Effect of High-Pressure Treatment on Lipoxygenase Activity

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Solutions of commercial soybean lipoxygenase (100 μg/mL) in 0.2 M citrate−phosphate and 0.2 M Tris buffer were subjected to pressures of 0.1, 200, 400, and 600 MPa for 20 min. The enzyme was stable at atmospheric pressure (0.1 MPa) over a wide pH range (5−9). In citrate−phosphate buffer, the enzyme had maximum stability over the pH range 5−8 in untreated samples and after treatment at 200 MPa, but with increasing pressure, the pH stability range become narrower and centered around pH 7−8. The enzyme was more sensitive to acid than alkali, and at pH 9, it lost virtually all activity after pressurization at 600 MPa for 20 min in both buffers. The activity of the crude enzyme extracted from tomatoes treated at 200 and 300 MPa for 10 min was not significantly different from that of the untreated tomatoes, while a pressure of 400 MPa for 10 min caused a significant decrease in activity and treatment at 600 MPa led to complete and irreversible activity loss. Compared to unpressurized tomatoes, treatment at 600 MPa gave significantly reduced levels of hexanal, *cis*-3-hexenal, and *trans*-2-hexenal, which are important contributors to 'fresh' tomato flavor, and this was attributed to the inactivation of lipoxygenase.

Keywords: *High-pressure treatment; tomato; lipoxygenase; pH; flavor*

INTRODUCTION

Consumer demand for fresh or minimally processed food of high nutritional and organoleptic quality has stimulated research into novel nonthermal or combined processes (Hoover et al., 1989). High-pressure treatment provides an alternative method of food processing since it can decrease microbial load and enzyme activity while retaining sensory and nutritional quality (Cheftel, 1992; Hoover et al., 1993; Galazka and Ledward, 1995). High-pressure treatment can increase or decrease the kinetics of enzyme-mediated reactions, depending on whether their reaction volumes are negative or positive, and may destroy enzyme activity entirely by modifying the enzyme structure. Inhibition of enzyme activity by high-pressure treatment depends on the properties of the medium (including its pH), temperature, and time of treatment (Knorr et al., 1992). There are several reports on the effect of high-pressure treatment on enzyme activity (e.g., Gomes and Ledward, 1996; Hernández and Cano, 1998; Ludikhuyze et al., 1998). Seyderhelm et al. (1996) classified enzymes, with respect to their sensitivity to high pressure (up to 900 MPa), as pressure-sensitive or pressure-tolerant. They classified lipoxygenase as pressure-sensitive, the activity decreasing noticeably after 2 min at 600 MPa in both pH 7 Tris buffer and soymilk at 25 °C and complete inactivation occurring in Tris buffer after 10 min at 600 MPa at 25 °C. Ludikhuyze et al. (1998) reported that pressure-induced, as well as thermal, inactivation of lipoxygenase could be explained by first-order kinetics. They also reported that, in the temperature range 10−64 °C, the enzyme was most resistant to pressure at temperatures slightly above room temperature.

Lipoxygenase (EC 1.13.11.12) plays an important role in the genesis of volatile flavor aroma compounds in many plant foods, including tomato, cucumber, and banana (Eskin et al., 1977). The enzyme degrades linoleic and linolenic acids to volatiles such as hexanal and *cis*-3-hexenal. The latter compound transforms to *trans*-2-hexenal, which is more stable. These compounds are thought to be the major volatile compounds that contribute to the 'fresh' flavor of blended tomatoes. (Kazeniac and Hall, 1970). The purpose of the present work was to obtain fundamental information regarding the effect of high pressure on the stability of lipoxygenase and to investigate how these effects modified the generation of volatile flavor compounds in tomatoes.

MATERIALS AND METHODS

Lipoxygenase type I and linoleic acid (~99% free acid) were obtained from Sigma Chemical Co. (Gillingham, U.K.). Other reagents were obtained from BDH (Lutterworth, U.K.). All chemicals used were analytical grade.

High-Pressure Treatment. A prototype Stansted Foodlab model high-pressure rig (Stansted Fluid Power Ltd., Stansted, U.K.) was used to pressure-treat the samples (Cheah and Ledward, 1996). A mixture of castor oil and ethanol (20:80) was used as the pressure transmitting medium, and all treatments were carried out at room temperature (~20 °C). Temperature changes in the pressure transferring medium were measured by a thermocouple, and during pressurization the temperature of the medium increased to a maximum of 38 °C at 200 MPa and 45 °C at 600 MPa within 1−2.5 min and returned to ambient within 4 min from the start of processing.

Pressurization of Samples. *Soybean Lipoxygenase.* Soybean lipoxygenase type I at a concentration of 100 μg/mL was prepared in 0.2 M citrate−phosphate buffer at pH 4, 5, 6, 7, 8, and 9 and 0.2 M Tris buffer at pH 6, 7, 8, and 9. A few samples were prepared in 0.2 M Tris buffer containing sodium chloride to give solutions having the same ionic strength as the citrate−phosphate ones. The enzyme solutions were sealed

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in polyethylene bags (Multivac, Swindon, U.K.), with no headspace or air bubbles, and were subjected to pressures of 200−600 MPa for 20 min in the high-pressure rig. After treatment, samples were chilled in ice water and lipoxygenase activity was measured within 1 h. To check for reversibility of inactivation, the solutions treated at 600 MPa were left at 4 °C for several days and their activities reassessed.

Whole Cherry Tomatoes. Red cherry tomatoes (*L. esculentum*) were purchased from a local supermarket. Samples (6−7 fruits) were sealed in polyethylene bags and subjected to pressures of 200−600 MPa for 10 min. After treatment, samples were immediately analyzed for lipoxygenase activity, and extracts from tomatoes pressurized at 600 MPa were also reassessed for activity after several days storage at 4 °C. For volatiles analysis, tomatoes were sealed under nitrogen and treated at 600 MPa for 10 min.

Tomato Enzyme Extracts. The cherry tomatoes (6−7 fruits) were cut into small pieces, and about 50 g was homogenized with 100 mL of 0.1 M phosphate buffer, pH 6.5, containing 1 mM EDTA and 0.1% (w/v) Triton X-100 (Smith et al., 1997). The suspension was filtered through a double layer of cheesecloth and centrifuged at 20000*g* for 40 min at 5 °C. The supernatant was diluted with double its volume of 0.1 M phosphate buffer, pH 6.5, before determining its activity.

Enzyme Assay. A substrate solution containing linoleic acid (2.5×10^{-3} M) and Tween 20 (0.20%) was prepared according to Ben-Aziz et al. (1970). For commercial soybean lipoxygenase, the stock solution was diluted to 4×10^{-5} M with 0.2 M citrate−phosphate buffer, pH 9. For the crude tomato extracts, the stock solution was diluted to 2.5×10^{-5} M with 0.2 M phosphate buffer, pH 6.5. The reaction was carried out in a quartz cuvette in a Perkin-Elmer (Beaconsfield, U.K.) Lambda 5 thermostated spectrophotometer. At zero time, 0.1 mL of enzyme solution was pipetted into the cuvette containing 2.4 mL of substrate solution. The cuvette was inverted to mix the solution and immediately placed in the spectrophotometer, the time between addition of the enzyme and the beginning of the measurement being less than 10 s. The absorbance at 234 nm was recorded every 30 s until a constant value was reached.

Gel Electrophoresis of Soybean Lipoxygenase Samples. Commercial soybean lipoxygenase type I (3 mg/mL) in 0.2 M Tris buffer, pH 7, was prepared for gel electrophoresis. Native polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) were carried out in gels containing 10% polyacrylamide, at a constant current of 50 mA and 150 V (Laemmli, 1970). Enzyme solutions were diluted 1:1 with sample buffer, and 15 μL of solution was applied to each well. Electrophoresis was carried out for 1 h at room temperature. Protein was fixed in 12% trichloroacetic acid for 1 h, then stained with Coomassie brilliant blue G-250 (Neuhoff et al., 1988). For native PAGE, the enzyme solution was mixed with an equal volume of 0.125 M Tris-HCl buffer, pH 6.8, containing 10% glycerol and 0.002% bromophenol blue, while for SDS−PAGE, the enzyme solution was mixed with an equal volume of 0.125 M Tris-HCl buffer, pH 6.8, containing 2% SDS, 10% glycerol, and 0.002% bromophenol blue. SDS−PAGE was also performed in the presence of 2% 2-mercaptoethanol. Before SDS−PAGE all solutions were heated in boiling water for 3 min.

Differential Scanning Calorimetry (DSC) of Soybean Lipoxygenase Samples. Calorimetric measurements were carried out on a Perkin-Elmer differential scanning calorimetry model DSC 7 instrument that had been calibrated using indium. Commercial soybean lipoxygenase type I at a concentration of 10 mg/mL was prepared in 0.2 M Tris buffer, pH 7; 10−12 mg of the enzyme solutions was weighed into aluminum pans and heated from 20 to 95 °C at a scan rate of 5 °C/min. An empty pan was used as reference. The peak temperatures (T_m) and total calorimetric enthalpies were recorded.

Analysis of Volatile Compounds from Tomato Samples. *Isolation of Volatile Compounds*. To minimize the effect of variation between individual fruits, for the blended samples six or seven fruits were blended together prior to analysis. Volatile compounds were isolated from both pressurized intact (4−5 fruits, ∼25 g total sample) and their blended tomato flesh (25 g) and compared to their unpressurized counterparts. The volatiles were collected on a Tenax TA trap (SGE, Milton Keynes, U.K.) by passing purified nitrogen at 40 mL·min^{-1} for 15 min over the sample held in a 250-mL flask at 37 °C and then through the trap. Following isolation, 1 μL of a solution of 1,2-dichlorobenzene (internal standard) in hexane (130.6 μg·mL^{-1}) was injected onto the trap, which was purged with nitrogen at 40 mL·min^{-1} for 5 min to remove moisture and most of the hexane.

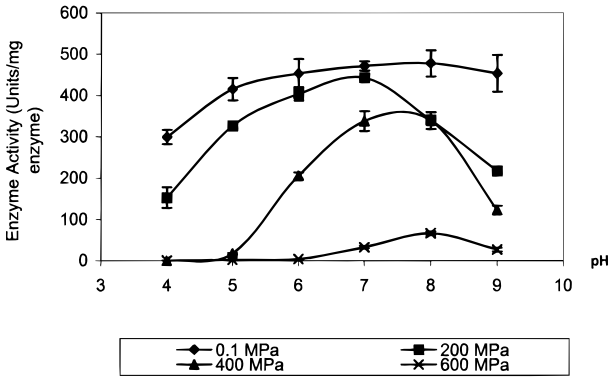

Figure 1. Effect of high-pressure treatment on the stability of commercial soybean lipoxygenase in citrate−phosphate buffer, pH 4−9.

Gas Chromatography−Mass Spectrometry (GC−MS). Volatile compounds were thermally desorbed from the trap by heating at 280 °C for 5 min while dry ice was used to precool the front of the GC column (50 m, 0.32-mm i.d., coated with a 0.5-μm layer of BPX-5, SGE). The column temperature was raised to 60 °C within 1 min, held at this temperature for 15 min, increased again to 250 °C at 4 °C·min^{-1}, and held at this final temperature for 10 min.

Identification of Volatile Compounds. The identification of compounds was achieved mainly by comparing their mass spectra with those of known compounds held in the NIST/EPA/NIH Mass Spectral Database (1992) as well as laboratory databases. Identifications were confirmed in most cases by comparing the linear retention indices (LRI) of authentic compounds run under the same conditions and on the same (or similar) stationary phase with those of the sample components.

Experimental Design. A randomized $a \times b$ factorial design was used for the commercial soybean lipoxygenase study, where a is the pH and b is the pressure applied. Completely randomized designs were used for the enzyme study in whole tomatoes and flavor study. The statistical program, SPSS version 7.5 (1996) (SPSS Inc., Chicago, IL), was used for data analysis, and the differences between treatments were analyzed by Duncan's multiple range test. The Wilcoxon signed-rank test, a nonparametric statistical method, was used to compare the differences in the amounts of volatile compounds between the unpressurized and pressurized samples (Mendenhall et al., 1981). All treatments were carried out in triplicate.

RESULTS AND DISCUSSION

Effect of High-Pressure Treatment on Commercial Soybean Lipoxygenase. *Citrate−Phosphate Buffer*. Lipoxygenase activity was stable at atmospheric pressure (0.1 MPa) between pH 5 and 9 (Figure 1). This result was similar to that of Al-Obaidy and Siddiqi (1981) who found that broad bean lipoxygenase had maximum stability between pH 4 and 8. However, our result shows a significant decrease in the activity at pH 4. After high-pressure treatment at 200−600 MPa for 20 min, the enzyme stability as a function of pH was a bell-shaped curve. As the pressure increased, the pH

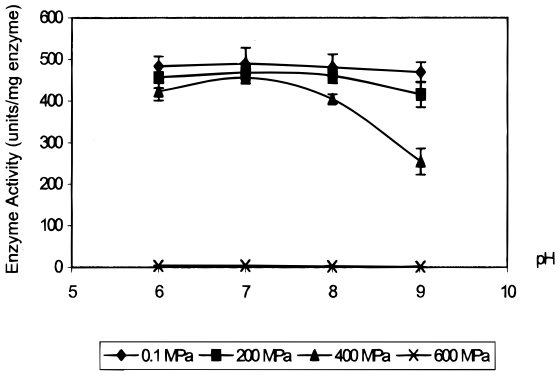

Figure 2. Effect of high-pressure treatment on the stability of commercial soybean lipoxygenase in Tris buffer, pH 6−9.

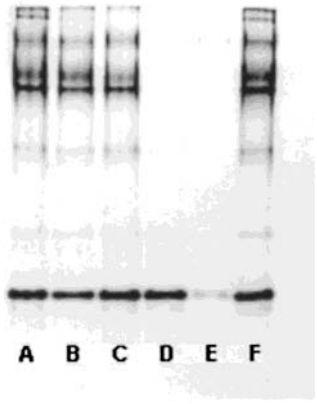

Figure 3. Native PAGE of lipoxygenase (3 mg/mL) before and after treatment at 200−600 MPa at ambient temperature for 20 min or heat treatment at 80 °C for 15 min. Lanes: A and F, native; B, 200 MPa; C, 400 MPa; D, 600 MPa; E, heated, 80 °C for 15 min.

range over which the enzyme was stable became narrower and the pH of maximum stability was shifted to a higher value. Thus, the untreated enzyme had maximum stability between pH 5 and 9, that treated at 200 MPa between pH 6 and 7.5 (maximum pH 7), that treated at 400 MPa between pH 7 and 8 (maximum pH 7.4), and that treated at 600 MPa between pH 7 and 9 (maximum pH 8). At 600 MPa, the enzyme lost more than 80% of its activity at pH 7−9. The enzyme appeared to be more affected by high-pressure treatment in acidic media than in alkaline conditions and was completely inactivated at pH 4−5 on treatment at 400−600 MPa. Ionization of the carboxylate group of citrate under pressure at pH 4−6 causes the pH of the solution to be lower than the buffer pH at atmospheric pressure. This may account for the apparent greater pressure sensitivity of lipoxygenase in acid compared to alkaline pH.

According to Heremans (1995), the pH change in weakly acidic solutions during pressure application is about 0.2 unit/100 MPa in acetic and citric acids, whereas a greater pH change, of 0.4 unit/100 MPa, is predicted in phosphate buffer. To correct for this pH shift, the pH was replotted by subtracting 0.2 unit/100 MPa from pH 4−6 (citrate buffer capacity range) and by 0.4 unit/100 MPa for pH 7−9 (phosphate buffer capacity range). Although the calculation to correct the pH of the buffer during pressure treatment was not precise, it did modify the pH of maximum activity for the enzyme after high-pressure treatment and suggested that the commercial soybean lipoxygenase had maximum stability in the pH range 5.3−6.5. The enzyme lost 10, 30, and 90% of its activity at these 'corrected' pH values after treatment at 200, 400, and 600 MPa, respectively.

Tris Buffer. The reaction was repeated in a barotolerant buffer, i.e., Tris, with a small ionization volume to see if ionization and the subsequent decrease in pH of the citrate−phosphate buffer under pressure was the possible cause of the shift in the optimum pH for lipoxygenase activity. The results in this buffer are shown in Figure 2 and indicate that the enzyme had high stability at atmospheric pressure between pH 6 and 9, as in citrate−phosphate buffer (Figure 1), and there was no difference in enzyme activity over the pH range examined. The pH for maximum stability was little affected after treatment at 200 and 400 MPa. However, activity was significantly reduced at pH 9 after pressurization at 400 MPa. The enzyme had its maximum stability around pH 7 after all pressure treatments, except 600 MPa, which caused loss of activity at all pH values examined. These findings agree with those of Seyderhelm et al. (1996) who reported that the activity of commercial soybean lipoxygenase was significantly decreased on treatment at 600 MPa, in Tris buffer at pH 7. In both citrate−phosphate and Tris buffers, untreated lipoxygenase has maximum stability near pH 7. However the enzyme is more pressure-sensitive in the former buffer, and in Tris buffer, there is no shift in the pH at which lipoxygenase has maximum stability on pressure treatment, as is found in the citrate−phosphate buffer. This supports the suggestion that ionization of the carboxylate and phosphate groups of the citrate−phosphate buffer causes a decrease in pH on pressure treatment, which modifies the apparent pH dependence (Heremans, 1995). However at 600 MPa and pH 8, the enzyme retained about 10% of its activity in citrate−phosphate buffer but lost all its activity in Tris buffer at this, and all other, pH's. Increasing the ionic strength of the Tris buffer, with sodium chloride, to that of the citrate−phosphate system had no significant effect on the results. Thus, the differences observed are due to the buffer system per se and not to the difference in ionic strength. These results agree with those of Ben-Aziz et al. (1970) who reported no difference in activity with ionic strength (0.05, 0.1, and 0.2 M citrate−phosphate buffer) at pH 9 and Ludikhuyze et al. (1998) who found that the pressure stability of lipoxygenase was buffer-dependent.

After storage at 4 °C for 4−9 days, there was no change in enzyme activity, in either buffer, after treatment at 600 MPa, indicating that inactivation was irreversible. This agrees with the results of Indrawati et al. (1998) and suggests that pressure induces a permanent modification of the protein structure.

Electrophoresis. The effect of high-pressure treatment on enzyme structure was studied by electrophoresis. Native PAGE (Figure 3) showed no protein bands after treatment at 600 MPa, presumably due to pressure-induced aggregation. In contrast, no difference could be observed between the unpressurized enzyme and enzyme after treatment at 200 or 400 MPa. Heat treatment (80 °C for 15 min) also gave no bands. SDS−PAGE (Figure 4) showed pressure treatment at 600 MPa and heat both modified the enzyme (mol wt 95−102 kDa) since no protein bands were seen. As with native PAGE, there were no differences in the SDS-soluble protein bands between the unpressurized samples

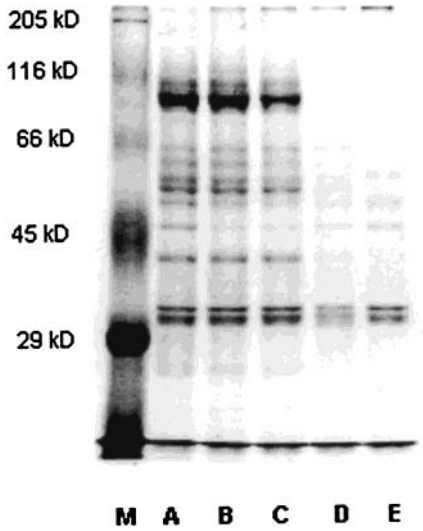

Figure 4. SDS−PAGE of lipoxygenase (3 mg/mL) in the presence of 2% SDS before and after treatment at 200−600 MPa at ambient temperature for 20 min or heat treatment at 80 °C for 15 min. Lanes: A, native; B, 200 MPa; C, 400 MPa; D, 600 MPa; E, heated, 80 °C for 15 min; M, marker.

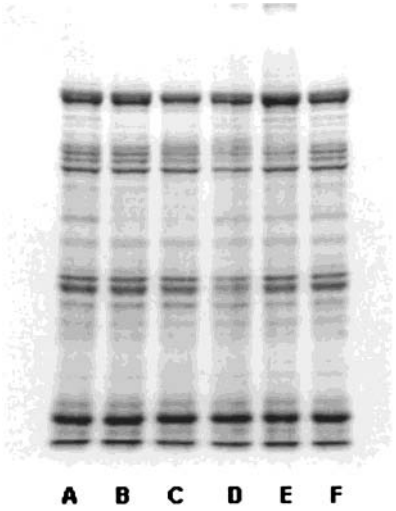

Figure 5. SDS−PAGE of lipoxygenase (3 mg/mL) in the presence of 2% SDS and 2% 2-mercaptoethanol before and after treatment at 200−600 MPa at ambient temperature for 20 min or heat treatment at 80 °C for 15 min. Lanes: A and F, native; B, 200 MPa; C, 400 MPa; D, 600 MPa; E, heated, 80 °C for 15 min.

and those treated at 200 and 400 MPa. In the presence of 2% 2-mercaptoethanol (Figure 5), SDS−PAGE for all treatments gave similar patterns, indicating that pressure and heat treatment both caused disulfide bond-induced aggregation of the enzyme.

DSC. The thermograms obtained for the native (0.1 MPa) and pressurized lipoxygenase are shown in Figure 6. The peak temperatures (T_m) were 68−69 °C. The calorimetric enthalpies for the native and pressure-treated enzyme at 400 MPa were the same but decreased markedly after treatment at 500 MPa. The DSC data thus support the electrophoretic and enzyme activity studies showing pressures above 400 MPa are necessary to inactivate the enzyme at neutral pH.

Effect of High-Pressure Treatment on Lipoxygenase Activity in Whole Cherry Tomatoes. Preliminary experiments showed that after treatment at 400 MPa for 20 min, lipoxygenase activity in the tomato

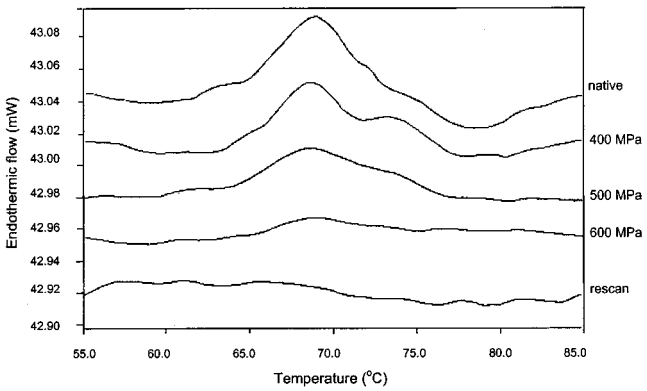

Figure 6. DSC thermograms of 10% lipoxygenase in 0.2 M Tris buffer, pH 7, after pressurization for 20 min at ambient temperature.

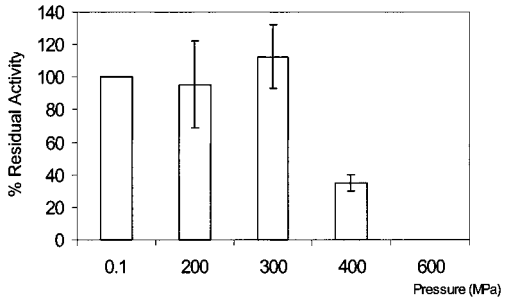

Figure 7. Effect of high-pressure treatment on the enzyme activity of crude lipoxygenase extracts from whole cherry tomatoes.

extracts was too low to measure. Thus, in this experiment the processing time was reduced to 10 min. The pH of the unpressurized blended cherry tomatoes was 4.0 ± 0.1. When whole tomato fruits were subjected to pressure in the range 200−600 MPa for 10 min, the lipoxygenase activity was significantly decreased at 400 MPa and completely lost at 600 MPa (Figure 7). The results at 600 MPa agree with those found with commercial soybean lipoxygenase (Figure 1) showing that, at this pressure and pH (4.0 ± 0.1), the enzyme is completely inactivated. However, the data show no statistical differences in lipoxygenase activity between tomatoes pressure-treated at 200 and 300 MPa and the untreated fruits, although at this pH some loss of activity might be expected, based on the data obtained for commercial soybean lipoxygenase (Figure 1). This discrepancy might be due to either (a) the natural variation among tomatoes being greater than the effect of high-pressure treatment below 400 MPa for 10 min or (b) lipoxygenase not being affected by pressure up to 400 MPa for 10 min in the whole tomato, due to the protective effect of other tomato components. On storage at 4 °C for 4 and 7 days, there was still no activity in the samples treated at 600 MPa, indicating that inactivation of lipoxygenase was irreversible.

Volatile Compounds. Numerous volatile compounds were separated by GC, making a complete and comprehensive analysis difficult. Thus, the present work focused on identification of the major volatiles, present at a level of at least 2 ng/25 g of tomato, with linear retention indices in the range 700−1200, the range for most of the tomato volatiles isolated. Table 1 shows the compounds identified. Aliphatic aldehydes occurred in the greatest numbers and in the highest amounts, with hexanal and *trans*-2-hexenal being among the most

Table 1. Volatile Compounds[a] in Unpressurized and Pressurized Baby Cherry Tomatoes from Blended and Intact Tomatoes

			concn (ng/25 g wet wt)			
			blended		intact	
compounds	exptl LRI	ref LRI	unpressurized	pressurized	unpressurized	pressurized
		Aldehydes				
pentanal	701	697	81	92	1[b]	10[a]
2-methyl-2-butenal	736	739	17[a]	12[b]	nd	nd
trans-2-pentenal	753	754	36[a]	10[b]	nd	3
3-methyl-2-butenal	783	781	31[a]	4[b]	nd	nd
hexanal	819	800	7329[a b]	1071[b]	7[b]	118[a]
cis-3-hexenal	*b*		*c*	3	nd	nd
trans-2-hexenal	858	854	737[a]	7[b]	nd	3
cis-4-heptenal	905	904	3	1	nd	nd
heptanal	906	903	24[b]	46[a]	7[b]	33[a]
trans-2-*trans*-4-hexadienal	917	912	33[a]	10[b]	nd	nd
cis-2-heptenal	964	957	40[b]	63[a]	nd	10
benzaldehyde	970	968	14	14	3	5
trans-2-*trans*-4-heptadienal	1002	998	5[b]	15[a]	nd	4
octanal	1007	1005	11[b]	16[a]	4[b]	11[a]
trans-2-octenal	1064	1060	20[b]	63[a]	nd	10
nonanal	1108	1107	14[b]	29[a]	12[b]	22[a]
trans-2-nonenal	1166	1172	13[b]	40[a]	17[b]	39[a]
cis-4-decenal	1198	1193	12[a]	nd[b]	nd	nd
decanal	1210	1204	19[b]	39[a]	19[b]	44[a]
trans-2-*trans*-4-decadienal	1302	1295	5	8	nd	nd
total			**8444**	**1543**	**70**	**312**
		Ketones				
1-penten-3-one	686	680	199[a]	22[b]	nd	nd
2-heptanone	896	898	3	2	6	2
5-ethyl-2(5)-furanone	972	954	52[a]	nd[b]	nd	nd
4-octen-3-one	982		7[b]	18[a]	nd	nd
6-methyl-5-hepten-2-one	990	985	50[b]	70[a]	3[b]	18[a]
acetophenone	1075		3	3	2	3
2-nonanone	1093		5	3	6	5
trans-6,10-dimethyl-5,9-undecadien-2-one	1453		15	13	3[b]	15[a]
total			**334**	**131**	**20**	**43**
		Alcohols				
2-methyl-1-butanol	742	744	47	60	2[b]	31[a]
1-pentanol	776	768	6[a]	3[b]	nd	1
3-hexen-1-ol	869	858	209[a]	nd[b]	nd	nd
1-hexanol	880	867	89[a]	2[b]	nd	nd
6-methyl-5-hepten-2-ol	998	992	5	3	nd	nd
1-octen-3-ol	985	982	2[b]	15[b]	1	4
2-ethyl-1-hexanol	1032		3	nd	nd	nd
total			**361**	**83**	**3**	**36**
		Furans				
2-ethylfuran	732	705	3	nd	nd	nd
2-pentylfuran	992	993	10[b]	22[a]	nd	4
total			**13**	**22**	**nd**	**4**
		Miscellaneous				
toluene	758	773	8	5	3	2
dimethyl disulfide	736	727	20	17	2	6
dimethyl trisulfide	974	974	17[a]	8[b]	nd	3
D-limonene	1033	1033	3	3	9	4
methyl salicylate	1205	1193	125[a]	3[b]	nd	nd
total			**173**	**36**	**14**	**15**
grand total			**9325**	**1815**	**107**	**410**

[a] The numbers are the mean from 3 replications. [a,b]The different superscripts mean significant difference between untreated and pressurized samples at $a = 95\%$. [b] *cis*-3-Hexanal was not separated from hexanal for this sample. [c] Coeluted with hexanal.

abundant. Ketones and alcohols were the other major groups of volatiles identified.

Blended and Intact Unpressurized Tomatoes. Blended tomatoes possessed higher concentrations of volatile compounds compared to the intact fruits (Table 1). The data confirm that volatile compounds in tomatoes form during blending, allowing enzyme and their substrates to come into contact, and that only low levels of most compounds are present in the intact fruits.

Various aldehydes, ketones, and alcohols (90, 4, and 4% of the grand total amount, respectively) were identified in blended unpressurized samples. Hexanal (eluting with *cis*-3-hexenal), *trans*-2-hexenal, 1-penten-3-one, 3-hexen-1-ol, and 1-hexenol were found in the greatest amounts. There are many studies on the volatile compounds present in tomatoes: e.g., Buttery et al. (1988), Petró-Turza (1986/87), and Linforth et al. (1994). More than 400 compounds have been identified, and *cis*-3-hexenal, *trans*-2-hexenal, hexanal, *cis*-3-hexen-1-ol, and hexanol are believed to be important flavor-contributing compounds. It is well-known that these aldehydes are produced by the lipoxygenase-induced oxidation of unsaturated fatty acids (mostly linoleic and linolenic acids) in the presence of oxygen.

Blended pressurized and Intact Tomatoes. Table 1 shows that pressurized intact tomatoes possess higher concentrations of volatiles than the unpressurized intact fruits, suggesting that cell disruption took place during pressurization thus encouraging lipoxygenase activity during the period before enzyme inactivation occurred. Certainly, cell damage in tomatoes occurs at pressures as low as 200 MPa (Tangwongchai et al., 1999). However, as well as being formed enzymatically, volatile compounds, such as hexanal, can be formed by chemical oxidation during both pressurization and blending. Cheah and Ledward (1996) have shown that, in meat, chemical oxidation is encouraged at pressures above 400 MPa and such a phenomenon may also occur in tomatoes. The data presented do not permit us to decide which mechanism, or both, is operating in pressurized intact tomatoes.

Blended pressurized tomatoes possessed higher concentrations of the volatile compounds, e.g., pentanal, hexanal, and *trans*-2-octenal, than pressurized intact fruits (Table 1). The blended pressurized tomatoes contained almost no *cis*-3-hexenal and significantly less hexanal than the blended unpressurized fruits, presumably due to inactivation of lipoxygenase by the treatment (600 MPa for 10 min). These compounds are all oxidation products of fatty acids. Kazeniac and Hall (1970) reported that the presence of oxygen during blending of fresh tomatoes has an effect upon the development of hexanal and *cis*-3-hexenal. Although blending could encourage lipoxygenase to react freely with substrates, the higher concentrations of compounds such as hexanal, in blended pressurized tomatoes, compared to pressurized intact ones, must be due to chemical oxidation during blending, since lipoxygenase was completely inactivated after pressure treatment. In addition, the pressure treatment may have inactivated other enzymes, such as alcohol oxidoreductase, since the corresponding alcohols of these aldehydes were not found after pressurization. These observations are similar to those of Sumitani et al. (1994), who found that pressure treatment of crushed peach at 400 MPa for 10 min led to lower amounts of alcohols and aldehydes (1-hexanol, *trans*-2-hexenol, hexanal, and *trans*-2-hexenal) compared to untreated crushed peach.

CONCLUSIONS

Commercial soybean lipoxygenase, in both citrate−phosphate and Tris buffers, was stable at atmospheric pressure between pH 6 and 9, and high-pressure treatment resulted in a narrower pH stability range. Treatment at 600 MPa for 20 min led to the enzyme losing all activity due to loss of secondary and tertiary structure leading to aggregation involving disulfide bond formation. Lipoxygenase activity in whole cherry tomatoes was markedly decreased following pressurization at 400 MPa for 10 min and was completely destroyed after treatment at 600 MPa for 10 min. Pressurization of whole cherry tomatoes at 600 MPa for 10 min resulted in large decreases in hexanal, *cis*-3-hexenal, and *trans*-2-hexenal concentrations. These compounds are important contributors to the 'fresh' flavor of tomato and are largely formed by enzymatic degradation of linoleic and linolenic acids. Furthermore, the loss of the corresponding alcohols of these aldehydes on pressure treatment suggests that alcohol oxidoreductases were also inactivated by treatment at 600 MPa for 10 min. Although treatment at 600 MPa for 10 min inactivates lipoxygenase, during treatment some oxidation (possibly both chemical and enzymatic) does occur due to cell disruption. Thus, pressure-treated intact tomatoes have higher concentrations of several volatiles than the untreated fruits. On subsequent blending the unpressurized tomatoes yielded far higher concentrations of volatiles due to lipoxygenase activity. However, due to autoxidation, some increases in concentration of certain volatiles were seen on blending the pressure-treated samples, indicating that such oxidations may also make some contribution to the overall flavor of fresh tomatoes.

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