EFFECTS OF RAW MATERIALS AND PROCESS VARIABLES ON THE HEAT PENETRATION TIMES, FIRMNESS, AND PECTIC ENZYME ACTIVITY OF DICED TOMATOES (HALLEY BOS 3155 *CV*)

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ABSTRACT

The effects of raw materials and process variables on the heat penetration times into diced tomatoes (Halley Bos 3155 cv) were evaluated. Variables included dice size (1.27 and 2.54 cm), maturity at harvest (red and red+2 weeks), and processing temperature (88 and 92C). Heat penetration times between dice sizes were significantly different, but not between maturities or processing temperatures. Tomatoes were also evaluated for firmness, pectin-methylesterase (PME) and polygalacturonase (PG) activities. Half-inch size diced tomatoes were processed at 88 and 92C, and evaluated for firmness using the shear-compression method. Firmness decreased to 60% of the initial raw firmness from 8.8×10^5 to 5.3×10^5 g-mm after 15 s at 88C, and to 50% from 8.8×10^5 to 4.4×10^5 g-mm after 15 s at 92C. Diced tomato firmness showed a slight firming trend after 150 s at both temperatures. PME was inactivated after 45 s, while 5% residual PG activity remained after 3 min.

INTRODUCTION

Diced tomatoes have been the focus of much attention since salsa replaced ketchup as the top-selling condiment in the United States in 1991 (Poole 1992). Approximately 80% of the diced tomatoes produced today are thermally processed and filled into cans, drums, and aseptic bags (Gould 1992) to be used at a later date for remanufacture into higher-value final products. This initial thermal processing subjects diced tomatoes to high temperatures and shear stresses through pumps, pipes, strainers, pressurized tanks, and fillers (Gould 1992). There is a resultant decrease in textural integrity (Porretta *et al.* 1995), which may affect the quality of finished tomato products.

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Although high temperature treatments contribute to decreased textural integrity, thermal treatment of diced tomatoes is necessary to kill spoilage microorganisms such as *Bacillus coagulans*, and to inactivate softening enzymes, in particular pectinmethylesterase (PME, pectin pectylhydrolase, EC 3.1.1.11), and polygalacturonase (PG, poly-α-1,4-galacturonide glycanohydrolase, EC 3.2.1.15; 3.2.1.67). PME and PG act together to break down pectic substances in the middle lamella and soluble cell wall pectin of tomato tissue. PME catalyzes the hydrolysis of methyl esters, thus allowing the deesterified galacturonic acid chain to become susceptible to the depolymerizing action of PG (Pressey and Woods 1992). Pectic substances are important constituents located in the middle lamella and cell walls and any changes can influence fruit and vegetable texture (Bourne 1989). Furthermore, tomato processors are concerned whether different harvest maturity requires different processing conditions. Presently, processors harvest the entire tomato plant, which may contain a range of red-ripened fruits (i.e. mature and overmature fruits). Color is not a sensitive indicator of red ripe tomato maturity, as fruit remain red even when overmature. Barrett and Garcia (1997) also conducted one of the few studies which document different physical attributes of red, red+2 weeks, and red+3 weeks maturity processing tomatoes.

Another question that tomato processors would like to address, is how changes in dice size may affect the length of processing time. According to the USDA Standards for Canned Tomatoes, processors must dice tomatoes into uniform cubes (USDA 1990). These cubes usually range from $3/8 \times 3/8 \times 1/2$ in^3 ($13 \times 9.5 \times 9.5$ mm^3) to $1 \times 1 \times 1$ in^3 ($25 \times 25 \times 25$ mm^3) in the California tomato industry.

Firming diced tomatoes using calcium salts during processing is a standard process in the industry (Gould 1992). The divalent calcium salts increase the rigidity of the middle lamella and cell wall (Grant *et al.* 1973), by binding PME-demethoxylated pectate chains to form calcium pectate or pectinate (Kertesz 1939a,b) and thus increasing resistance of pectin to PG attack (Buescher and Hobson 1982).

The objective of this experiment was: to document the effects of processing temperature, dice size, and maturity on heat penetration times, firmness, and PME and PG activities in processed diced tomatoes.

MATERIALS AND METHODS

Raw Materials

Halley Bos 3155 cultivar tomatoes, grown at the Vegetable Crops Experimental Station at the University of California-Davis, were used based on a previous study comparing various California processing tomato cultivars

(Barrett and Garcia 1997). Halley Bos 3155 exhibits good firmness, large fruit size, and good peelability.

Tomatoes were tagged using twist ties at the USDA pink stage (stage 4), which is defined as when the surface of the tomato fruit is at least 30% but no more than 60% pink or red. Uniformly sized tomatoes without visible defects were tagged and harvested from the second set of trusses on each plant. One half of the tagged fruits were harvested one week later at approximately the USDA red stage (stage 6). Two weeks following the red stage harvest, the remainder of the tagged tomatoes were picked and labeled as 'red +2 weeks'.

All chemicals were obtained from Fisher Scientific (Fair Lawn, NJ), except for pectin (from citrus fruits), polygalacturonic acid, disodium 2,2' bicinchoninate, L-serine, and galacturonic acid ordered from Sigma Chemical Co. (St. Louis, MO).

Processing Operations

Tomato fruit were washed in industrial use water, submerged for 40 s in boiling water, and immediately transferred for 20 s into ice water to induce cracking in the tomato skins. The tomatoes were hand peeled and diced into 1.27 cm (0.5 inch) or 2.54 cm (1 inch) cubes using two serrated knives spaced at the desired fixed distance. Intact solid pieces with no missing locular or core material were selected from those cut for experimental use.

Five insulated copper-constantan thermocouples (type T-24 gauge) with soldered ends were inserted into the center of five individually diced tomatoes to measure the internal temperature of each cube, following the method of Chang and Toledo (1990). The wire was bent to form a tight U-shape, which served as a physical restraint in preventing the thermocouple from penetrating the diced tomato past the center point. The soldered end was inserted into the center of the dice tomato, and the U-shaped portion was tied firmly to it using cheesecloth. The free ends of the five thermocouples were attached to a datalogger (Molytek Co./Partlow-West Co., New Hartford, NY), which recorded the individual internal diced tomato temperature every 16 s. A sixth thermocouple was used to monitor the water bath temperature, which was either 88 or 92C. The five diced tomatoes and attached thermocouples were dipped into a 10% calcium chloride solution maintained at 35C ($\pm$ 2C) for 1 min.

Diced tomatoes were cooked to a final geometric center temperature of either 88 or 92C in an insulated and circulating water bath (model PC+20B; Julabo USA, Kutztown, PA) containing deionized water pumped at 2 L per min. A clear rectangular fiberglass lid was designed to fit over the water bath to insulate the temperature, as well as hold the thermocouple and diced tomatoes in place. Five holes, each the size of a No. 6 rubber stopper, were cut out into the four corners and center of the fiberglass lid. Lengthwise cuts were made

halfway into five No. 6 stoppers, and each thermocouple with an attached diced tomato was inserted lengthwise into a rubber stopper. Each diced tomato with attached thermocouple was placed through its designated hole in the fiberglass lid, and the lid was then placed over the water bath. The internal temperature profiles of all five diced tomatoes were recorded using a Molytek datalogger (Partlow-West Co., New Hartford, NY).

The heat penetration time into the geometric center of diced tomatoes were evaluated at two processing temperatures using tomatoes of two maturities cut into two dice sizes (Table 1). Each process combination was carried out in triplicate. Analysis of variance tests were calculated to find significant differences ($p < 0.05$) between dice sizes, harvest maturities, and processing temperatures.

TABLE 1.
EXPERIMENTAL DESIGN FOR HEAT PENETRATION INTO HALLEY BOS 3155 CV DICED
TOMATOES VARYING MATURITY, DICE SIZE, AND PROCESSING TEMPERATURE

Maturity	Dice Size		Processing	Processing
	(cm)	(in)	Temperature (°C)	Replicates
Red	1.27	0.5	88	3
Red	1.27	0.5	92	3
Red	2.54	1.0	88	3
Red	2.54	1.0	92	3
Red + 2 Weeks	1.27	0.5	88	3
Red + 2 Weeks	1.27	0.5	92	3
Red + 2 Weeks	2.54	1.0	88	3
Red + 2 Weeks	2.54	1.0	92	3

After all heat penetration times were determined, approximately 800 g diced tomatoes were dipped in 0.5% $CaCl_2$ for 1 min and processed in 15 s increments from 0 s (control) up to 3 min at either 88 or 92C. The process treatments were randomized and conducted in triplicate.

Approximately 50 g were frozen in liquid nitrogen immediately after processing for enzyme activity assays. The rest of the diced tomatoes were immediately cooled on a tray in a freezer (-18C) for 3 min and then tested for firmness.

Firmness Tests

Processed red maturity diced tomatoes (1.27 cm) were cooled to room temperature and firmness was tested in triplicate using the TA.XT2 Texture Analyzer (Texture Technologies, Corp., Scarsdale, NY) and the Kramer shear-compression method. Two hundred grams of diced tomatoes were

compressed at 5.0 mm/s (crosshead speed) to 90% strain level. Firmness was measured as the area under the curve, and was recorded in kilogram force.

PME Activity Assay

PME activity in diced tomatoes was assayed using a NaOH titration method modified from Bartley et al. (1994) and Rouse and Atkins (1955). Modifications made included using 4.3-5.0 g of tomato pulp and standardized NaOH (0.001-0.1 N) (AOAC 1984). The volume of NaOH was recorded every 4 min up to 20 min total. Three replicates per processed sample were analyzed. One unit of PME activity, (PE u)-g, was calculated as:

(volume of NaOH used X Normality of NaOH X10^4)/(assay time X g of tomatoes)

PG Activity Assay

Extraction. The method used for PG extraction was modified from Pressey (1986). The modifications made included homogenizing 5 g of tomatoes in 10 mL cold deionized water for 1 min at 20,000 rpm with a Brinkmann Polytron (Kinematica AG, Switzerland). The Polytron head was washed with an additional 4 mL of cold deionized water, which was added to the tomato homogenate before adjustment to pH 3 with 0.1 M HCl. The homogenate was centrifuged for 20 min at 10,000 × g (4C). The pellet was resuspended in 10 mL cold water at pH 3.0 before homogenizing for 30 s, washing the Polytron head with 4 mL of cold water (pH 3.0), stirring, and centrifuging as before. The pellet was resuspended in 10 mL of cold 1.2 M NaCl, pH 6.5, stirred for 30 min on ice, and then centrifuged for 20 min at 10,000 × g (4C). The supernatant containing the crude PG extract was assayed using a rapid colorimetric method developed by Garcia et al. (1993) and Doner and Irwin (1992), which uses BCA to measure the total amount of reducing groups formed.

Incubation. An enzyme blank was prepared by boiling 500 μL of enzyme extract for 5 min and then cooling in ice water. The following were added, mixed, and incubated in a test tube at 37C: 250 μL of 1% polygalacturonic acid (PGA) solution, 100 μL of 1 M NaCl and 550 μL water. Exactly 100 μL of either boiled enzyme blank or crude enzyme extract was added to the equilibrated substrate mixture and incubated for 30 min at 37C. Three replicates per crude enzyme extract and per inactivated enzyme blank were incubated. The reaction was stopped by transferring the tubes into a boiling water bath for 5 min, followed by cooling in ice water.

Reductometric Assay. Modifications made to the methods of Garcia et al. (1993) and Doner and Irwin (1992) included using 50 μL of incubation mixture

or enzyme blank plus water to complete the volume to 1 mL. Two replicates per incubated sample and enzyme blank were assayed. The final absorbance was read at 560 nm using a Shimadzu UV-2101 PC Model Spectrophotometer (Kyoto, Japan), and compared to a galacturonic acid standard curve. The standard curve was prepared using the BCA method of Doner and Irwin (1992). The amount of galacturonic acid released by the enzyme was expressed in micromoles of galacturonic acid/min/g tomato.

Predicted Heat Transfer Time Measurement

The predicted times for heat penetration into the center of a 1.27 cm and 2.54 cm cube were calculated from the time-temperature charts for an infinite slab, using a standard procedure by Singh and Heldman (1993) to measure the heat transfer coefficient. Aluminum cubes were used as a model for diced tomatoes.

The heat transfer coefficient (h) for the water bath was measured using an aluminum cube (1.27 or 2.54 cm per side), and heated from 0C to 90C. Holes were drilled into the center of the aluminum cubes, the thermocouples (24 gauge, type T) were inserted, and the holes covered with a sealant. Each cube was immersed in an ice water bath until the internal temperature reached 0C, and then immediately transferred to water bath at 90C. The slope from each time-temperature curve was determined, and the heat transfer coefficient was calculated for each profile:

$$h = [(slope) \, \rho c_p V]/A$$

where ρ was the density of the aluminum cube ($2712.6 kg/m^3$); c_p the specific heat of aluminum (962.9 J/kg°C); A, the surface area of the cube; and V, the volume of the cube. Three replicate values of h were averaged to give a final value for each cube size. The heat transfer coefficient was used to calculate the respective Biot number (N_{Bi}) for each dice size:

$$N_{Bi} = (h \, D)/k$$

where D was the radius of cube, and k the thermal conductivity for tomatoes (0.675 W/mK) (Singh and Heldman 1993). The temperature ratio for an infinite slab (TR_{IS}), was calculated by:

$$(TR_{IS}) = [(T_m - T)/(T_m - T_o)]^{1/3}$$

where T_m was the water bath temperature (90C); T_o, the initial internal tomato temperature (35C); and T the internal target temperature for the diced tomato (88 or 92C). Using the calculated Biot number and the temperature ratio, the Fourier number (N_{FO}) was approximated from time-temperature charts (Singh and Heldman 1993). The predicted time (t) to reach the center of a diced tomato

cube with a thermal diffusivity (α) of 1.48×10^{-7} m^2/s (Singh and Heldman 1993) was calculated by:

$$t = [(N_{Fo})D^2]/\alpha$$

RESULTS AND DISCUSSION

Heat Penetration Times

The heat penetration to the geometric center of the 1.27 cm diced tomato was much faster than for the 2.54 cm diced tomato for both the red (Fig. 1) and red + 2 weeks maturity stages. There were no significant differences ($p < 0.05$) in heat penetration times between the two maturities for the same dice size and processing temperature. One of the reasons why there were no heating time differences between maturities may be due to high temperature induced structural changes in the tomato tissues. Bourne (1989) found that high temperature processing of tomatoes resulted in decreased hydrostatic pressure and a loss of cell turgor. Furthermore, during tomato ripening, major changes include solubilization of cell wall polysaccharides (Seymour 1987), which leads to fragile walls in very ripe fruit. Exposure of diced tomatoes to high heat may have caused sudden physical changes in both maturities.

Seven of the eight process treatments had significantly longer actual than predicted times ($p < 0.05$) (Table 2). This was possibly due to the differences between the aluminum material and the tomato tissue. The aluminum material represented a model for the ideal cube, however, the tomato tissue introduced some variables, especially during cutting and processing. For example, the softness and elasticity of the tomato material made it difficult to cut into a perfect cube. Also, many of the 2.54 cm diced tomatoes contained soft locular gel as well as core material due to the small core width in the tomato fruit. The locular gel sometimes separated from the core material during high temperature processing, leaving an imperfect cube shape. These tomato tissue properties could have caused the variability in heat penetration times.

However, the 2.54 cm diced tomatoes were closer to their predicted times than the 1.27 cm dice (Table 2). This was possibly because of the relatively thick thermocouple thickness to dice size ratio, thus leading to greater conduction error in the smaller dice piece. ANOVA and LSD tests showed that there were no significant differences in heat penetration times due to processing temperature for same sized diced tomato ($p < 0.05$). Thus, a 4C difference in processing temperature did not make a difference in the heating time.
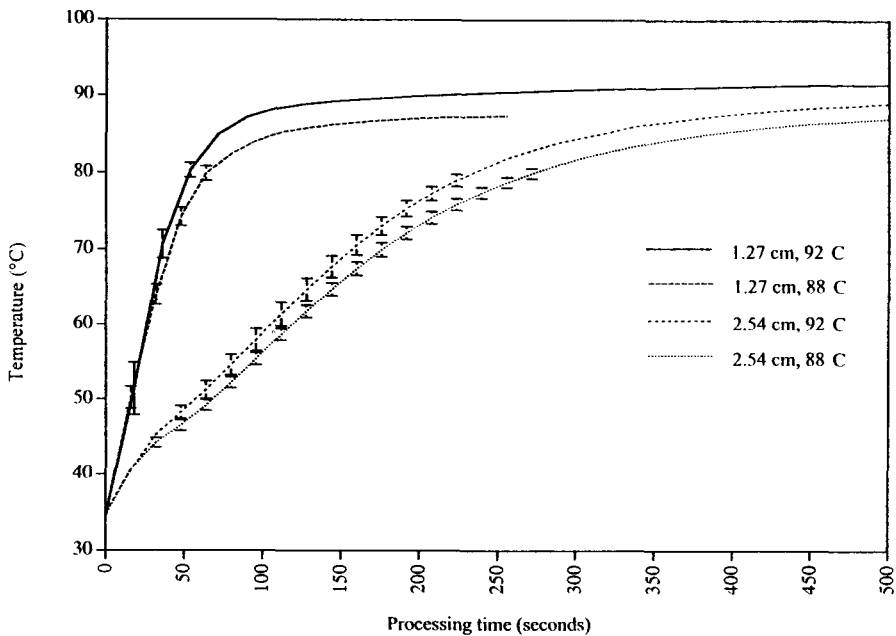
FIG. 1. HEAT PENETRATION TIME-TEMPERATURE DATA OF RED MATURITY DICED
TOMATOES (HALLEY BOS 3155 CV) PROCESSED AT 88 OR 92C

Firmness

Diced tomato firmness decreased significantly ($p < 0.05$) to 60% of its initial raw level within the first 15 s of processing at 88C, and to 50% at 92C (Fig. 2). The asterisk (*) indicates significant differences in diced tomato firmness at both temperatures between *-marked samples and the sample immediately preceding it. Jackman and Stanley (1995) reported that turgor-related changes were responsible for 25-30% of raw tomato softening. However, Bourne and Comstock (1986) found that high temperature processing of plant tissue reduces the hydrostatic pressure responsible for maintaining cell turgor even more, resulting in softer samples than the raw tissue. The results of this experiment agreed with their findings.

Samples between 15-660 s were further evaluated by ANOVA LSD ($\alpha = 0.05$) to determine any further significant differences by reducing the mean square error (MSE). Firmness decreased significantly ($p < 0.05$) between 30 and 45 s of processing, possibly due to further heat induced turgor loss at both temperatures.

TABLE 2.
HEAT PENETRATION TIMES THROUGH DICED TOMATOES HARVESTED AT TWO MATURITY LEVELS AND PROCESSED
TO A TARGET GEOMETRIC CENTER TEMPERATURE OF 88C OR 92C[1]

Dice Size (cm per side)	Red (seconds)						Red + 2 Weeks (seconds)					
	88°C	SE	Predicted[2]	92°C	SE	Predicted[2]	88°C	SE	Predicted[2]	92°C	SE	Predicted[2]
1.27	389	67	165	576	36	166	299	10	165	459	57	166
2.54	693	44	632	981	79	653	709	22	632	650	3	653

[1] Each time is an average of 15 pieces (5 replicate diced tomatoes per process replicate; 3 process replicates per sample).

[2] Model heat penetration time of aluminum cube. Calculated from $t=N_{Fo}(D^2)/a$, where a (thermal diffusivity)$=1.48 \times 10^{-7}$ m²/s, D= 0.00635 m² or 0.01270 m², and N_{Fo}(Fourier number)$=0.60$ and 0.61 for 1.27 cm and 2.54 cm diced tomatoes.
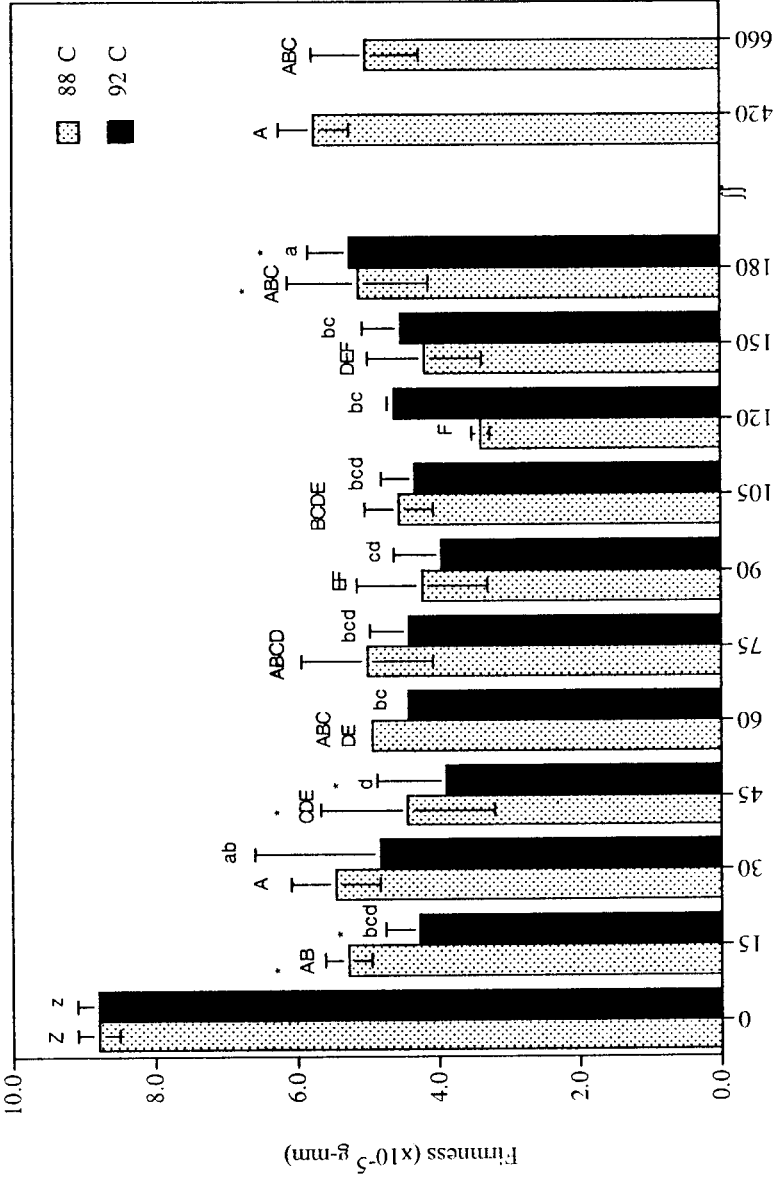
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FIG. 2. FIRMNESS OF RED MATURITY DICED TOMATOES (1.27 CM PER SIDE, HALLEY BOS 3155 CV) PROCESSED AT 88 OR 92C.
An asterisk (*) indicates significantly different firmness in samples (α=0.05, LSD) at both temperatures processed at that cook time, and the time immediately preceding it.

Diced tomatoes maintained a similar degree of firmness ($p < 0.05$) throughout processing from 45 s onwards, with the exception of a slight firming trend at the longer processing times (between 150 and 180 s). This gradual firming effect was possibly due to continual calcium diffusion through the heat ruptured diced tomato tissue until further diffusion equilibrium was reached in the middle lamella as well as cell wall pectin. This was supported by the fact that diced tomatoes maintained this firmness up to 660 s of processing at 80C.

PME and PG Activities

Figure 3 shows that PME activity decreased significantly to 40% of the initial raw level after dipping in calcium chloride solution ($p < 0.05$). This decrease may be due to the presence of calcium salts. There was no change in PG activity. Roeb and Stegemann (1975) found that concentrations of 0.01M (0.09%) $CaCl_2$ and higher actually suppressed tomato PME activity. Even though the concentration used in this experiment was 0.05% calcium chloride, there may have been some partial suppression.

PME activity continued to decrease significantly ($p < 0.05$) to 20% after 15 s, 5% after 30 s and was totally inactivated after 45 s of processing at 90C (Fig. 3). Note that firmness had also decreased significantly between 30 and 45 s, and had remained constant after 45-150 s. It was possible that PME was still actively demethoxylating pectin chains to form calcium pectate, and therefore maintained firmness in diced tomatoes up to 45 s as shown in Fig. 2. Diced tomato firmness was maintained from 45 s until 180 s, which may have been possibly due to the effects of further calcium diffusion through the middle lamella and cell walls. Firmness at 88C had a r^2 correlation of 0.841 to PME activity, while the r^2 correlation at 92C was 0.742. No previous reports on PME inactivation in Halley Bos 3155 cultivar were found in literature.

Unlike PME activity, PG activity in the control diced tomatoes (dipped in calcium chloride) was not significantly different ($p < 0.05$) from the raw diced tomatoes (no calcium chloride) (Fig. 3). PG activity decreased rapidly to 50% after 15 s processing, and then to 10% after 30 s ($p < 0.05$), and remained constant at approximately 5% up to 180 s. This 5% residual activity agrees with the results found by Garces and Luh (1972), where 5% crude PG activity remained in steam-cooked tomato macerates of 'VF-145' cultivar tomatoes after 58 s at 90C. Pressey and Avants (1973) found that PG1, the more thermostable of two isozymes in ripe tomatoes (no cultivar specified), was inactivated after 5 min at 90C. Their study suggested it was very likely that there was residual PG1 activity remaining in the diced tomatoes after 180 s of processing at 90C. It is possible that the remaining 5% residual PG activity may be that of the PG1 isozyme. Further research is needed to determine this point.
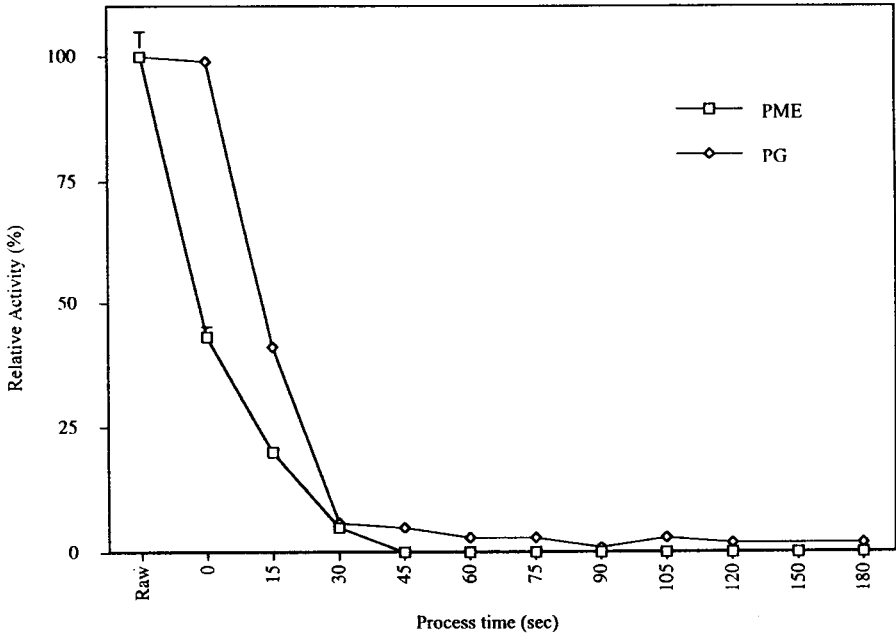
FIG. 3. RELATIVE ACTIVITY OF PECTINMETHYLESTERASE (PME) AND
POLYGALACTURONASE (PG) IN RED MATURITY DICED TOMATOES
(HALLEY BOS 3155 *CV*) PROCESSED AT 90C

CONCLUSIONS

The 1.27 cm dice size tomatoes heated significantly faster than the 2.54 cm
dice size, but processing temperature (88 vs 92C), and harvest maturity (red vs
red + 2 weeks) did not significantly affect the heat penetration time into the
geometric center of the diced tomatoes. The actual heat penetration times were
much slower than the predicted times, which indicated that aluminum cubes
represented an ideal case for the highly variable tomato tissue composition and
its subsequent cube dimensions.

Diced tomato firmness (1.27 cm dice size) decreased significantly to 60%
of its initial firmness level within the first 15 s of processing at 88C and 50%
at 92C, possibly due to heat induced cell disruption and subsequently the rapid
loss in cell turgor. Diced tomatoes maintained a similar degree of firmness
throughout processing after 45 s, with the exception of a slight firming trend
after a longer processing time (180 s at 92C and 120 s at 88C). This was
possibly due to the gradual calcium diffusion into the diced tomatoes.

PME activity decreased to 40% of the initial raw level after dipping in
calcium chloride solution, possibly due to the presence of calcium salts. PME
activity continued to decrease significantly to 20% after 15 s, 5% after 30 s, and
was totally inactivated after 45 s of processing at 90C.

There were no significant changes in PG activity between the initial raw and the control diced tomatoes. PG activity decreased to 50% after 15 s of processing, 10% after 30 s ($p < 0.05$), and remained constant at approximately 5% up to 3 min of processing. It is possible that the remaining 5% residual activity is due to the PG1 isozyme. Further research is required to resolve this question.

ACKNOWLEDGMENTS

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