

Polygalacturonase, Pectinesterase, and Lipoxygenase Activities in High-Pressure-Processed Diced Tomatoes

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High-pressure processing (HPP) can inactivate pathogenic microorganisms and degradative enzymes without the use of heat, thereby minimizing the destruction of flavors, nutrients, and other quality attributes. Lipoxygenase plays a role in the off-flavor production of tomatoes, whereas pectinesterase and polygalacturonase impact tomato texture. The purpose of this study was to determine HPP's ability to inactivate lipoxygenase, pectinesterase, and polygalacturonase in diced tomatoes. Processing conditions used were 400, 600, and 800 MPa for 1, 3, and 5 min at 25 and 45 °C. The magnitude of applied pressure had a significant effect on inactivating lipoxygenase and polygalacturonase ($p < 0.05$), with complete loss of activity occurring at 800 MPa. Pectinesterase was very resistant to pressure treatment. Percent soluble solids, pH, titratable acidity, and color *a/b* values did not differ significantly among the high-pressure-processed samples as compared to the control, but color *L* values increased. This change in *L* values was not considered of practical importance. Apparent protein content decreased in the pressure-processed samples, due possibly to protein denaturation, loss of solubility, and/or a decrease in dye binding sites to assay protein content.

Keywords: *Tomato; high-pressure processing; polygalacturonase; pectinesterase; lipoxygenase*

INTRODUCTION

The growth of novel food-processing research has been driven by the food industry's desire to produce safe, nutritious, and minimally processed foods of higher quality than their thermally processed counterparts. Because high-pressure processing (HPP) of foods can occur at room temperature, the degradation in food quality as seen with traditional thermal processing is avoided. There is minimal destruction of nutrient, flavor, and color because only relatively weak chemical bonds are affected by pressure (1). Pressures >300 MPa can inactivate yeast, mold, and most vegetative bacteria, including food-borne pathogens (2). Spore-forming bacteria are more resistant to pressure; thus, much of the HPP research has been limited to high-acid foods (pH <4.6). Although HPP has tremendous promise to deliver very high quality products, many areas still need to be investigated before high pressure can become a widely accepted food-processing method.

This project focused on the pressure effects on enzyme activity. Pressure results in various changes in proteins, including irreversible or reversible structural modifications leading to protein denaturation, aggregation, or gelation (1, 3). Enzymes that undergo conformational changes due to pressure may have a complete loss, a reduction, or an increase in activity. Influencing factors such as temperature, pH, and solids and protein concentrations must be considered when pressure effects on enzymes are analyzed (4). Studies performed in model systems are often not representative of true food systems in which various constituents can misconstrue the effects of pressure. In addition, many plant enzymes

are membrane bound and thereby cannot be simulated adequately by studies of enzymes in solution.

Lipoxygenase (LOX), pectin methylesterase (PME), and polygalacturonase (PG) are enzymes important to the quality of tomato fruit. PG and PME act to break down pectin, which is critical to tomato texture. LOX catalyzes the oxidation of polyunsaturated fatty acids, leading to the development of volatiles central to tomato flavor. Research on HPP of tomato products has concentrated on juices or purees (5–7) and on cherry tomatoes (8). The objective of this study was to determine the effect of pressure processing on these three enzymes in diced tomatoes and to determine the pressure–temperature–time combination necessary for their inactivation.

MATERIALS AND METHODS

Roma tomatoes were purchased from a local wholesaler and stored at 4 °C until processed. They were washed, diced with a mechanical dicer (half-inch cubes), vacuum-sealed in nylon/EVA bags, and kept on ice until pressure processed, with a maximum of 7 h between dicing and processing. All chemicals used in analysis procedures were purchased from Fisher Scientific (Pittsburgh, PA) unless otherwise noted.

An ABB Quintus Food Processing Cold Isostatic Press (ABB Autoclave Systems, Inc., Columbus, OH) with a 1 L capacity and a vessel heating/cooling mechanism was used. The average rate of pressure increase of all the experimental runs was 5.3 MPa/s. The chilled pressure-transmitting medium was an aqueous propylene glycol solution.

The tomato samples were processed at 400, 600, or 800 MPa for 1, 3, or 5 min. The temperature of the pressure vessel, samples, and pressure medium were controlled to achieve 25 or 45 °C at processing pressures. A split-plot experimental design was used with day and temperature being the blocked variables. Pressure and time combinations were randomly assigned within each temperature block, and each day was a

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Table 1. Quality Parameters of Diced Tomatoes Treated with Various Levels of Pressure, $n = 18$

pressure (MPa)	soluble solids (g/mL)	pH	titratable acidity (mg/g)	color L , a , b	
				L	a/b
0.1	4.70 ± 0.20	4.40 ± 0.07	0.39 ± 0.01	33.14 ± 0.69	1.70 ± 0.04
400	4.78 ± 0.08	4.36 ± 0.04	0.44 ± 0.02	37.41 ± 1.04	1.59 ± 0.02
600	4.75 ± 0.08	4.39 ± 0.01	0.41 ± 0.02	38.04 ± 0.48	1.58 ± 0.04
800	4.73 ± 0.08	4.42 ± 0.02	0.41 ± 0.02	37.81 ± 0.85	1.60 ± 0.05

replicate of the full factorial design (18 pressure/time/temperature combinations). The tomatoes were processed on three consecutive days.

Statistical Analysis. Results were analyzed using Minitab version 12.21 (Minitab, Inc., State College, PA) with $\alpha = 0.05$. ANOVA and Tukey's test were utilized to determine significance of the factors and their interactions and differences among the treatments, respectively. Dunnett's test was applied to determine the differences between the treatments and the non-pressure-processed control. In cases when a residual analysis of the ANOVA indicated nonuniformity and non-normality of variances, a square root transformation of the data was performed.

Quality and Protein Analysis. Color, soluble solids, pH, and titratable acidity were measured on the day of processing; samples for enzyme analysis were stored at -80°C . L , a , and b values were measured in triplicate using a Hunter colorimeter, and percent soluble solids was measured in duplicate at 25°C using an Abbe refractometer. Titratable acidity, expressed as milligrams of citric acid per gram of sample, was determined by a fixed end point titration (pH 8.1) using a computer-aided titrator and 0.1 N NaOH. Duplicate titratable acidity and pH measurements were made of a homogenized sample.

Protein analysis was performed on the two enzyme extracts using the Bio-Rad protein assay based on the Bradford (9) method. Bovine serum albumin was used as a standard, and absorbance was measured at 595 nm using a Hewlett-Packard 8453 spectrophotometer.

Polygalacturonase and Pectinesterase Extraction. The diced tomato sample was homogenized for 1 min with a Braun hand mixer. A 10 g sample of the tomato homogenate was added to 20 mL of extraction buffer consisting of 0.1 M 4-morpholine ethanesulfonic acid (MES) and 1.2 M NaCl at pH 6.1 and then homogenized again for 1 min. This mixture was homogenized for 1 min, stirred for 3 h, and then centrifuged at 15000g for 30 min with the supernatant being retained. The supernatant was brought up to 25 mL total volume with extraction buffer. A 2.5 mL sample of the extract was run through a PD-10 disposable column (Amersham Pharmacia Biotech, Piscataway, NJ) containing Sephadex G-25 M with 0.1 M sodium acetate at pH 4.5. The total volume of the purified extract collected off the column was 4.0 mL. The entire extraction procedure was performed at 4°C .

Polygalacturonase Assay. The measurement of PG activity was based on a method described by Gross (10). A 50–200 μL aliquot of purified extract was added to small vials containing 200 μL of a reaction solution composed of 0.1 M sodium acetate, 0.1 M NaCl, 0.01% (w/v) BSA, and 1.0% (w/v) polygalacturonic acid (Sigma, St. Louis, MO). The vials were then incubated in a water bath at 37°C for 30 min. The reactions were terminated with 2.0 mL of cold 0.1 M borate buffer at pH 9.0. Added to the vials was 200 μL of 1.0% (w/v) 2-cyanoacetamide (Aldrich, Milwaukee, WI) solution. The samples were agitated and then immersed in a boiling water bath for 10 min. After equilibration to room temperature, the absorbance of the samples was measured at 276 nm. On the basis of a standard curve using galacturonic acid, activity was reported as 1 unit of activity equal to 1 μmol of reducing groups produced per minute.

Pectinesterase Assay. The assay for pectinesterase was modified from a method for measuring orange juice PE described by Rouse and Atkins (11). A 0.5 mL aliquot of the purified extract was added to 40 mL of substrate solution containing 1.0% pectin (Sigma) and 0.1 M NaCl. The pH of the system was adjusted close to 7.5 with diluted NaOH. A

fixed end point titration was performed at pH 7.5 with 0.05 N NaOH for 5–10 min. The volume of NaOH used was recorded, and 1 unit of activity was equal to 1 μmol of methanol produced per minute.

Lipoxygenase Analysis. The analysis of lipoxygenase was based on a combination of procedures described by Ben-Aziz et al. (12), Smith et al. (13), and Tseng (14). All extraction procedures were performed at 4°C . Diced tomato samples were homogenized for 1 min with a Braun hand mixer. A portion of the mixture (25 g) was added to 25 mL of 0.5 M Tris-HCl buffer (pH 8.0) containing 2 mM EDTA, 1.0% (w/v) insoluble polyvinylpyrrolidone (PVP), and 0.1% (w/v) Triton X-100. The sample was homogenized again for 1 min, stirred for 10 min, and filtered through four layers of cheesecloth. After centrifugation at 15000g for 40 min, the supernatant was retained and brought up to 50 mL of total volume with deionized water.

The assay was performed with a working substrate that was the combination of 1 part stock substrate and 25 parts 0.2 M sodium phosphate buffer at pH 6.5. The stock substrate contained 7.5 mM linoleic acid (Sigma) and 0.5% (w/v) Tween 20. A 2.5 mL volume of the working substrate at 30°C was added to 0.1–0.5 mL of the LOX extract. The change in absorbance was measured at 234 nm for 3 min at 15 s intervals, with 1 unit of activity equal to a 0.001 change in absorbance per second.

RESULTS AND DISCUSSION

Quality Results. The results of the quality measurements at each pressure level across all repetitions (day), temperatures, and processing times were compared (Table 1). The average results for combined pressure-processed samples were as follows: 4.76% soluble solids, pH 4.39, 0.42% acid content, 37.75 L value, and 1.59 a/b value. Percent soluble solids did not change significantly from the control in any of the pressure-processed samples. The day of processing was a significant factor, with day 3 samples having a greater soluble solids content than days 1 and 2 (Table 2). This effect may possibly be attributed to the natural ripening process. Note that while day-to-day variation existed in pH, as well as soluble solids and color L value, this variation was taken into account in the statistical analysis as the experiment was blocked by day. Although the ANOVA in Table 2 indicates several processing factors to have a significant effect on pH, none of the processed samples were significantly different from the control, as determined via the Dunnett's test. However, compared to other pressure treatments, a significant increase in pH occurred as pressure increased. The pH shift, although small (increase of 0.01–0.1), must be considered when processing due to the possibility of growth of *Clostridium botulinum* spores.

The effects of pressure processing on titratable acidity of diced tomatoes correlated with the pH results. Only two samples were considered to be significantly different from the control—the 400 MPa samples treated for 5 min at 25°C and for 3 min at 45°C . Both of these samples had greater acid contents than the control sample. The acid contents of the 800 and 600 MPa treated samples were found to be significantly lower than those of the 400 MPa processed samples.

Table 2. Analyses of Variance Results for the Different Responses Measured Including Degrees of Freedom, *F* Ratios, and Mean Square Errors (MSE)

	<i>F</i> ratios ^a									
	pressure (P)	time	temperature (temp)	day	P × time	P × temp	time × temp	P × time × temp	day × temp	MSE
polygalacturonase activity	570.58***	16.28***	1.01	2.29	10.44***	6.79**	0.69	4.56**	0.10	0.003
lipoxygenase activity	1485.59***	21.76***	7.62*	2.56	11.80***	4.99*	0.84	0.80	2.21	34.8
pectinesterase activity	9.66**	0.27	7.01*	8.86**	2.09	5.72**	0.02	0.16	0.48	102.5
apparent protein content	44.22***	13.38***	27.92***	2.17	0.35	0.40	0.31	0.04	2.96	1.925
<i>L</i> value	1.16	0.60	0.84	6.98**	1.19	2.38	0.96	1.76	0.07	1.581
<i>a/b</i> value	0.43	0.10	0.20	2.55	0.17	0.01	0.82	1.00	0.50	0.007
percent soluble solids	0.75	0.25	0.11	11.29***	0.57	1.75	0.25	2.32	0.14	0.016
pH	17.76***	1.64	0.07	4.95*	3.89*	1.48	1.27	0.94	2.28	0.001
titratable acidity	7.35**	2.18	0.03	0.75	2.39	1.17	1.03	2.34	1.37	0.001
degrees of freedom	2	2	1	2	4	2	2	4	2	32

^a *, significant at $p < 0.05$; **, significant at $p < 0.01$; ***, significant at $p < 0.001$.

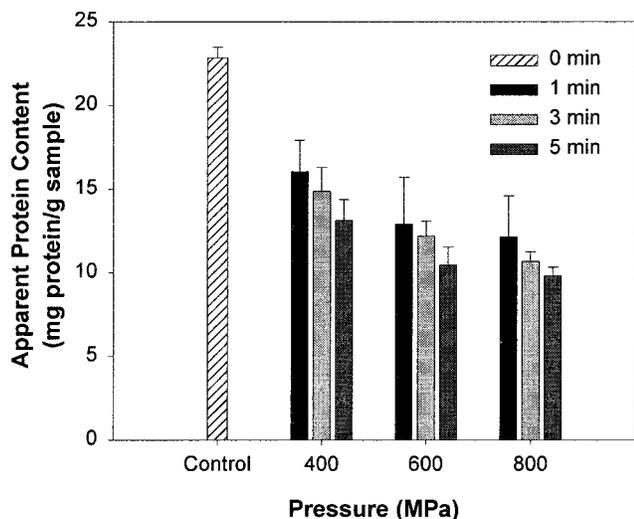


Figure 1. Apparent protein content of HPP diced tomato samples. The content was determined after the extraction as outlined for LOX analysis had been performed. Error bars represent one positive standard deviation.

All pressure-processed samples had significantly greater *L* values than the control samples. Pressure-induced changes to the cell structure could change the light reflectance properties of the tomato fruit. The average *L* values for the control and processed samples were 33.14 and 37.75, respectively. Although found to be statistically significant, this small difference may not be practically relevant.

Protein Concentrations. Protein content was determined in both the polygalacturonase and pectinesterase extractions and the lipoxygenase extractions. Pressure, time of processing, and temperature significantly affected the apparent protein content of LOX extracts (Table 2). As each of these factors increased, protein content decreased (Figure 1). All processed samples had apparent protein concentrations that were significantly less than the control samples. The coefficient of variation for the protein results ranged from 2 to 22%.

The results have been presented as "protein content"; however, this should not be misinterpreted as an actual decrease in the protein present in the samples. The decrease observed in both the PG/PE extracts and the LOX extracts is likely to be an artifact of the protein analysis method and the pressure modification of the protein. The dye, Coomassie Brilliant Blue G250, used in the Bradford method forms noncovalent complexes with the basic groups on proteins (15). A denaturation and subsequent aggregation of the protein shielding

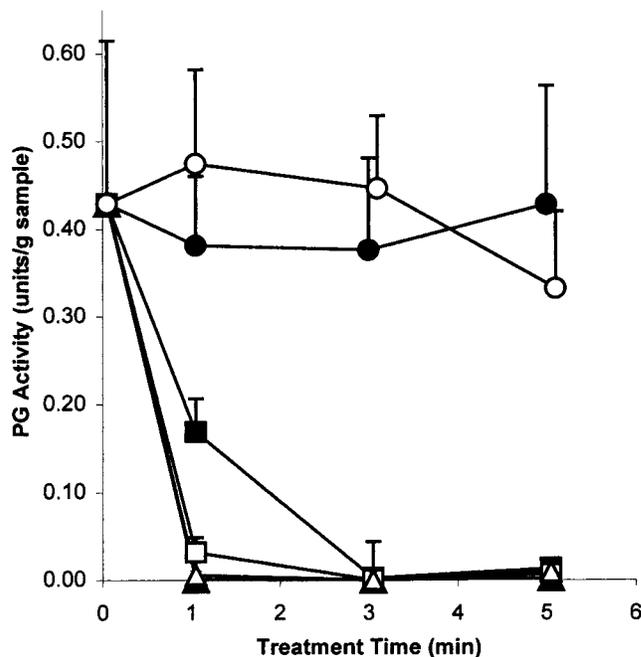


Figure 2. PG activity of HPP diced tomatoes: (●) 400 MPa at 25 °C; (■) 600 MPa at 25 °C; (▲) 800 MPa at 25 °C; (○) 400 MPa at 45 °C; (□) 600 MPa at 45 °C; (△) 800 MPa at 45 °C. Error bars represent one positive standard deviation. Each point represents an average of three samples.

these basic groups from complexing with the dye could lead to an apparent decrease in protein content. Alternatively, protein could be interacting with carbohydrates, thereby reducing the dye binding. In either case, one should view the protein data as an "apparent" loss of protein.

In reporting enzyme activity, traditionally specific activity units based on the milligrams of protein present in the sample are used. The pressure-induced apparent protein loss can skew enzyme activity results reported in this manner. Therefore, the following enzyme activity results are reported as fresh weight activity (units of activity per gram of sample) to give a comprehensive view of these changes in HPP diced tomato samples.

Polygalacturonase Activity. PG activity was affected by a variety of processing factors in relation to HPP (Table 2). All samples treated at 600 and 800 MPa had significantly less activity than the control (Figure 2). Time of processing significantly affected PG activity levels for the 400 and 600 MPa treated samples. The only exception to this observation was the samples treated at 400 MPa for 5 min at 25 °C, which had a greater amount of activity than other 400 MPa pro-

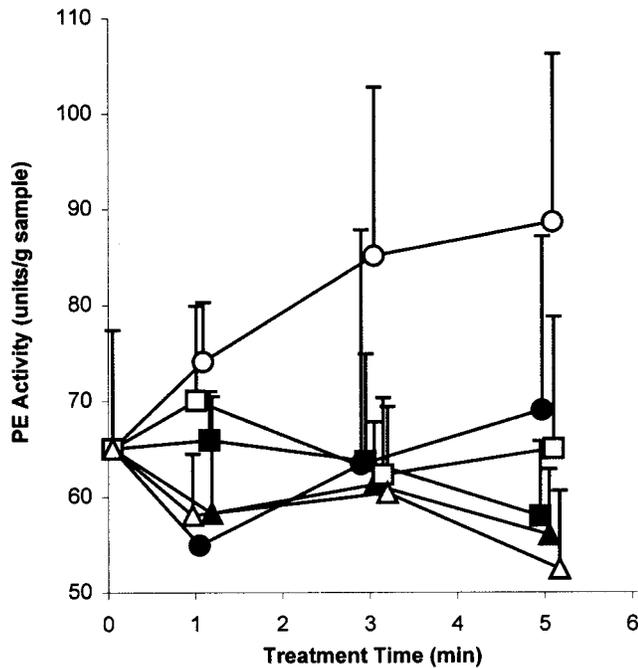


Figure 3. PE activity of HPP diced tomatoes: (●) 400 MPa at 25 °C; (■) 600 MPa at 25 °C; (▲) 800 MPa at 25 °C; (○) 400 MPa at 45 °C; (□) 600 MPa at 45 °C; (△) 800 MPa at 45 °C. Error bars represent one positive standard deviation. Each point represents an average of three samples.

cessed samples. At 800 MPa, almost no PG activity remained in the samples for all time and temperature combinations (Figure 2). Similar PG inactivation behavior has been observed in whole cherry tomatoes, although the pressure dwell time was considerably longer (20 min) and the temperature at pressure was uncontrolled and not isothermal during pressurization (16). In the present study, special care was taken to control the starting temperature of the samples, the pressure transmitting fluid, and the press temperature such that the adiabatic temperature rise upon pressurization resulted in the sample temperature reaching the target press temperature. In this manner, samples were exposed to isothermal/isobaric treatments at each pressure/temperature/time combination.

Pectinesterase Activity. Pressure, temperature, and day of processing significantly affected PE fresh weight activity (Table 2). The treatment of 400 MPa at 45 °C caused significant activation (Figure 3) and was the cause for the significant pressure–temperature interaction (Table 2). No samples were considered to have significantly different fresh weight activities from those of the controls, due in part to the large amount of variation in the control samples. Although citrus PE is sensitive to high pressures (17, 18), results of the present study on diced tomatoes and as well as other studies indicate that tomato PE is clearly insensitive to pressure. Tangwongchai et al. (8) measured PE activity in whole cherry tomatoes following pressurization up to 600 MPa for 20 min and found no significant inactivation relative to the nonpressurized control.

Lipoxygenase Activity. LOX in HPP diced tomatoes was significantly affected by a variety of processing factors (Table 2). Activity decreased as pressure magnitude and dwell time increased (Figure 4). At 400 MPa, LOX inactivation was greater at 25 °C than at 45 °C. Pressure dwell time effects were dependent upon the pressure used. Complete inactivation of LOX occurred

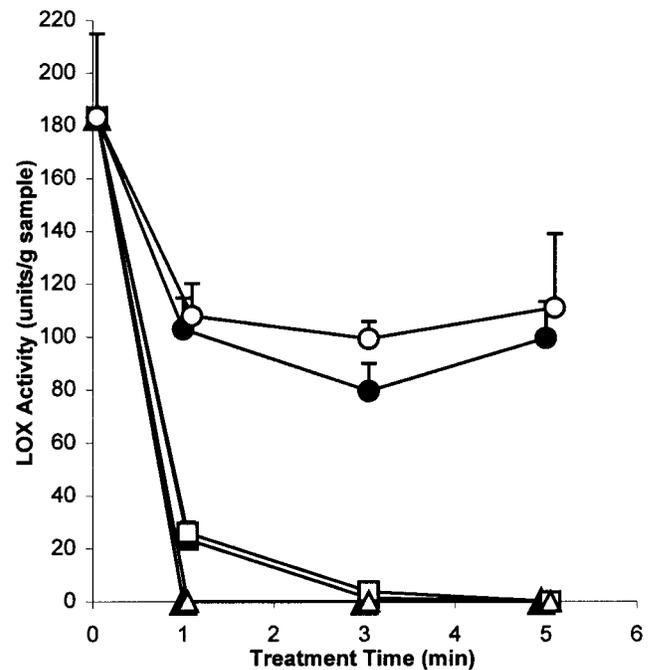


Figure 4. LOX activity of HPP diced tomatoes: (●) 400 MPa at 25 °C; (■) 600 MPa at 25 °C; (▲) 800 MPa at 25 °C; (○) 400 MPa at 45 °C; (□) 600 MPa at 45 °C; (△) 800 MPa at 45 °C. Error bars represent one positive standard deviation. Each point represents an average of three samples.

at 800 MPa across all pressure dwell times. At 600 MPa, a pressure dwell time of 5 min was necessary for complete inactivation of this enzyme. All pressure-processed samples had significantly less lipoxygenase activity than the control. Work performed by others on pressure inactivation of LOX has demonstrated its pressure sensitivity. Tomato LOX was completely inactivated by 20 min at 600 MPa (16).

Numerous studies have focused on identifying the pressure/temperature effects on the inactivation rate of soybean and green bean LOX (19–22). Studies on soybean LOX found that increasing temperature had a mixed effect on first-order inactivation rates. Inactivation rates of soybean LOX were positively affected by temperature at pressures up to 550 MPa, but above that pressure, temperature had a negative effect on soybean LOX inactivation rates. That is, below 550 MPa, increasing temperature increased first-order reaction rates, whereas above 550 MPa, increasing temperature reduced the reaction rates (21). Similar results have been reported for green bean LOX (20). These findings are supported by the present study on diced tomatoes (Figure 4 and Table 2). Increasing temperature from 25 to 45 °C had a significantly protective effect on pressure inactivation of LOX in fresh tomatoes.

Conclusion. HPP was effective for inactivating lipoxygenase and polygalacturonase in diced tomatoes but ineffective at inactivating pectinesterase. The magnitude of the applied pressure had the greatest contribution toward enzyme inactivation, whereas processing time and temperature had less significant effects. The pH, titratable acidity, and color *L* values of the tomato samples were slightly affected by pressure treatments. Although these results are promising, further research needs to be performed on whether the flavor and texture quality of HPP diced tomatoes are maintained following processing and during storage.

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