Effect of High-Pressure Treatment on the Texture of Cherry Tomato

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The effect of high-pressure treatment (200−600 MPa for 20 min) on the texture of cherry tomatoes and on the key softening enzymes (pectinmethylesterase and polygalacturonase) was investigated. When subjected to high-pressure treatment whole cherry tomatoes showed increasing textural damage with increasing pressures up to 400 MPa. However, treatment at pressures above 400 MPa (500−600 MPa) led to less apparent damage than treatment at 300 and 400 MPa; the tomatoes appearing more like the untreated samples. These visual changes were reflected in the texture (firmness) and amount of cell rupture in the tomatoes, with the least firmness and the most cell rupture being seen after treatment at 400 MPa. Light and scanning electron microscopy supported these observations. Although a sample of purified commercial pectinmethylesterase was partially inactivated at pressures above 200 MPa, irrespective of pH (4−9), in the whole cherry tomatoes no significant inactivation was seen even after treatment at 600 MPa, presumably because other components in the tomato offered protection or the isoenzymes were different. Polygalacturonase was more susceptible to pressure, being almost totally inactivated after treatment at 500 MPa. It is concluded that the textural changes in tomato induced by pressure involve at least two related phenomena. Initially, damage is caused by the greater compressibilty of the gaseous phase (air) compared to liquid−solid components, giving rise to a compact structure which, on pressure release, is damaged as the air rapidly expands, leading to increases in membrane permeability. This permits egress of water, and the damage also enables enzymatic action to increase, causing further cell damage and softening. The major enzyme involved in the further softening is polygalacturonase, which is inactivated at 500 MPa and above, and not pectinmethylesterase, which in the whole fruit, is barotolerant.

Keywords: *High-pressure treatment; pectinmethylesterase; polygalacturonase; tomato; texture; structure*

INTRODUCTION

High-pressure treatment (100−1000 MPa) is an alternative to thermal processing of food. It is claimed that the process is clean and energy-efficient compared to many conventional processes. High pressure affects primarily noncovalent bonds. Therefore, the flavor and nutritional quality of the food is usually unaffected, although microorganisms and enzymes may be inactivated. High-pressure effects on enzyme activity depend on the type and source of the enzyme, the nature of the substrate, pressure, holding time, and processing temperature (Cheftel, 1992). Thus, to aid the development of high-pressure treatment of food, it is necessary to understand the effect of high pressure on food enzyme activity. There are many reports relating to studies of high-pressure treatment on enzyme activity, including some on pectinmethylesterase in citrus juice (e.g., Ogawa et al., 1990; Irwe and Olsson, 1994; Gomes and Ledward, 1996; Gomes et al., 1997, 1998). However, there are few reports of its effect on the enzymes in tomato products (Porretta et al., 1995; Hernández and Cano, 1998).

Pectinmethylesterase (EC 3.1.1.11) and polygalacturonase (EC 3.2.1.15) have been found in all species of higher plants and also in a number of plant pathogenic fungi and bacteria. These two enzymes are found together in tomatoes. Pectinmethylesterase de-esterifies the methyl group of pectin (methyl ester of polygalacturonic acid), converting it into low methoxy pectin or pectic acids, whereas polygalacturonase hydrolyses the glycosidic linkages in pectic substances, which result in significantly decreased viscosity. The activities of these two enzymes result in softening of plant tissues and cause cloud separation in citrus juice and tomato products (Pilnik and Voragen, 1991). It has been shown that pectinmethylesterase in citrus juice is more barotolerant than most microorganisms in these juices (Irwe and Olsson, 1994). A conventional method used to achieve pectinmethylesterase inactivation requires high temperatures, leading to flavor loss and off-flavor formation (Basak and Ramaswamy, 1996). Therefore, high-pressure treatment may be a better way to inactivate these enzymes. Goodner et al. (1998) suggested that a heat-labile form of pectinesterase was sensitive to pressure but not a heat-stable form. Irwe and Olsson (1994) stated that pressures below 400 MPa for up to 2 min did not result in inactivation of pectinesterase, even when combined with mild heating of 40−60 °C. However, pressures of 400 and 600 MPa for 2 and 1 min, respectively, decreased the enzyme activity by 90%. An increase in temperature led to the same results at shorter holding times. Denès et al. (1998) suggested that in the absence of pectate, apple pectinmethylesterase lost 37% and 39% of its initial activity at 100 and 640

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MPa, respectively. A protective effect of pectate against pressure inactivation was found for apple pectinmethylesterase but not for orange pectinmethylesterase. Parish (1998) reported that orange juice pressurized at 500 MPa for 90 s lost its cloudiness during storage at 4 °C due to insufficient inactivation of pectinmethylesterase. Basak and Ramaswamy (1998) also studied the effect of pressure on pectinmethylesterase activity (Basak and Ramaswamy, 1996) and reported that pressure had two influences on product texture. First, initial loss in texture was ascribed to the expeditious action of pressure. The second and subsequent effect was a more gradual loss with holding time, at each pressure level.

As well as affecting enzymes, high pressure can also damage plant tissues due to permeability changes in the cell membrane. Pressures of 75−110 MPa for 10 min caused the permeability of the tonoplast of cultured plant cells to increase, sequentially releasing acidic and toxic substances into the cytoplasm, whereas the permeability of the outer cell membrane was affected at 250−350 MPa (Knorr, 1995).

The aim of the present study was to investigate the effect of high-pressure treatment on cherry tomato texture and to relate any effects to pectinmethylesterase and/or polygalacturonase activity and changes in cell structure.

MATERIALS AND METHODS

Pectinesterase, pectin (from citrus), polygalacturonic acid, D-galacturonic acid, and polyacrylamide gel were obtained from Sigma Chemical Co., Poole, UK. 2-Cyanoacetamide was obtained from Aldrich Chemical Co., Gillingham, UK; NaCl was bought from Fisher Scientific UK Ltd., Loughborough, UK; and the other reagents were obtained from BDH, Poole, UK. All chemicals used were analytical grade.

Pressurization of Samples. *Whole Tomatoes.* Red, ripe cherry tomatoes (*Lycopersicon esculentum*) grown in the UK were purchased from a local supermarket and seven or eight of the fruits were sealed in polyethylene bags, expelling as much air as possible. The samples were subjected to pressures of 200−600 MPa for 20 min at ambient temperature (ca. 20 °C). After treatment the enzymes were extracted within 1 h and their activities determined.

Commercial Pectinmethylesterase (from Tomato). Solutions of commercial pectinmethylesterase from tomato at a concentration of 1.5 μg/10 mL were prepared in distilled water, and adjusted to the desired pH (pH 4−9) with 0.1 N NaOH or 1% HCl. The enzyme solutions were sealed, freed from air bubbles, in polyethylene bags and subjected to pressures of 200−600 MPa for 20 min at room temperature (ca. 20 °C) in a prototype Stansted "Food-lab" model high-pressure rig (Stansted Fluid Power Ltd., Stansted, UK). A mixture of castor oil and ethanol (20:80) was used as the pressure medium. The pressure build-up time varied from 1 to 2.5 min, and the depressurization time varied from 10 to 30 s. Temperature changes in the pressure transferring medium were measured by a thermocouple. During pressurization, the temperature rose to a maximum of 40 °C at 200 MPa and 52 °C at 600 MPa, but decreased to ambient temperature (ca. 20 °C) within 4 min of the start of the holding time. After treatment, the enzyme solution was chilled in ice water and its activity measured within 20 min, using a titrimetric method (Kertesz, 1955). To check for the reversibility of the enzyme activity, samples were left at 4 °C for 7 days and their activity reassessed.

Enzyme Extraction. *Pectinmethylesterase (Hagerman and Austin, 1986).* Tomatoes were cut into four pieces and 50 g samples were homogenized in 100 mL of cold 8.8% (w/v) NaCl. The homogenates were centrifuged at 20000*g* for 25 min. The supernatants were collected, adjusted to pH 7.5 with 1 N NaOH, and assayed for PME activity using the spectrophotometric assay.

Polygalacturonase (Pressey, 1986). Cut tomatoes (50 g) were added to 150 mL of cold distilled water and blended for 1 min. The homogenate was adjusted to pH 3 with concentrated HCl, stirred for 15 min, and centrifuged at 20000*g* for 40 min. The pellet was resuspended in 150 mL of cold distilled water at pH 3, stirred for 15 min, and centrifuged at 20000*g* for 40 min. The pellet was resuspended in 100 mL of cold 1 M NaCl. The pH was adjusted by the addition of 1 N NaOH, maintained at pH 6 for 30 min, stirred overnight, and centrifuged at 20000*g* for 40 min. The supernatant was adjusted to pH 4.4 and assayed for polygalacturonase activity. All extraction procedures were carried out at 4 °C.

Enzyme Assays. *Pectinmethylesterase Assays. Titrimetric Method (Kertesz, 1955).* A substrate, 1% (w/v) solution of citrus pectin, was prepared in 0.1 M NaCl by dissolving the pectin in 0.1 M NaCl at 60−70 °C and stirring with a magnetic stirrer. If necessary, the mixture was heated (70−75 °C) with continuous stirring to obtain a homogeneous solution. The solution was cooled to room temperature and adjusted to the final volume of 500 mL with 0.1 M NaCl. During monitoring of the pH and stirring, the pectin solution (20 mL) was equilibrated to 30 °C and the pH adjusted to 7.5 with 0.02 N NaOH, before addition of 1 mL of the enzyme solution (pH 7.5). The reaction was maintained at pH 7.5 by the addition of 0.02 N NaOH, and the volume of alkali used over 10 min was recorded. The pectinmethylesterase activity was defined as the microequivalents of acid from pectin per minute at pH 7.5 and 30 °C.

Spectrophotometric Assay (Hagerman and Austin, 1986). A 0.5% (w/v) solution of citrus pectin was prepared in 0.1 M NaCl. A 0.01% (w/v) solution of bromothymol blue was prepared in 0.003 M potassium phosphate buffer, pH 7.5. To achieve a constant starting pH for the reaction, pectin solutions, indicator dye, and water were adjusted to pH 7.5 with 1 N NaOH. The extent of the reaction was monitored at 620 nm in a Lamda 5 Perkin-Elmer recording spectrophotometer maintained at 30 °C with a circulating water bath. Pectin solution (2 mL) was mixed with 0.2 mL of bromothymol blue and 0.78 mL of water, and the initial absorbance at 620 nm (A_{620}) was measured against an appropriate blank, to ensure that no nonenzymatic reaction occurred. Pectinmethylesterase extract (20 μL) was added to the mixture and the decrease in A_{620} (A_{620}/min) recorded. D-Galacturonic acid solutions, up to 0.5 μmol, were used to obtain a standard curve.

Polygalacturonase Assay. The assay for polygalacturonase activity was based on the hydrolytic release of reducing groups from polygalacturonic acid and quantifing these with 2-cyanoacetamide (Gross, 1982). The reaction mixture (0.4 mL) containing 50 mM Na-acetate (pH 4.4), 0.2% polygalacturonic acid, and enzyme (100 μL) was incubated at 30 °C for 1 h. The reaction was stopped by adding 2 mL of cold 100 mM borate buffer, pH 9, followed by 0.4 mL of 1% 2-cyanoacetamide to the chilled mixture. The mixture was immersed in boiling water for 10 min and cooled in ice water. The absorbance of the solutions was measured at 276 nm after equilibration to room temperature (ca. 20 °C) and within 30 min of heating. Blanks were prepared using heat denatured enzyme extracts. D-Galacturonic acid, up to 250 nmol, was used to obtain a standard curve.

Gel Electrophoresis. Commercial pectinmethylesterase (from tomato) (6 mg/mL) in 0.2 M Tris buffer, pH 7, was prepared for gel electrophoresis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE) was carried out in gels containing 12.5% polyacrylamide, at a constant current of 50 A and 150 V (Laemmli, 1970). The unpressurized and pressurized enzyme solutions were diluted 1:1 with buffer (0.125 M Tris-HCl buffer, pH 6.8, containing 2% SDS, 10% glycerol, and 0.002% bromophenol blue), and 10 μL of solution was applied to each well. SDS−PAGE was also performed in both the presence and absence of 2% 2-mercaptoethanol, for 90 min at room temperature. Before electrophoresis, all solutions were heated in boiling water for 3 min. Protein was fixed in 12% trichloroacetic acid for 1 h and stained with Coomassie Brilliant Blue G-250 (Neuhoff et al., 1988).

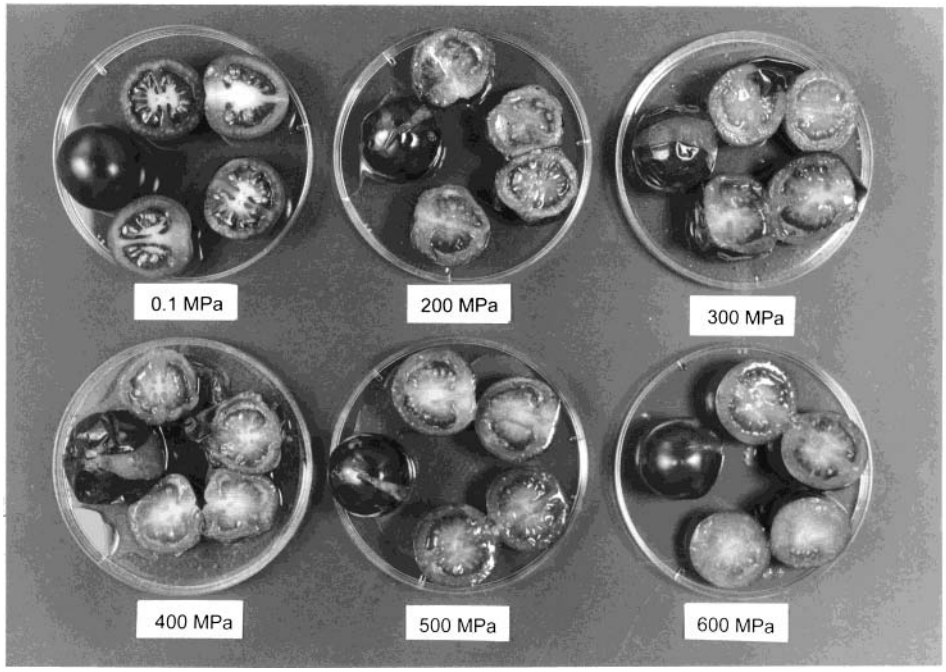

Figure 1. Photograph of cherry tomatoes either unpressurized (0.1 MPa) or after pressure treatment at 200−600 MPa at ambient temperature.

Texture Analysis. After pressurization, the samples were left to stand at atmospheric pressure for 2 h at 8 °C, and their textures were assessed at room temperature. The firmness of the tissues was estimated by a compression test using a TX2 TA texture analyzer (Stable Micro System, Godalming, UK). The samples were compressed to 30% deformation in a single compression−decompression cycle at a steady speed of 5 mm min^{-1} using a 50 mm diameter circular flat plate. After treatment, due to their different compressibilities, the flesh of the pressure-treated tomatoes separated from the skin. Thus, in the unpressurized tomatoes, the skin was lightly lacerated using a razor blade to minimize its effect on the compression (firmness) measurement. Maximum force (grams) was used as an index of firmness.

Estimation of Cell Damage. The tomatoes were cut into quarters and the seeds and jelly-like matrix discarded. The assessment of cell damage was as described by Khan and Vincent (1996). Tomato flesh was weighed into polyethylene bags and pressurized at 200−600 MPa for 20 min at ambient temperature (ca. 20 °C). The unpressurized and pressurized tomatoes were centrifuged at 140*g* for 40 min at 20 °C. The amount of extracted fluid was recorded and used as an indicator of cell rupture.

Scanning Electron Microscopy. Tomato tissue was cut into 4 × 4 mm cubes and fixed in 4% (v/v) glutaldehyde for 2 h in sealed bottles at room temperature. The fixative was decanted, and the samples were washed (3 × 15 min) in 0.1 M potassium phosphate, pH 7. The samples were dehydrated using graded acetone:water in the sequence 30, 50, 70, 90, 95, 100% acetone, 30 min for each solution, and dehydrated (2 × 40 min) in absolute acetone. Critical point drying was carried out in a Tousimis Samdri-780 critical point dryer. Following drying, the sample was mounted on SEM cylinder stubs (10 × 10 mm) using silver conducting paint (TAAB Laboratories, Reading, UK), stored in a desiccator and coated with gold. Approximately 40 nm of gold was sputtered onto the sample using an Emscope SC-500 sputter coater. The sample was observed in a JEOL JSM T20 scanning electron microscope operating at 20 kV.

Light Microscopy. A thin slice of tomato flesh was removed with a razor blade and placed on a glass slide. The cell structure was observed using an Olympus BH-2 light microscope. If necessary, distilled water was used to moisten the specimen, to prevent dehydration.

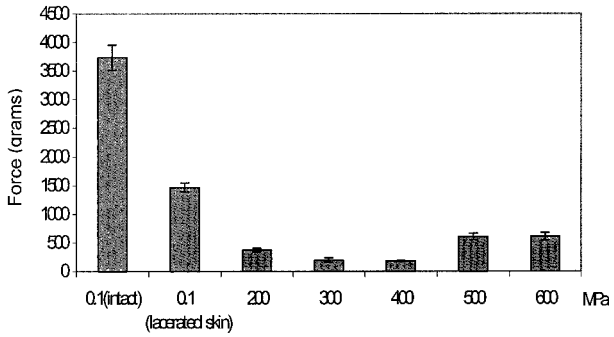

Figure 2. Firmness of untreated and pressure treated tomatoes (200−600 MPa for 20 min) (each data point is the mean ± standard deviation of 24 determinations).

Statistical Data Analysis. A randomized *a* × *b* factorial design, where *a* is the pH and *b* is the pressure applied, was used for the enzyme stability studies, and a completely randomized design was used for the crude extract enzymes and firmness studies. All data were analyzed using ANOVA and the differences between treatments were compared by Duncan's multiple range tests, using the statistical program SPSS v 7.5.

RESULTS AND DISCUSSION

Effect of High-Pressure Treatment on Tomato Texture. The texture of the tomatoes became softer with some evidence of free water after pressure treatment. Less free water was seen after treatment at 500 MPa than after treatment at 300 and 400 MPa (Figure 1). Free water after treatment at 600 MPa was less than seen after treatment at 500 MPa (Figure 1). Visually, the tomato tissues became softer as the pressure increased to 400 MPa, but at 500 and 600 MPa, they looked more like the fresh samples. The firmness and percentage cell rupture results indicated that high pressure caused softening due to cell damage (Figures 2 and 3), with increasing cell rupture causing decreased

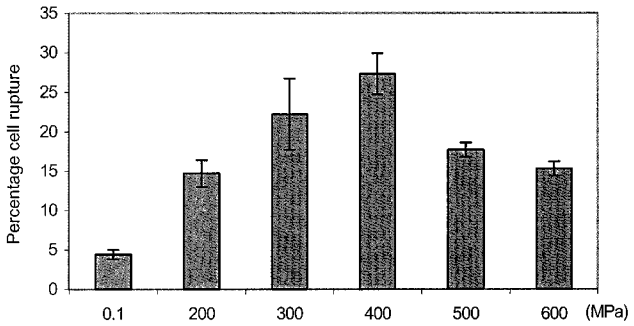

Figure 3. Effect of high-pressure treatment (200−600 MPa for 20 min) on percentage cell rupture in cherry tomatoes (each data point is the mean ± standard deviation of 12 determinations).

firmness. There was some cell damage in the untreated samples, due to the centrifugal force applied. At 200−400 MPa, the samples were more seriously damaged, exhibiting less firmness and greater percentage cell rupture. The percentage cell rupture increased and firmness decreased up to 400 MPa, but cell rupture decreased and firmness increased after treatment at 500 and 600 MPa. The untreated tomatoes with intact skin had double the maximum compressive force of those whoseskin had been lacerated (Figure 2).

Effect of High-Pressure Treatment on the Histological Structure of Tomato Tissue. The SEM micrographs show that high-pressure treatments above 200 MPa were detrimental to the tomato tissues (Figure 4). At 200 MPa, the structure of tomato tissue was similar to that of the unpressurized samples, although it showed some cell injury. At higher pressures, more damage was seen; however, the differences were small at pressures above 300 MPa (Figure 4C−F). It should be noted that for the SEM investigation the tissues were dried prior to analysis, thus the location of the water and its contribution to the texture cannot be evaluated. The micrographs clearly reveal the folding and corrugation of the cell wall due to collapse, causing loss of turgor and firmness. These results agree with those of Préstamo and Arroyo (1998), who found that the parenchyma cell of spinach leaf, which has a soft structure, is completely disrupted after pressure treatment at 400 MPa for 30 min at 5 °C. They also reported that high-pressure treatment of cauliflower changed cell permeability and caused movement of water from inside to outside the cell, resulting in a drenched appearance. High-pressure treatment caused more extensive changes in the structure of spinach than in cauliflower, which has a harder and less elastic cell structure. Préstamo and Arroyo (1998) agreed with the suggestion of Chong et al. (1985) that pressure induced the inactivation of (Na, K)-ATPase from dog kidney and eel electroplax and caused the collapsed appearance of the cell membranes.

Light micrographs of untreated and pressure treated samples are shown in Figure 5. Histological damage was observed at all pressures applied. Many large bubbles were trapped in the tissues after treatment at 200 and 300 MPa (Figure 5B,C) but such bubbles were not seen after treatment at pressures of 500 and 600 MPa, when the intercellular spaces became much broader. It is likely that, at moderate high pressure (200−400 MPa), air in the tissues was compressed much more than the liquid and solid materials, and on depressurization these air bubbles expanded rapidly and aggregated into larger bubbles, resulted in misshapen cells and the formation of cavities. At higher pressures (500−600 MPa), these air bubbles probably escaped through the intercellular space during depressurization, resulting in the larger intercellular void between the cells. This would lead to a reduction in total cell adhesion and reduced firmness. Préstamo and Arroyo (1998) also found that some cavities formed between the cells of spinach leaf after pressure treatment at 400 MPa for 30 min at 5 °C.

Effect of HPT on the Stability of Commercial Tomato Pectinmethylesterase. Figure 6 shows the resultant pectinmethylesterase activity after treatment at different pH values and pressures. It is seen that commercial tomato pectinmethylesterase was reasonably barotolerant at all pH values studied (pH 4−9). The enzyme lost about 10, 20, and 40% of its activity after treatment at 200, 400, and 600 MPa, respectively. Seyderhelm et al. (1996) reported that pectinmethylesterase lost 30% of its activity in Tris buffer at pH 7, after treatment at 600 MPa for 10 min. Data analysis indicated that there was no effect of pH or the interaction of pressure and pH in the current study. Thus, inactivation resulted from the effect of the pressure applied and not pH. However, the effect of pressure treatment in the model study differed from that found in the food system, since Basak and Ramaswamy (1996) found that pressure treatment (100−400 MPa) at pH 3.2 caused greater inactivation of the enzyme in orange juice than treatment at pH 3.7. They also suggested that an inverse of the effect might be obtained at pressures greater than 600 MPa. After storage at 4 °C, there was no change in the enzyme activity after pressurization, indicating that inactivation was irreversible. Ogawa et al. (1990) also reported that pectinmethylesterase in orange juice was partially and irreversibly inactivated after pressurization at 300−400 MPa for 10 min.

SDS−PAGE. SDS−PAGE (Figures 7 and 8) showed a band corresponding to a molecular weight of about 33 kDa, which is presumably pectinmethylesterase (Markovic and Jörnvall, 1986). Under both reducing and nonreducing conditions this band was still clearly seen after pressure treatment (200−600 MPa for 20 min), suggesting that no major changes in the structure were induced by pressure. This protein band was not seen after heat treatment (95 °C, 10 min), under nonreducing conditions. Therefore, the decrease in commercial tomato pectinmethylesterase activity due to pressure possibly resulted from modification of the active site. Gomes et al. (1997) reported that a reduction in the activity of papain on pressure treatment was due to modification at the active site and not to major conformational changes leading to aggregation of the enzyme. It is seen that a contaminant protein of molecular weight 45 kDa was pressure sensitive in the range of 400−600 MPa (Figures 7 and 8). It is also seen that, in the heat-treated sample, the enzyme is aggregated by disulfide bonds, at least to some extent, since under reducing conditions the band at 33 kDa is still observed (Figure 8).

Effect of HPT on Pectinmethylesterase and Polygalacturonase in Cherry Tomato. Pectinmethylesterase and polygalacturonase are the key texture-softening enzymes found in tomatoes. From a preliminary study, the titrimetric method gave slightly higher values for pectinmethylesterase activity than the spectrophotometric assay. Both methods were very reproducible with standard deviations of <2% and <5% for

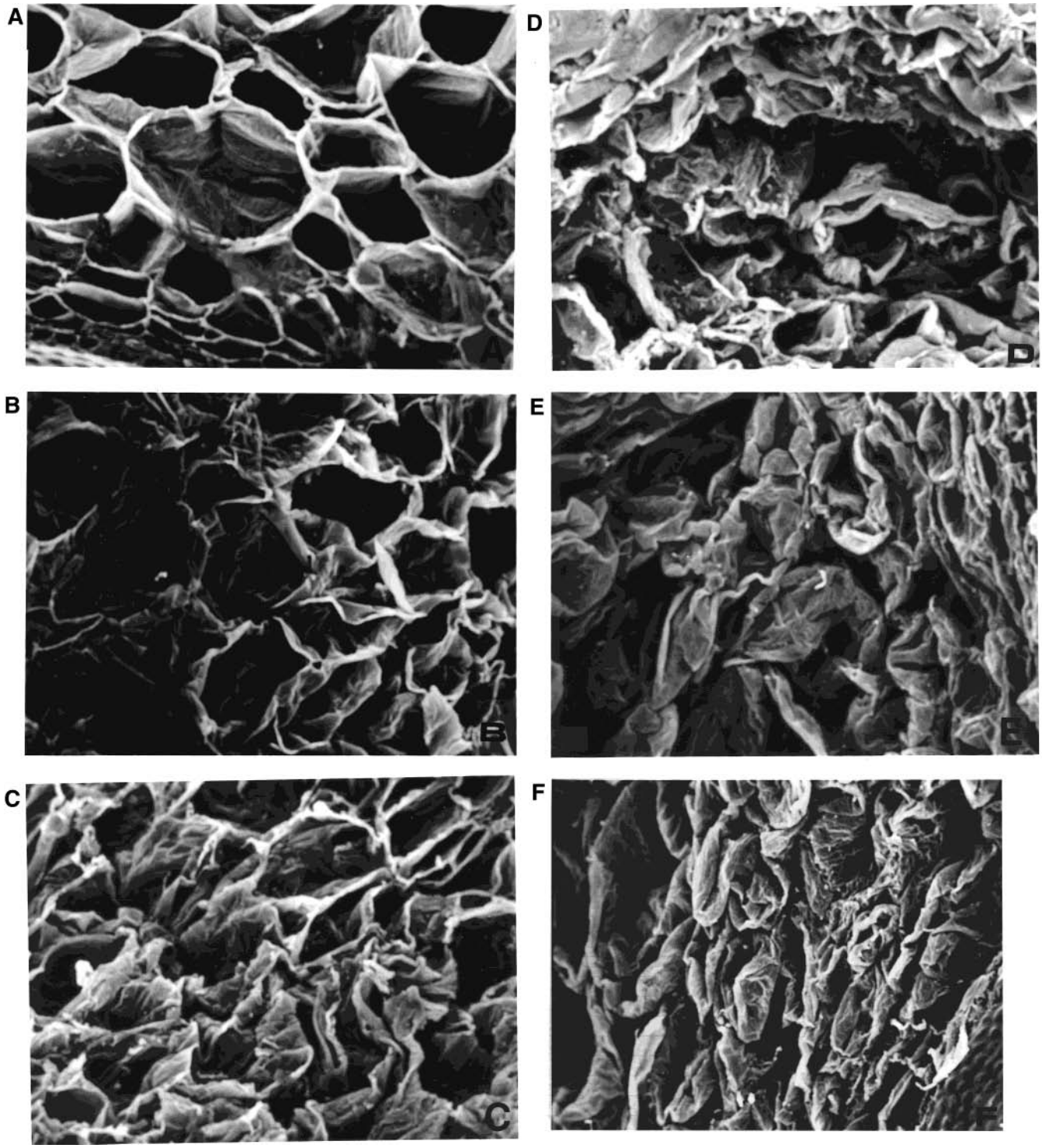

Figure 4. Scanning electron micrographs of untreated and pressure treated tomato tissue (100 μm): A, 0.1 MPa; B, 200 MPa; C, 300 MPa; D, 400 MPa; E, 500 MPa; F, 600 MPa for 20 min.

the titrimetric and spectrophotometric assays, respectively (data not shown). Due to its convenience, specificity, and sensitivity, the spectrophotometric assay was used for further work.

Pectinmethylesterase in cherry tomatoes was more barotolerant than the purified commercial pectinmethylesterase from tomatoes (Figures 6 and 9), since even treatment at 600 MPa led to no loss of activity in the former material. These results agree with those of Crelier et al. (1998), who found that pectinmethylesterase activity in tomato was not affected by pressures up to 500 MPa for 15 min, even at 60 °C. The lack of effect seen in the whole fruit may be due to the protective effect of other components of the tomato, or the commercial pectinmethylesterase could have been extracted from tomatoes with a different ratio of isoenzymes from those in the cherry tomato. However, since other workers have reported protection in juice, the presence of other components seems most likely. For example, Ogawa et al. (1990) and Seyderhelm et al. (1996) reported that pectinmethylesterase increased its barostability in orange juice containing high soluble solid content, and Pollard and Kierser (1951) reported that enzyme inactivation in raw juice was different from

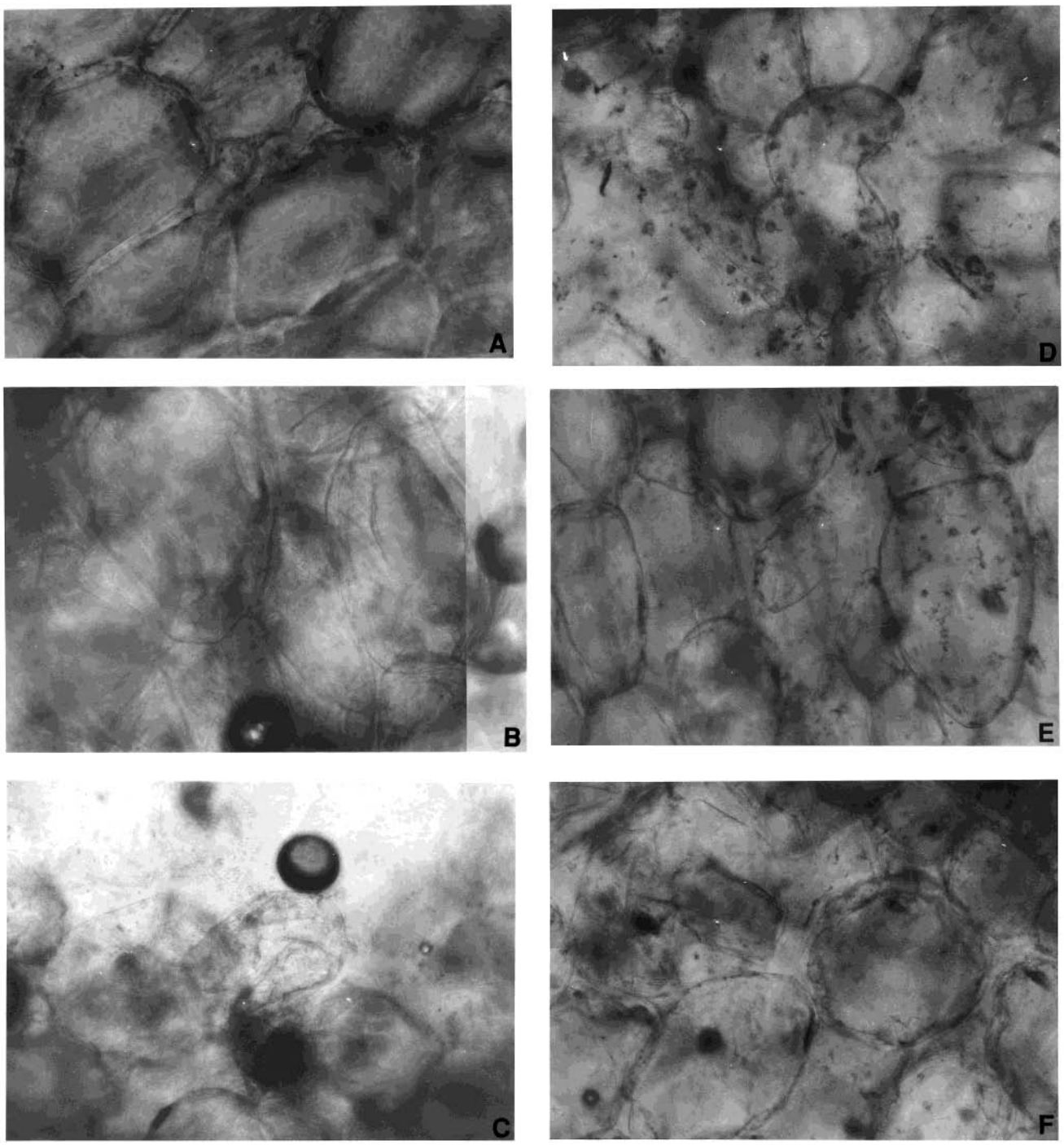

Figure 5. Light micrographs of untreated and pressure treated tomato tissue (50 μm): A, 0.1 MPa; B, 200 MPa; C, 300 MPa; D, 400 MPa; E, 500 MPa; F, 600 MPa for 20 min.

that found for the purified enzyme. Knorr et al. (1992) commented on the importance of both food composition and pressure medium with regard to the inactivation of enzymes. However, different ratios of isoenzymes may also be important, since Irwe and Olsson (1994) found that the presence of different isoenzymes of pectinmethylesterase in orange juice led to different degrees of inactivation after pressurization. Also, Goodner et al. (1998) reported that heat-sensitive pectinmethylesterase in orange and grapefruit juice was instantly inactivated at pressures >600 MPa, but the heat-stable form of the enzyme, even at dwell times >15 s and pressures >600 MPa, was not inactivated.

It is apparent that, in the present study, pectinmethylesterase in whole cherry tomato behaved very differently from the behavior reported in tomato puree (Hernández and Cano, 1998). Again, this might be because either different ratios of pectinmethylesterase isoenzymes were involved or because the enzyme was present in different environments.

However, polygalacturonase was more susceptible to pressure in the cherry tomatoes (Figure 10), the enzyme losing 30% of its activity after treatment at 400 MPa and almost all its activity after treatment at 500 and 600 MPa. Crelier et al. (1998) also found that polygalacturonase was completely inactivated at 500 MPa at 60 °C for 15 min. Thus, inactivation of polygalacturonase at pressures of 500 and 600 MPa may contribute to the changes in texture observed on pressure treatment.

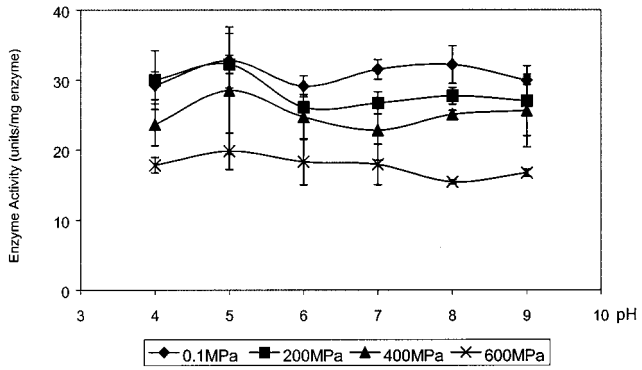

Figure 6. Effect of high-pressure treatment (200−600 MPa for 20 min) at different pH values on the stability of commercial pectinmethylesterase (from tomato).

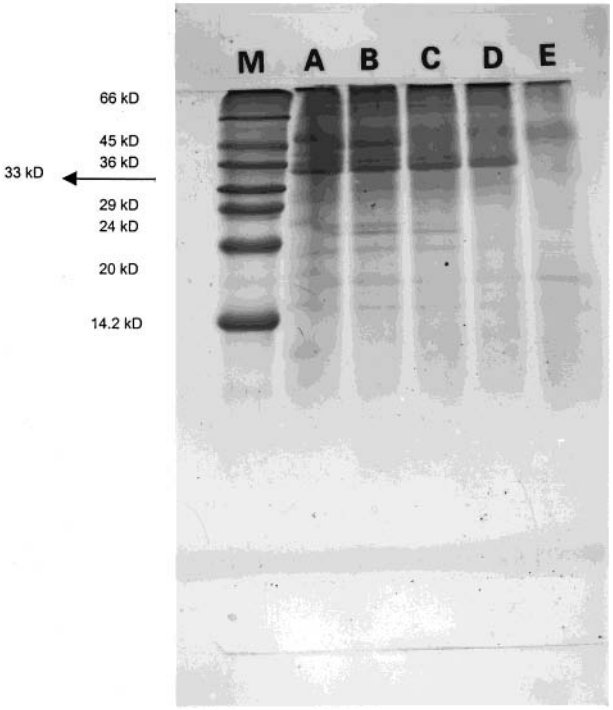

Figure 7. SDS−PAGE of pectinmethylesterase (6 mg/mL) in the presence of 2% SDS before and after pressure treatment at ambient temperature for 20 min or heat treatment at 90 °C for 10 min: lane A, native; lane B, 200 MPa; lane C, 400 MPa; lane D, 600 MPa; lane E, heated at 90 °C for 10 min; and lane M, marker.

Basak and Ramaswamy (1998) suggested that inactivation of pectinmethylesterase in some fruits and vegetables during high-pressure treatment resulted in the low methoxy pectin forming a gel network with divalent ions, e.g., Ca and Mg, leading to hardening of the tissue. This explanation can account for the textural changes observed for cherry tomatoes in the study, since the pectinmethylesterase was still active after treatment at all pressures. It is possible that pectinmethylesterase plays an important role in the increase in tomato firmness seen after treatment at 500 and 600 MPa. Low methoxy pectin produced by this enzyme forms a gel and thus helps retain the water in its structure and increase the firmness (Figure 2). However at pressures <500 MPa, polygalacturonase is still active and will hydrolyze the low methoxy pectin to galacturonic acid, which is soluble in water. This will result in more free water being seen and a concomitant decrease in firmness.

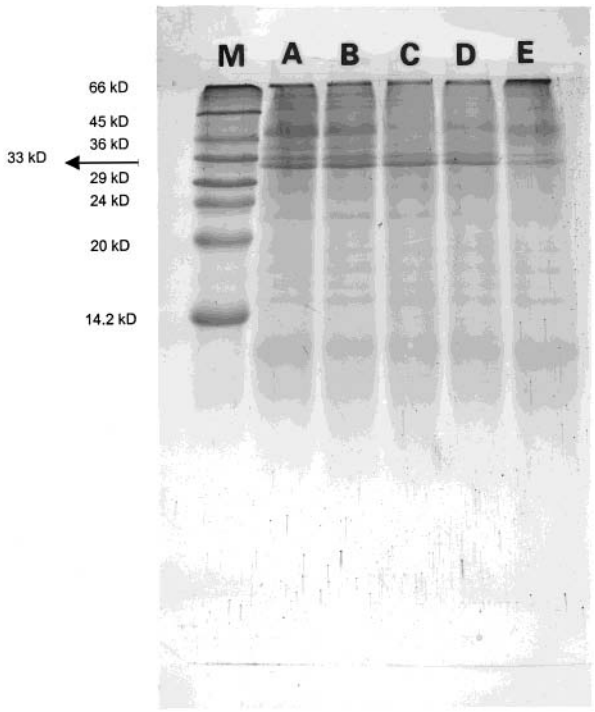

Figure 8. SDS−PAGE of pectinmethylesterase (6 mg/mL) in the presence of 2% SDS and 2% 2-mercaptoethanol before and after pressure treatment at ambient temperature for 20 min or heat treatment at 90 °C for 10 min: lane A, native; lane B, 200 MPa; lane C, 400 MPa; lane D, 600 MPa; lane E, heated at 90 °C for 10 min; and lane M, marker.

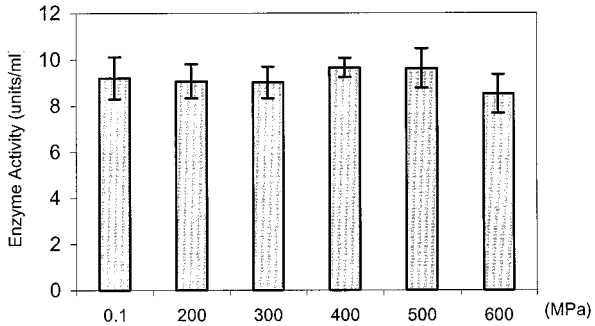

Figure 9. Effect of high-pressure treatment (200−600 MPa for 20 min) on the activity of crude pectinmethylesterase in extracts from pressure-treated whole cherry tomatoes.

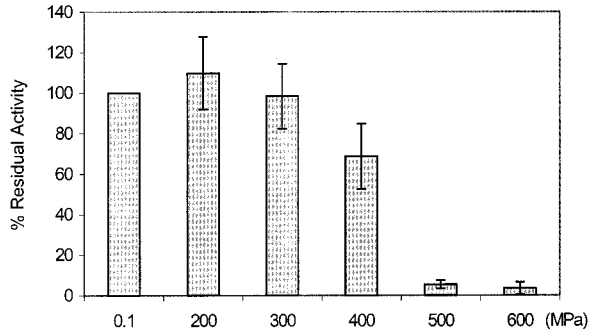

Figure 10. Effect of high-pressure treatment (200−600 MPa for 20 min) on the activity of crude polygalacturonase in extracts from pressure-treated whole cherry tomatoes.

Conclusion. In summary, it would appear that the textural changes observed on pressure treatment of cherry tomatoes are due to both physical disruption of the tissue and enzyme action. First, the much greater compressibility of the gaseous (air) phase compared to

the liquid and solid components in the tomato means that, on pressure release, the rapid expansion of the air in the compressed matrix of the fruit causes distortion and the accumulation of large air bubbles and, at higher pressure, voids, in the matrix. Such changes will also contribute to pressure-induced changes in the cell membrane, leading to increased permeability. These changes will permit the texture-modifying enzymes, pectinmethylesterase and polygalacturonase, to appear more active, causing textural changes. However, the additive effect of these changes ceases at pressures >400 MPa, since the polygalacturonase, but not the pectinmethylesterase, is inactivated, and thus the texture after treatment at 500 and 600 MPa is firmer than that seen after treatment at lower pressures.

This study suggests that pressure treatment of tomatoes is not a viable process since pectinmethylesterase is not inactivated and high-pressure itself causes physical damage to such soft plant tissues as tomatoes. However, such a process may be suitable for other products such as juice, puree, and paste or harder plant tissues such as carrot, cauliflower, and radish.

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