

Improved Firmness in Calcified Diced Tomatoes by Temperature Activation of Pectin Methyl-esterase

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ABSTRACT: The effects of temperature and calcium on pectin methyl-esterase (PME) activity and texture in tomato pericarp material were examined. Heating thin slices of pericarp to temperatures between 50 °C and 75 °C led to the rapid evolution of methanol from the material, indicating an activation of PME. This activity was further stimulated when CaCl₂ (up to 2.0% w/v) was added. When applied to half-inch diced tomato pericarp, the same conditions that led to the activation of PME also improved firmness. Diced tomatoes treated for 5 min with 0.5% CaCl₂ at 70 °C were 2.5 times firmer than diced tomatoes treated with CaCl₂ at room temperature. This improvement in texture by treating with CaCl₂ at elevated temperatures was only apparent when the tomatoes received a subsequent 100 °C treatment. Heating tomatoes to 70 °C either before or after the CaCl₂ treatment also improved firmness through a subsequent high-temperature treatment, but to a lesser extent than heating during the CaCl₂ treatment. These results are consistent with the model that heating to 70 °C greatly increases PME activity, leading to extensive pectin de-esterification and increased calcium cross-linking of the pectins in the middle lamella. Production of thermally processed diced tomatoes with improved firmness should be possible by increasing the temperature during and after calcium treatment.

Keywords: tomato, diced, texture, pectin methyl-esterase, calcium

Introduction

A substantial portion (approximately 25%) of the 10 to 12 million metric tons of processing tomatoes grown in California are processed into high-value products such as diced tomatoes. Most of these diced tomatoes are thermally processed and aseptically packaged in bulk containers for use as ingredients in the manufacture of other products such as salsa and pizza sauce. The high temperatures needed to inactivate spoilage organisms, along with the shear stresses involved in pumping the material through the thermal processing unit, can seriously deteriorate the quality of the finished product. Maintaining a firmer texture and greater particle integrity will allow for better yields and improved product quality.

To improve the firmness of diced tomatoes, calcium salts are routinely added. In a typical commercial processing scheme, peeled tomatoes are cut into either 1/2- or 3/4-inch dice, drained of excess liquid, and then immersed in a calcium chloride solution. The calcium concentrations used and the duration of this dip vary, but typical values are 0.5% CaCl₂ for about 1 min. As was first shown many years ago (Loconti and Kertesz 1941), the mechanism for calcium-firming is through the interaction of calcium with the pectins in the cell wall. Calcium salts bind to blocks of free carboxylic acid groups along the polygalacturonic acid backbone of the pectin to form cross-links between pectin chains (Grant and others 1973). This increased cross-linking in the middle lamella leads to greater adhesion between cells and to a firmer texture.

The extent to which added calcium can improve firmness is determined by the degree of methyl-esterification of the pectin chains. In

tomatoes, as in other fruits and vegetables, the degree of pectin methyl-esterification is controlled by the enzyme pectin methyl-esterase (PME). Increasing the level of this activity will increase the number of calcium-binding sites in the pectins and thus allow for increased calcium cross-linking and better texture. It has been shown that adding PME, purified either from tomatoes (Castaldo and others 1995) or a microbial source (Grassin 2002), to diced tomatoes enhances the ability of calcium to improve firmness. A decrease in the degree of pectin methylation can also be achieved by activating the endogenous PME. One way to do this is to raise the pH of the diced tomatoes to 7 by adding base to the surrounding solution. At pH 7, PME is many times more active than at pH 4.5, typically found in tomato juice. Adjusting the pH in this way during the calcification process was shown to increase the firmness of diced tomatoes (Castaldo and others 1996). An increase in the endogenous PME activity can also be achieved by heat activating the enzyme. It has long been known that preheating vegetables to above 50 °C, in a so-called low-temperature blanch, activates PME and results in a firmer final product through subsequent high-temperature processing. This effect has been extensively studied and shown to occur in a number of vegetables (Bartolome and Hoff 1972; Van Buren 1979; Greve and others 1994; Stanley and others 1995; Ng and Waldron 1997; Stolle-Smits and others 2000; Ni and others 2004).

Only 1 published report indicates that temperature activation of PME occurs in tomatoes. Hsu and others (1965) showed that the firmness of calcified canned tomatoes was improved by applying a combination of a short blanching treatment (30 to 120 s at 100 °C), followed by a hold at room temperature (5 to 20 min). Analysis of extracted pectins showed that tomatoes, treated with the combination of blanch and hold times that gave the firmest texture, had pectins with the lowest degree of methyl-esterification. The explanation for this was that the short 100 °C blanch followed by a long

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hold caused internal portions of the tomato to reach some intermediate temperature that activated PME activity. The effect of blanching at temperatures less than 100 °C was not reported.

The effects of different times, temperatures, and calcium concentrations during a calcium dip of diced tomatoes on subsequent firmness have been reported (Floros and others 1992). Through response surface methodology, optimum conditions were found to be 0.43% CaCl_2 for about 3.5 min at ambient temperature. These authors found that increasing the temperature of the calcium dip up to 65 °C had no beneficial effect. This is surprising because one would expect that these elevated temperatures would activate PME, increasing calcium binding and improving firmness. In this study, firmness was measured immediately after the calcium dip, without subjecting the material to a subsequent high-temperature treatment. An important aspect of low-temperature blanching is that the improvement in texture is usually apparent only after additional high-temperature processing (Van Buren 1979). Because a high-temperature treatment is typically part of diced tomato processing, we have reevaluated the effects of elevated temperature during the calcium dip by looking at firmness following a subsequent high-temperature treatment, rather than immediately after the dip. We show here that temperature activation of PME does occur in tomatoes and that improved firmness can be achieved by increasing the temperature of the calcium dip.

Materials and Methods

Tomatoes

Heinz 9665 tomatoes, grown at the Vegetable Crops Experimental Station, Univ. of California, Davis, were used throughout. Tomatoes were harvested weekly and used within 3 d of harvest.

Determination of methanol production

Pectin de-esterification by PME activity was measured by determining the production of methanol (Anthon and Barrett 2004). Thin slices (1 to 2 mm thick) of tomato pericarp were obtained from near the middle of the tomato. In a 20-mL serum bottle, 18 mL of water was preheated to the desired temperature in a water bath, then 2 g of the sliced pericarp material was added. The bottle was closed with a rubber serum stopper and incubated at the desired temperature on a shaking water bath. At various time points, 0.1-mL aliquots of the water were removed with a Hamilton syringe, transferred to small sealed vials, and held on ice. To determine methanol content, 50 μL of this blanch water was then assayed with alcohol oxidase and Purpald as described (Anthon and Barrett 2004). To determine the endogenous methanol content of the tomatoes, PME activity was inactivated by heating before homogenization. Tomato halves were sealed in a Zip-Lock bag, then heated in a microwave oven (1200 W) for 1 s/g fresh weight. After cooling to room temperature, the material was homogenized, clarified by centrifugation, and the methanol content determined.

Diced tomato heat treatments

Tomatoes were immersed in boiling water for 1 min then immediately transferred to ice water and held for 2 min. The tomatoes were hand-peeled and diced into 1.27-cm (0.5-inch) pieces using a pair of serrated knives spaced 1.27 cm apart. Only the pericarp of the tomato was used. For each treatment, 20 pieces were placed in a wire basket, then immersed in a water bath at various temperatures containing various CaCl_2 concentrations as indicated. After 5 min, or other times as indicated, the tomatoes were removed and immersed in a room temperature water bath for 5 min. The diced tomatoes were then subjected to a high-temperature treatment by

immersing them in a boiling water bath for 5 min. After this, they were again cooled to room temperature by immersion for 5 min in a room temperature water bath. Texture was determined immediately after heat treatment.

Texture

Firmness of the treated diced tomatoes was measured with a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, N.Y., U.S.A.) in a compression test using a 25-mm-radius probe (TA-25). For each test, 3 pieces were placed under the probe and compressed to a final height of 3 mm and the maximum compression force determined. For each treatment, at least 6 replicate texture measurements were made for which the mean and standard deviation are given in the figures.

Statistical analysis

Statistical analysis of variance (ANOVA) was performed using Microsoft Excel. Comparison of means were performed using a least significant difference (LSD) procedure with significance defined as $P < 0.05$.

Results and Discussion

PME activity

Heating intact pieces of many types of vegetables to between 50 °C and 70 °C greatly increases PME activity and leads to an accumulation of methanol in the tissue (Bartolome and Hoff 1972; Stanley and others 1995). Because this methanol is produced outside of the cell in the cell wall, it will readily diffuse out of the vegetable tissue and into the surrounding solution. As we have previously shown, when pieces of vegetable material are immersed in water and heated to temperatures that activate PME, methanol rapidly accumulates in the surrounding solution (Anthon and Barrett 2004). A measurement of methanol accumulation in the surrounding solution is thus a simple way to monitor the increase in PME activity in intact tissue during heating.

When thin slices of tomato pericarp were heated to 70 °C, a rapid evolution of methanol was observed. A typical time course for methanol accumulation in the surrounding solution is given in Figure 1. After the rapid increase in the 1st 10 min, very little additional methanol was produced. Both the initial rate and final extent of methanol production were increased if the tomatoes were heated in 0.5% CaCl_2 instead of water. Initial methanol levels in these tomatoes were determined to be about 1.5 nmols/mg fresh weight. This indicates that the methanol we measured during heating was being produced during the incubation and was not the release of some preexisting pool. While we have no direct evidence that this

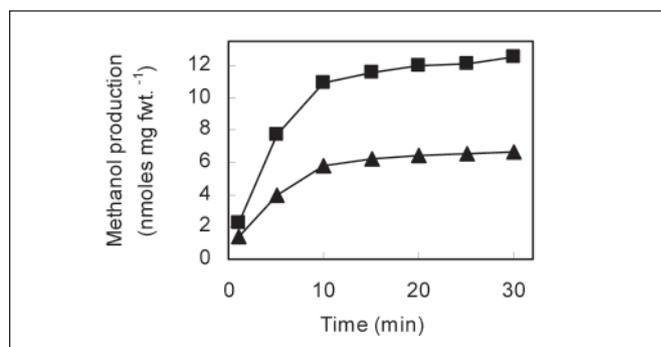


Figure 1—Production of methanol by heating tomato slices to 70 °C. Incubation in water (▲) or 0.5% CaCl_2 (■)

methanol production was due to PME activity, we know of no reaction other than pectin demethylation that has been shown to be a significant source of methanol in plants. The time course shown in Figure 1 is for a single tomato. The initial rate and final amount of methanol produced varied somewhat between different tomatoes. This is not surprising because methanol production would depend on the level of PME activity and the initial degree of pectin methylation, both of which would vary between different tomatoes.

The approximate initial rate of methanol production, estimated by the amount of methanol accumulated in the 1st 5 min of incubation, was determined as a function of the calcium concentration (Figure 2). Methanol production increased with the calcium concentrations up to 0.5% CaCl_2 (30 mM calcium). The activity of PME, purified from tomatoes and acting on pure pectin, is known to be increased by added calcium, but with a narrow optimum above which additional calcium inhibits (Lee and Macmillan 1968). We did not observe this inhibition by high calcium for the methanol production by tomato slices at 70 °C (Figure 2). The properties of PME in intact tissue, acting on the endogenous pectins, apparently differ from those of the purified enzyme acting on purified pectin substrates.

By measuring a suitably short time course, the initial rate of methanol production, and therefore an estimate of the PME activity, can be determined at different temperatures. Plotting this rate against temperature shows a significant increase at temperatures above 50 °C, indicating a large increase in PME activity above this temperature (Figure 3). As we have shown previously, in tomato juice, thermal inactivation of PME becomes significant at temper-

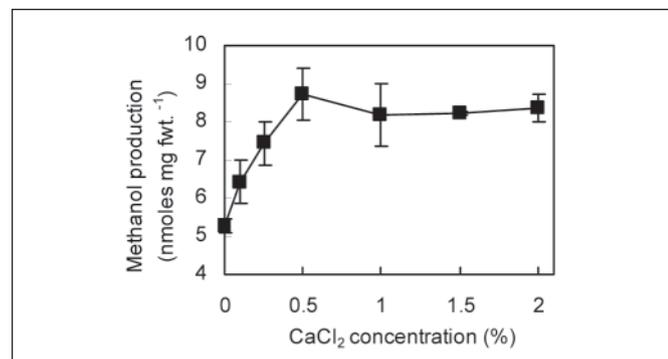


Figure 2—Calcium concentration dependence for stimulation of methanol production. Each point is the average of 3 determinations. Methanol production at each calcium concentration was significantly different ($P < 0.05$) from that in tomatoes with no added calcium.

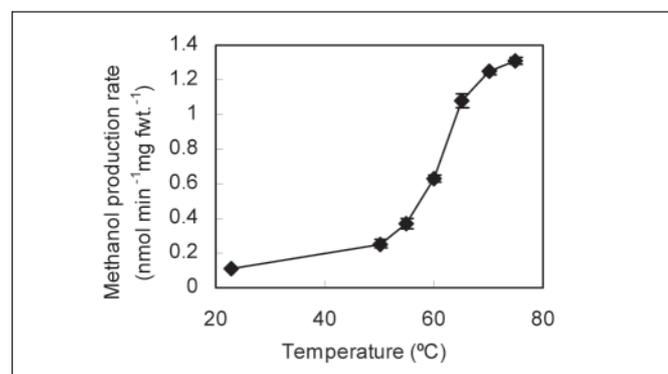


Figure 3—Temperature-dependence of methanol production.

atures above 70 °C (Anthon and others 2002). Partial inactivation of PME would explain why the measured initial rates at 70 °C and 75 °C are only slightly higher than that measured at 65 °C. Above 75 °C, the initial rate could not be measured due to the rapid inactivation of the enzyme. The large increase in PME activity above 50 °C is consistent with the temperature activation of PME observed in a number of other plant materials (Bartolome and Hoff 1972; Greve and others 1994a; Stanley and others 1995; Stolle-Smits and others 2000; Ni and others 2004).

Samples incubated in the presence of calcium produced a greater total amount of methanol after 30 min than samples incubated in water (Figure 1). A complication in this data is that at the temperature used, 70 °C, enzyme inactivation may be occurring. It is possible that the relatively small increase in total methanol accumulation after the 1st 10 min is simply the result of PME inactivation. To eliminate this complication, we repeated this time course experiment but at a lower temperature. At 65 °C, a sample incubated with calcium once again reached a higher level of total methanol, but at this lower temperature, 20 min were required for methanol production to be complete (Figure 4). If, however, calcium was added after 30 min, to a sample initially incubated in water, a burst of additional methanol was produced, raising the total level to about that of the sample containing calcium from the outset. This burst of activity indicates that the PME enzyme was still active after 30 min at this incubation temperature, and thus the lack of additional methanol production after the 1st 20 min was due to something other than loss of enzyme activity. The amount of methanol measured in the surrounding solution increased very rapidly following the addition of calcium, indicating that changes in PME activity within the tomato slices are rapidly reflected in the methanol content of this surrounding solution, supporting our contention that the measurement of this released methanol is a reasonable measure of the PME activity in the tissue.

The final level of methanol produced, and therefore the final level of methyl-esterification, was determined by the amount of calcium present during heating. This is inconsistent with a direct activation of the PME enzyme by calcium. Were the effect of calcium a direct enzyme activation, one would have expected samples with and without calcium to produce methanol at different rates but ultimately reach the same final level of methanol accumulation as all the available substrate was exhausted. The different final levels of methanol suggest that more substrate is available when calcium is added. This is consistent with the model that the effects of calcium and other cations on PME activity are through the interactions of the cations with the pectin substrate rather than a direct

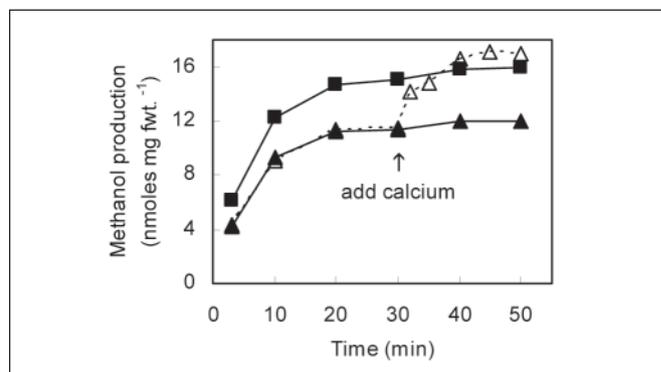


Figure 4—Methanol production at 65 °C. Tomato slices were incubated in water (Δ), in 0.5% CaCl_2 (■), or in water with CaCl_2 added after 30 min (▲).

effect on the PME enzyme (Lineweaver and Ballou 1945; Moustakas and others 1991). In this model, as PME de-esterifies the pectin, the number of free carboxyl groups in the pectin increases, causing the enzyme to become tightly bound to the pectin and thus inactive. Added cations such as calcium compete for these binding sites, reducing the binding of the enzyme to the pectin, and allowing for additional de-esterification to occur. The results in Figure 4 appear to be consistent with this model.

Texture effects

The effect of treating diced tomatoes at a series of temperatures, both with and without added calcium, on subsequent firmness was examined. Diced tomatoes were incubated for 5 min at various temperatures in either water or 0.5% CaCl₂. This relatively short incubation time was initially selected because longer times would be unrealistic for a commercial process. At all temperatures, the addition of 0.5% CaCl₂ led to a firmer diced tomato, consistent with the well-known firming effect of added calcium (Figure 5). Incubating the tomatoes at elevated temperatures, particularly above 60 °C, greatly improved the texture above that of the tomatoes that remained at room temperature. The optimum temperature for this effect was about 70 °C. While the increase in firmness from an elevated temperature treatment occurred in tomatoes incubated both with and without added calcium, the effect was always larger and more consistently observed in tomatoes treated with calcium. In the absence of added calcium, a peak of firmness was always observed at about 70 °C, but the difference in firmness between tomatoes treated at room temperature and at 70 °C was usually small.

PME activation (Figure 3) and texture improvement (Figure 5) showed similar temperature responses, which is consistent with the proposal that increased pectin de-esterification due to the action of PME results in better calcium cross-linking between cells and thus firmer texture. The 2 temperature responses were not exactly the same, however. For instance, by 60 °C, the activation of PME is substantial while the increase in firmness is still small. This difference may be partially due to the fact the PME measurements were made with 1- to 2-mm-thick pieces of tissue with rapid come-up times, whereas the texture measurements required larger 1.27-cm cubes with longer come-up times. The total time at the indicated temperatures is thus slightly less in the diced material, which may account for the reduced response below 60 °C. The extents to which

a 70 °C treatment increases in PME activity and improves firmness also differ. Treatment at 70 °C resulted in a 10-fold increase in PME activity over that at room temperature but only a 3-fold increase in firmness. This smaller increase in firmness versus PME activation is easily explained by the fact that a significant portion of the pectins are already de-esterified before any treatment. The high level of PME activity at 70 °C might lead to only a relatively small increase in the total amount of de-esterified pectin and thus only a small increase in firmness. There is also a difference between the 2 effects at 75 °C. In this case, methanol production was as high as at 70 °C, but firmness was significantly less. This may indicate that temperature-induced softening is greater at 75 °C, counteracting some of the firming effect of PME activity at this temperature. The time course for texture improvement by incubating in 0.5% CaCl₂ at ambient and elevated temperatures is indicated in Figure 6. At treatment times as short as 2 min, the tomatoes treated at 70 °C were significantly firmer ($P < 0.05$) than those treated at room temperature. The beneficial effect of the high-temperature treatment became even more apparent at longer treatment times. There was little additional effect of a calcium dip longer than 10 min at either temperature. The calcium concentration dependence for a 5-min treatment (Figure 7) indicated that at both ambient and elevated temperatures, added calcium, up to 0.5% CaCl₂, improved firmness with no apparent additional effect by higher calcium levels. This is similar to the concentration dependence of calcium in stimulating PME activity. Figure 6 and 7 illustrate the substantial increase in firmness obtained by treating with calcium at 70 °C over that obtained by treating with calcium at room temperature.

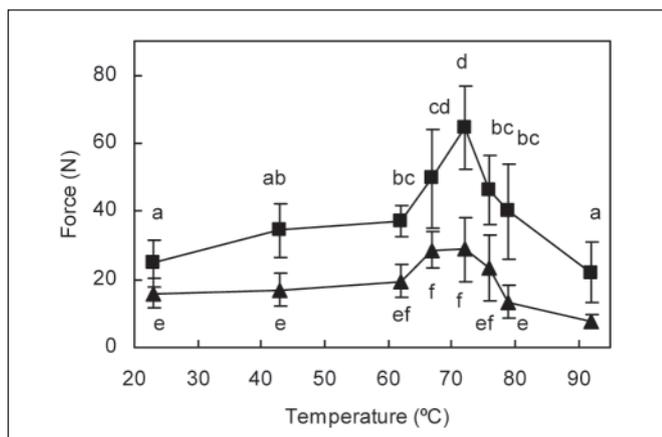


Figure 5—Diced tomato firmness plotted against pretreatment temperature. Tomatoes were pretreated for 5 min at the temperature indicated either in water (▲) or in 0.5% CaCl₂ (■). Points with same letter are not significantly different ($P < 0.05$).

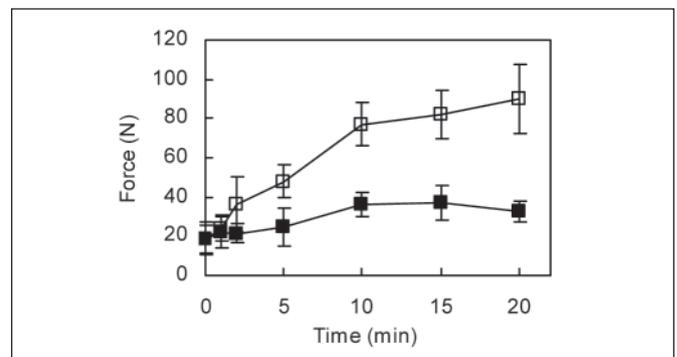


Figure 6—Diced tomato firmness plotted against treatment time in calcium. Samples were incubated in 0.5% CaCl₂ for the times indicated either at 23 °C (■) or at 70 °C (▲).

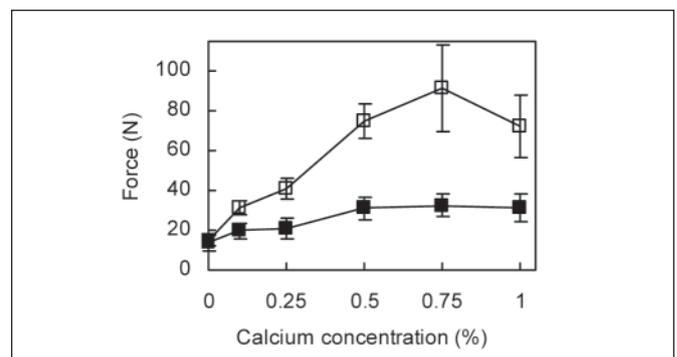


Figure 7—Diced tomato firmness plotted against calcium concentration. Samples were incubated for 5 min at the CaCl₂ concentration for the times indicated either at 23 °C (■) or at 70 °C (▲).

During diced tomato processing, tomatoes are typically mixed with acidified tomato juice after the calcium dip but before sterilization and aseptic packaging. This step can take several minutes and offers an additional point in the processing line at which elevated temperatures might be applied. To see whether heating at this, or some other step in the process, might be beneficial, we examined the effects of heating to 70 °C either before or after the calcium dip. Consistent with other experiments, the combination of 70 °C and 0.5% CaCl₂ gave a much firmer texture than that obtained with tomatoes receiving either treatment alone or receiving no pretreatment (Figure 8). Treating sequentially with a 0.5% CaCl₂ dip and then 70 °C in water gave an intermediate level of firmness. The same combination, but in the reverse order, a 70 °C treatment in water followed by a 0.5% CaCl₂ dip also gave an intermediate, although somewhat lower, level of firmness. Both sequential treatments indicate that heating either before or after the calcium dip would be beneficial. Heating to 70 °C in the presence of calcium was more effective than heating 1st and then adding calcium (Figure 8). This is easily explained by the fact that calcium enhances the de-esterification reaction by PME and thus more calcium binding sites are created when calcium is present during the heating. Calcium also inhibits polygalacturonase activity (Buescher and Hobson 1982). It is possible that if heating is done in the absence of calcium, some of the de-esterified pectin will be cleaved by polygalacturonase activity, reducing cell wall strength and thus decreasing firmness.

Our results show that a calcium treatment at elevated temperatures is far more effective than a calcium treatment at room temperature in improving firmness. This is in contrast to the conclusion of Floros and others (1992) that the temperature of the calcium treatment has no effect. The principal difference between our methods and theirs is that we subjected our tomatoes to a high-temperature treatment after the calcium dip whereas they did not. To determine the importance of this difference, we pretreated diced tomatoes with 0.5% CaCl₂ at various temperatures and then heated half of them for 5 min at 100 °C and held the other half at room temperature. The textures of the 2 groups were then compared. The 100 °C treated tomatoes showed a peak of firmness at a calcium pretreatment temperature of about 70 °C (Figure 9), in agreement with the results in Figure 5. The tomatoes not receiving the 100 °C treatment still showed a peak in firmness at a calcium pretreatment temperature of 70 °C, but this peak was obscured by the overall decline in firmness with increasing calcium pretreatment temperature. As a consequence, in the tomatoes not receiving

the 100 °C treatment, the firmness of the sample pretreated with calcium at 70 °C was now lower than the firmness of the sample pretreated with calcium at room temperature. Thus, if one looks at the firmness of the tomatoes before high-temperature processing, rather than after the final high-temperature processing step, the beneficial effect of elevated temperatures in the calcium dip will be missed. This would explain the earlier conclusion that the temperature of the calcium dip does not affect firmness.

Loss of texture during heating of vegetables has been shown to occur in several phases. A fast phase, which occurs in about 1 min at temperatures near 100 °C, can be attributed to a loss of turgor resulting from the breakdown of the cellular membranes (Greve and others 1994b). A 2nd slower phase results from changes in cell wall structure, particularly from β -eliminative cleavage of pectins in the middle lamella (Greve and others 1994a; Ng and Waldron 1997; Stolle-Smits and others 2000). Under the conditions used here, it is likely that turgor loss is the principal factor responsible for the texture loss in the diced tomatoes. We observed that the texture of our diced tomatoes was reduced to about 1/3 of the original level after 1 min at 100 °C with very little additional change over the next 4 min (data not shown). This agrees with earlier results in which diced tomatoes, held at 88 °C, showed a greatly reduced firmness in the 1st min of heating and then remained almost unchanged over the next 10 min (Ma and Barrett 2001). Turgor loss at temperatures less than 100 °C would explain the declining texture with increasing pretreatment temperature of the tomatoes not receiving the 100 °C treatment (Figure 9).

With low-acid vegetables, such as carrots and green beans, it has been shown that pectin cleavage by β -elimination is a major contributor to texture loss during high-temperature processing (Greve and others 1994a; Ng and Waldron 1997; Stolle-Smits and others 2000). Reducing the extent of pectin methylesterification by activating PME in a low-temperature blanch reduces the extent of this reaction because β -elimination can occur only at esterified galacturonic acid residues (Krall and McFeeters 1998). Reduced β -elimination has been proposed as the principal mechanism by which a low-temperature blanch (LTB) improves texture in canned green beans and carrots (Greve and others 1994b; Stolle-Smits and others 2000). There are several reasons to believe that this is not the case with tomatoes. The β -elimination reaction is highly pH-dependent and would be expected to occur at a very low rate at the pH of tomatoes (Krall and McFeeters 1998). In the studies of green beans and carrots, the material was subjected to higher temperatures for

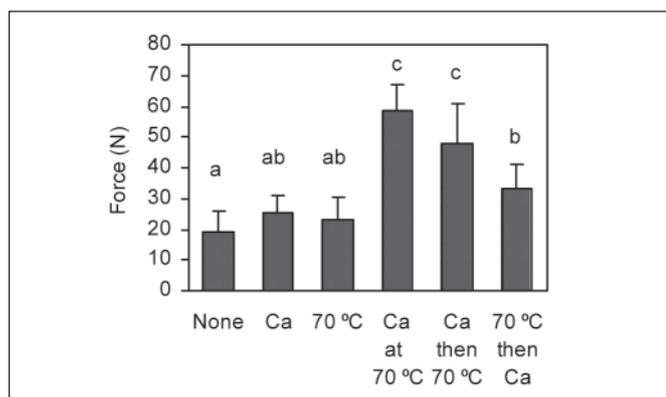


Figure 8—Treatment with calcium either at room temperature or at 70 °C. Calcium treatments were done either before, during, or after heating to 70 °C. Treatments with same letter are not significantly different ($P < 0.05$).

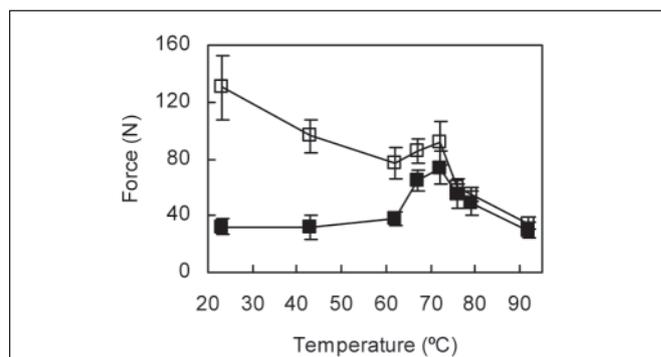


Figure 9—Diced tomato firmness plotted against pretreatment temperature. Tomatoes were pretreated in 0.5% CaCl₂ at the temperatures indicated for 5 min and then cooled to room temperature. Texture was measured without any further heat treatment (□) or after heating to 100 °C for 5 min (■).

longer periods than were used with tomatoes in this study. This harsher temperature treatment would also favor more β -elimination. If a reduction in β -elimination was a significant factor in the texture improvement by an LTB of diced tomatoes, one would expect to see a significant improvement in texture in the absence of added calcium when in fact only a small (Figure 5 and 8) or no (Figure 7) improvement was seen. It is also significant that there was very little difference in texture between raw and cooked tomatoes that received pretreatment temperatures above 75 °C (Figure 9). This shows that, at these temperatures, the heating in the pretreatment alone was sufficient to cause almost all the texture loss. Further texture loss by β -elimination in the subsequent 100 °C treatment does not appear to be significant.

Conclusions

The improvement in firmness of diced tomatoes, caused by the addition of calcium, was greatly enhanced when the calcium dip was done at elevated temperatures rather than at room temperature. At elevated temperatures, a large efflux of methanol from the material was also observed. These results are consistent with the model that says that at temperatures of approximately 70 °C, PME activity greatly increases and leads to a substantial decrease in the degree of methylation of the pectins. This creates an increased number of calcium binding sites, allowing for increased calcium cross-linking of the pectin of the middle lamella. Greater cell-to-cell adhesion results and improves the firmness of the diced tomatoes. The greatest improvement in texture was achieved when the tomatoes were heated to 70 °C in the presence of 0.5% CaCl_2 for 10 min or more. While such a long hold time in the calcium bath may not be practical for a typical processing plant, shorter hold times still improve texture. Furthermore, heating after the calcium dip also improved texture (Figure 8). As a practical matter, it might be possible to use a relatively short-duration calcium dip (1 to 2 min), as is current practice, but at an elevated temperature. If the tomatoes were then held at the elevated temperature through subsequent mixing with juice, additional improvement in texture would likely occur.

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