Impact of Heating on Carrot Firmness: Contribution of Cellular Turgor†

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The firmness of carrot root disks was measured after various periods of heating in boiling water. Tissues lost more than half of their initial firmness within a few minutes. A second, much slower, phase of firmness loss continued for 10–15 min. The early phase of firmness loss could be mimicked by plasmolyzing carrot tissues in mannitol solution, and plasmolyzed disks showed only the second, slower, phase of firmness loss during cooking. Measurements of cellular turgor (turgor pressure probe) and membrane integrity (vital dye) indicate that turgor and membrane function are quickly lost during heating. When disks were incubated in a range of mannitol solutions, tissue firmness and cell turgor were linearly related.

Keywords: *Texture; carrot; turgor; plasmolysis*

INTRODUCTION

Texture is an important component of the food quality of fruits and vegetables. Loss of texture (firmness, crispness, etc.) during processing of horticultural commodities is often dramatic and has been the subject of a great amount of research. At the practical level, research has been directed toward modifying processing techniques so that more of the fresh product's texture can be retained. At the fundamental level, research has been directed toward understanding which characteristics (chemical and structural) of plant cells and tissues contribute to texture and how processing affects these. It is expected that the results of research at each level can provide useful insights at the other.

While both cellular turgor and the integrity of cell walls are thought to be the important components of the rigidity or firmness of plant materials (Ilker and Szczesniak, 1990), only the latter has been the focus of research on the effects of processing on texture. When carrots are processed at high temperature (>90 °C), tissue firmness is lost—rapidly in the first few minutes and then more slowly over the duration of the processing period (Bourne, 1989; Greve et al., 1994). Bourne (1989) has interpreted the biphasic pattern to be an indication that texture loss occurs by two distinct processes, each of which has its own rate constant. In this paper we report the results of experiments designed to test the role of cell turgor in carrot firmness loss during heating.

MATERIALS AND METHODS

Plant Materials. Carrots of the cultivar Danvers and the USDA breeding line B9304 (Simon et al., 1990) were grown near Modesto, CA. Roots were harvested at 5.5–7.5 months in the early morning and transported rapidly to Davis. Carrots not used immediately were cleaned and held at 0 °C until used (Greve et al., 1994). If a particular carrot type is not indicated, B9304 was used.

Carrot Heating. Carrot disks 1 cm in thickness and 2.1–3.0 cm in diameter were used in all heating experiments. Disks were routinely pretreated overnight in water or osmoticum (4 °C) prior to heating in boiling water or mannitol solution. After heating, disks were rinsed and cooled on ice. Firmness was measured (Greve et al., 1994) with an Instron Universal Testing Machine in the penetrometer mode.

Measurement of Cell Turgor Pressure and Cell Vitality. The pressure microprobe (Hüsken et al., 1978) was used to measure the turgor of parenchyma cells located in the central plane of the carrot disk, both in the core region, which contained vascular elements (inner tissue), and in the phloem region (outer tissue). To reach these cells, which were typically 40–120 μm in overall diameter, a disk was cut in half along its diameter and the pressure microprobe inserted to a depth of 0.6–1.5 mm below the cut surface. Water evaporation from all but the cut surface was minimized by wrapping with moistened tissue paper, and turgor measurements were performed under laboratory conditions (diffuse fluorescent light and 25–30 °C air temperature) and were completed within 30 min of removal of the disks from incubation. Over this period of time, there was no detectable change in cell turgor. Tissue penetration and meniscus behavior were observed at 200× through a vertically illuminated microscope with a long distance objective (Shackel et al., 1987). The methodology used was essentially the same as that described by Cosgrove and Cleland (1983), in which the cells near the cut surface were sacrificed to establish an observable meniscus external to the tissue. As was found by Cosgrove and Cleland (1983), after meniscus establishment, the measured turgor of sequentially penetrated cells (a minimum of three was used) was quite uniform (typically +0.2 bar). Microcapillary tips were pulled (Koph Model 750 micropipet puller) and widened and sharpened with a modified jet-stream microbeveler (Ogden et al., 1978; Shackel et al., 1987).

Cell vitality was evaluated using fluorescein diacetate (FDA) as described by Heslop-Harrison and Heslop-Harrison (1970). Longitudinal sections of 0.5–2 mm thickness were cut along a disk diameter and were incubated for 25 min in a water or 0.5 M sucrose solution of 5×10^{-6} M FDA. In all cases FDA staining results were the same using either solution. Stained sections were washed in distilled water for 5–10 min and examined under both transmitted and fluorescent light conditions. Vital cells were easily identified by a bright fluorescence.

RESULTS AND DISCUSSION

Because changes in carrot pectin characteristics begin relatively late during heating (after 6 min; Greve et al.,

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Table 1. Freeze–Thaw Treatment Effect on Carrot Firmness[a]

	B9304 genotype		Danvers genotype	
	inner tissue	outer tissue	inner tissue	outer tissue
control (no freezing)	2180 ± 110	2200 ± 151	2300 ± 220	2180 ± 83
treated	880 ± 89 (60%)	420 ± 28 (81%)	1090 ± 110 (53%)	415 ± 31 (81%)

[a] Firmness was measured in carrots subjected to a freeze–thaw treatment to disrupt cellular membranes. Data are in grams force (± standard deviation) for three measurements/treatment. Values in parentheses indicate the percentage reduction in firmness caused by each treatment.

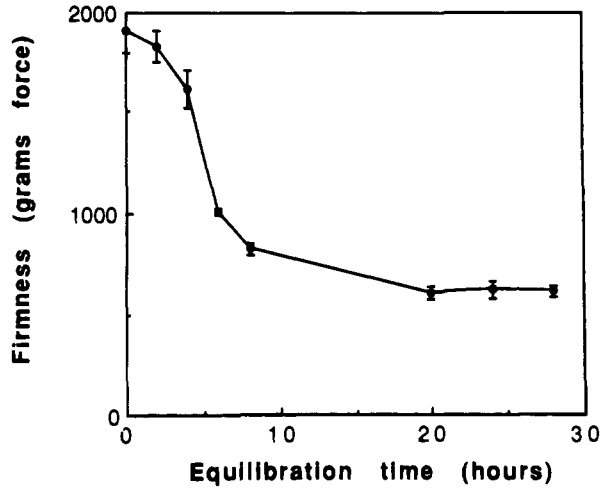

Figure 1. Changes in outer (phloem) tissue firmness of B9304 carrot disks immersed in 1 M mannitol and allowed to equilibrate over 24 h at 4 °C. Data are grams force ± standard deviation for three measurements.

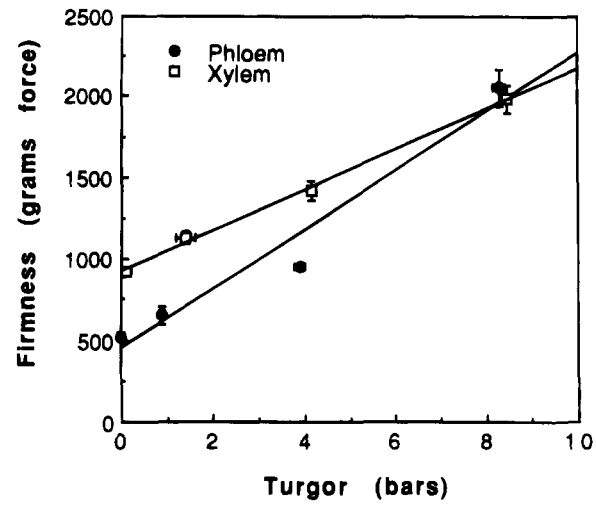

Figure 2. Changes in firmness (grams force) associated with various levels of cell turgor (bars) in B9304 carrot disks. Different levels of turgor were established by equilibrating disks in 0, 0.3, 0.6, and 1.0 M mannitol solutions for 24 h at room temperature. Each point represents the mean turgor and firmness (±1 standard deviation, respectively) for each solution, with separate linear regressions shown for xylem and phloem tissues. Regression equations were, for inner tissue, $Y = 924 + 124X$ ($r^2 = 0.99$) and, for outer tissue, $Y = 448 + 182X$ ($r^2 = 0.96$).

1994), a time after the rapid phase of firmness loss has occurred, we asked whether cooking effects on membrane integrity (and, therefore, cellular turgor) could contribute to firmness loss. Turgor can be eliminated by a freeze–thaw cycle or reversibly reduced by treatment with an impermeable osmoticum. The impacts of each of these treatments on uncooked carrot firmness were tested.

The freeze–thaw treatment reduced the firmness of outer tissues to a greater extent than it affected firmness of inner tissues (Table 1). This may be because the inner tissue contains the xylem which is likely to have a greater amount of secondary cell wall-containing cell types (vessel elements and fibers) than the phloem region. Because a freeze–thaw cycle might affect solubility of cell wall components (Reid et al., 1986) in addition to eliminating turgor, we also reduced turgor osmotically. Prior to reducing turgor osmotically, we measured the osmotic potentials of inner and outer tissues that had been disrupted by a freeze–thaw cycle using a vapor pressure osmometer (inner = 0.545 M; outer = 0.437 M) to be certain of the osmoticum concentrations to use. Samples of tissue disks were then placed in solutions containing different concentrations of mannitol. The firmness of disks treated with 1 M mannitol was measured periodically (Figure 1). It took more than 20 h for a new "equilibrium" level of firmness to be reached. Presumably, disks had reached a stable, near-zero level of turgor. This was tested by placing sets of disks in various concentrations (0, 0.3, 0.6, and 1.0 M) of mannitol for 24 h. Tissue firmness and turgor were then measured (Figure 2).

Again, the firmness of outside tissues was affected more greatly than that of inner tissues. In this case, turgor could be restored by placement of osmotically dehydrated disks in water for 16 h. Firmness values at least equal to those of controls were recorded. (Often turgor values exceeded those of controls; presumably because carrots fresh from storage were not fully turgid.)

Data in Figure 3 show firmness loss in carrots subjected to a typical cooking treatment. The "breakpoint" which marks the two phases of firmness loss comes in the region of 1000 g and is reached after 3–6 min of cooking. This is not much different from the component of tissue firmness that can be removed by reducing turgor to zero either by freezing (Table 1) or osmotically (Figure 2).

It is clear from the data in Figure 2 (involving treatments that do not affect wall integrity) that a great deal of the early (and steep) component of heating-induced firmness loss could be turgor dependent. We tested this by cooking carrot disks whose turgor pressure and, hence, firmness had been reduced by treatment in mannitol. Untreated carrots and those with intermediate reductions in turgor rapidly lost firmness—dropping to a typical breakpoint within 1 min (Figure 3), a time at which tissue internal temperature had reached only 50 °C [see Greve et al. 1994]. Disks that had no turgor (i.e., 1.0 M mannitol pretreated) showed no breakpoint. The slower phase of firmness loss for all sets of carrot disks followed the same (typical) time course (Figure 3). Microscopic examination of uncooked carrot disks treated with the vital dye fluorescein diacetate showed living cells throughout the tissue; no living cells could be identified after cooking of unplasmolyzed tissues for 1 min.

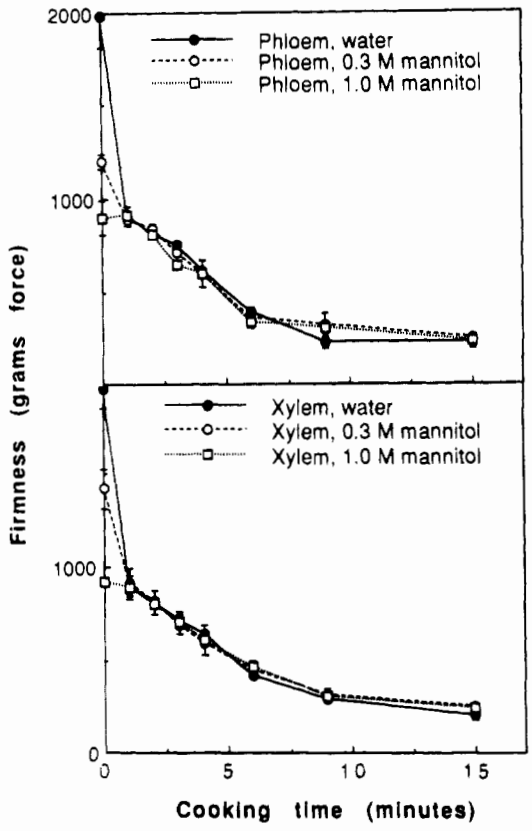

Figure 3. Changes in phloem and xylem tissue firmness of B9304 carrot disks heated in boiling water after an overnight equilibration in water or 0.3 or 1.0 M mannitol at 4 °C. Disks were cooled on ice and then held at room temperature prior to firmness measurement. Data are grams force ± standard deviations for three measurements.

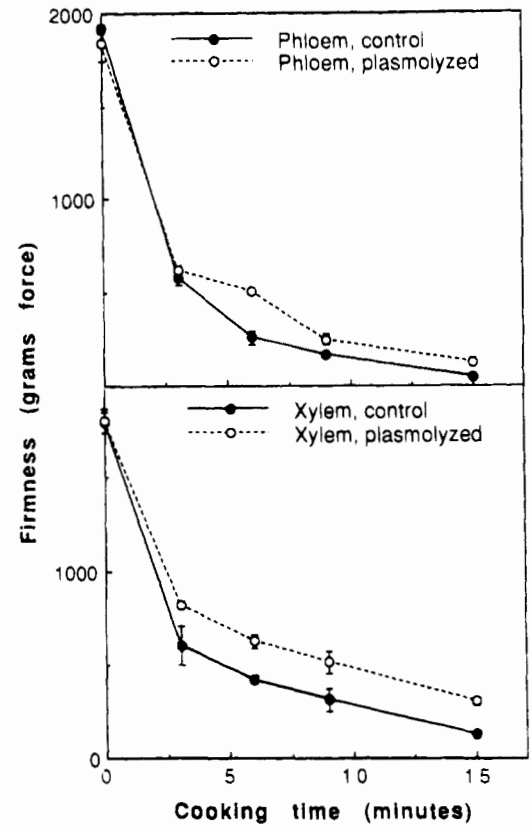

Figure 4. Changes in phloem and xylem tissue firmness of B9304 carrot disks equilibrated overnight in 1 M mannitol or water, boiled in the same solution, and then held in water overnight (4 °C) prior to firmness measurement at room temperature. Data are grams force ± standard deviations for three measurements.

We have observed that the firmness and turgor pressure of carrots vary with time in storage. To obtain more uniform results from a batch of carrots, disks were routinely cut the evening prior to cooking and held overnight in cold (4 °C) water. Tissue turgor and firmness generally increase during this period. Because turgor in the carrot cells is high prior to heating and the vital dye results indicated a rapid cooking-induced disruption of membranes, we asked whether heating under plasmolyzed conditions might "protect" membranes and, thus, alter the impact on firmness. Carrot disks were cut and divided into matched sets. One set was held overnight in the cold at high humidity. The other was treated in 1 M mannitol overnight. Disks were cooked as usual and chilled immediately after cooking. They were then placed in water (16 h, 4 °C) to rehydrate prior to firmness measurement (Figure 4). The firmness of tissues that had been cooked while plasmolyzed was, at all points, greater than that of untreated carrots. The turgor pressure microprobe was used to determine the effect of cooking. There was no turgor pressure in cells from untreated carrots that had been cooked for 3 min; cells of plasmolyzed tissues that had been rehydrated following 3 min of cooking gave low, but detectable, turgor measurements (0.15–0.3 bar), and some turgor could be measured even after 6 min of cooking. No turgor could be detected after 9 min of treatment.

CONCLUSION

The early, rapid phase of cooking-induced firmness loss in carrots is a consequence of membrane disruption which eliminates the turgor component of texture. The fact that tissues which have been cooked with reduced turgor tend to retain firmness better than "native" tissues suggests that texture retention performance could be modified by osmotic treatment prior to cooking.

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