

$$J_c = 4(\sin \beta_1 - \beta_1 \cos \beta_1)/(2\beta_1 - \sin 2\beta_1)$$

β_1 is estimated for a given value of J_c from a curve demonstrating the relationship between J_c and β_1 (26) as shown in Figure 11. β_1 is

substituted into Eq. (b) to calculate K, which is then substituted into Eq. (a) to calculate T_m.

Figure 11. The relationship between J_c and β_1. A graphical solution for the equation $J_c = 4(\sin \beta_1 - \beta_1 \cos \beta_1)/(2\beta_1 - \sin \beta_1)$


3.4
3.0
2.6
2.2
1.8
1.4
1.0
0.6
B_1
$J_c = 4(\sin B_1 - B_1 \cos B_1)/(2B_1 - \sin B_1)$
1.0
1.2
1.4
1.6
1.8
2.0
J_c

The T_{ct}'s tabulated below are the results for an apple fruit 6.7 cm in diameter with T_o = 67.9°F cooled in a solution at 40.1°F for 40 min. The T_m's of this fruit are calculated to illustrate the usage of the equations in the preceding page.

t	T_{ct}	$T_{ct} - T_1$	t	T_{ct}	$T_{ct} - T_1$
0	67.9	27.8	22	52.3	12.2
2	67.9	27.8	24	51.1	11.0
4	67.3	27.2	26	50.0	9.9
6	66.1	26.0	28	49.0	8.9
8	64.5	24.4	30	48.2	8.1
10	62.2	22.5	32	47.4	7.3
12	60.6	20.5	34	46.8	6.7
14	58.5	18.4	36	46.2	6.1
16	56.9	16.8	38	45.7	5.6
18	55.1	15.0	40	45.2	5.1
20	53.7	13.6			

$T_{ct} - T_1$ vs t is plotted on a semi-log graph paper as show in Figure 12. The regression equation for log $(T_{ct} - T_1)$ vs t is resolved for the straight line portion of the cooling data, i.e., from t = 8 to t = 32. The intercept of the regression is 37.52 which is T_a.

Substituting T_a = 37.52 into Eq. (c);

$$J_c = T_a/(T_o - T_1)$$

$$J_c = 37.52/(67.9 - 40.1) - 1.35$$

Referring to Figure 12, at J_c = 1.35, β_1 has the value of 1.76.

Substituting β_1 = 1.76 into Eq. (b);

$$K = 3/\beta_1^3 (\sin \beta_1 - \beta_1 \cos \beta_1)$$

$$K = 3/1.76^3 (\sin 1.76 - 1.76 \cos 1.76) = 0.7226$$

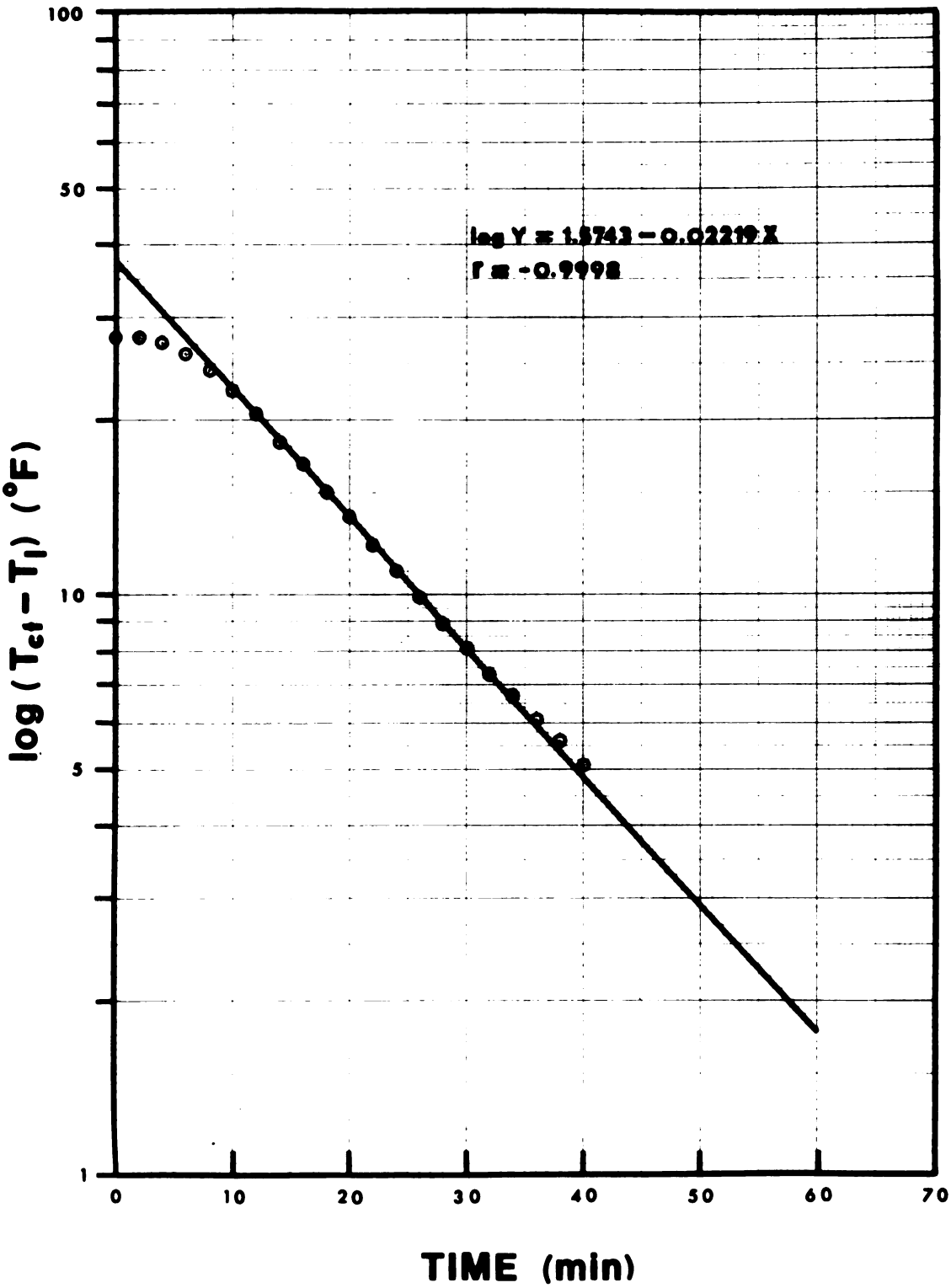
Substituting K = 0.7226 into Eq. (a);

$T_m = T_1 - K(T_1 - T_c)$, for T_c = 62.2, i.e., T_{ct} when t = 10.

$$T_m = 40.1 - 0.7226(40.1 - 62.2) = 56.36$$

The T_m's and ΔT_m's for all the cooling treatments at solution temperature (T_1) of 40.1°F are tabulated below for reference. All the

Figure 12. A typical cooling curve of 'Jonathan' apple fruit. T_o = 69.7°F, T_1 = 40.1°F.


log Y = 1.5743 − 0.0221 X
r = −0.9998
log (Tct − Ti) (°F)
TIME (min)
100
50
10
5
1
0
10
20
30
40
50
60
70

temperatures are given in °F. The ΔT_m's are converted to °C and then correlated with mean fruit weight gains in Table 5.

	Diameter of fruits (cm)								
	6.4		6.7		7.0				
	rep 1	rep 2	rep 1	rep 2	rep 1	rep 2	Ave	ΔT_m	ΔT_m(°C)
T_o = 50									
t = 10	45.50	46.26	46.75	46.09	46.82	46.07	45.50	4.50	2.50
t = 20	42.92	43.61	44.09	43.63	44.51	44.02	43.05	6.95	3.86
t = 30	41.55	42.18	42.52	42.10	42.92	42.62	41.55	8.45	4.69
T_o = 59									
t = 10	50.07	50.96	51.64	50.82	51.32	51.16	50.44	8.56	4.75
t = 20	45.74	45.85	46.68	47.15	47.16	46.82	46.02	12.98	7.21
t = 30	43.13	43.37	44.08	44.72	44.40	44.15	43.42	15.58	8.65
T_o = 68									
t = 10	56.02	54.58	56.36	55.76	56.34	55.86	56.09	11.91	6.62
t = 20	49.35	47.72	49.93	49.66	49.86	49.50	49.60	18.40	10.22
t = 30	45.33	44.33	45.95	45.78	46.00	45.49	45.75	22.25	12.36

Appendix V. The estimation of ΔV from ΔT_m.

The specific gravity of mature 'Jonathan' apple fruit was reported to be 0.807 (25). The soluble solid content of mature 'Jonathan' apple fruit is 12 to 17%. The specific gravity of 12 and 17% sucrose solution are 1.0465 and 1.0678, respectively (13), averaging 1.15715. The volume of 100 g fruit is estimated as:

$$100 \text{ g}/0.807 \text{ g cm}^{-3} = 123.916 \text{ cm}^3$$

The volume of the flesh of a 100 g fruit is estimated as:

$$100\text{g}/1.05715 \text{ g cm}^{-3} = 94.594 \text{ cm}^3$$

The difference between these two values should provide a reasonably good estimation of the volume (V_1) of the intercellular spaces plus seed cavity, which is:

$$123.916 \text{ cm}^3 - 94.594 \text{ cm}^3 = 29.322 \text{ cm}^3$$

The volume of the seed cavity is also considered, because it is physically connected to the CIS (14).

The ΔV's, the maximum available volume for solution infiltration, are calculated according th $\Delta V = \Delta T V_1/T_1$ (Appendix II) and tabulated as the following:

$T_1 = 283$		$T_1 = 288$		$T_1 = 293$	
ΔT_m (°C)	$\Delta V/(\mu l)$	ΔT_m(°C)	$\Delta V(\mu l)$	ΔT_m(°C)	$\Delta V(\mu l)$
2.50	259	4.75	486	6.62	622
3.86	400	7.21	734	10.22	1022
4.69	486	8.65	880	12.36	1236

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