High-Pressure and Temperature Effects on Enzyme Inactivation in Tomato Puree

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A study was made of the effect of high hydrostatic pressure treatment (50−500 MPa) combined with heat treatment (20−60 °C) on peroxidase (POD), polyphenol oxidase (PPO), and pectin methylesterase (PME) activities of tomato puree. Assays were carried out on fresh made tomato puree, and a 15 min treatment time was selected. Pressurization/depressurization treatments caused a continuous denaturation of soluble proteins at room temperature (20 °C). Also, ultrahigh hydrostatic pressure (UHP)/mild heat treatments produced a significant reduction (32.5%) of PME activity when a combination of 150 MPa/30 °C treatment was employed, while some activation was observed for treatments carried out at 335−500 MPa and different temperatures. A reduction of POD activity (25%) was obtained in tomato purees treated at 350 MPa/20 °C, but a combination of higher pressures and mild temperatures (30−60 °C) produced an enhancement of this activity. PPO activity did not show any significant change due to UHP/mild-temperature treatments in tomato product. Only a combination of 200 MPa/20 °C seemed to produce a significant loss (10%) in PPO activity.

Keywords: *High pressure; tomato; polyphenol oxidase; peroxidase; pectin methylesterase; proteins*

INTRODUCTION

High hydrostatic pressure treatment reduces microbial counts and enzyme activity and affects product functionality (Farr, 1990; Hoover et al., 1989; Cheftel, 1990). This provides a good potential basis for development of new processes for food preservation or product modifications (Mertens and Knorr, 1992). The first commercial products made using high-pressure treatments have been almost exclusively plants or products containing plants (Knorr, 1995).

Effects of high-pressure treatments on enzymes could be related to reversible or irreversible changes in protein structure (Cheftel, 1992). However, loss of catalytic activity can differ depending on the enzyme, the nature of the substrates, and the temperature and length of processing (Cheftel, 1992; Kunugi, 1992; Cano et al., 1996). These studies of the effects of pressure on biopolymers such as proteins and enzymes have increased interest in the application of high-pressure treatment for food preservation.

The demand for minimally processed tomato products of rich flavor and high consistency has risen markedly in recent years. In this way, some authors (Porreta et al., 1995) reported the effects of ultrahigh hydrostatic pressure (UHP) treatments on the quality of tomato juice. This product (juice) was prepared using a pH adjustment, but combinations of UHP/temperature were not employed. Also, in this work no enzymatic inactivation studies were made. Other authors (Dönenburg et al., 1996) studied the effects of high-pressure treatment on the activities of some enzymes of plant cell culture from *Lycopersicon esculentum*. In these experiments, tomato suspension cultures were treated with pressures from 20 to 110 MPa, and the results indicated that the cell response to high-pressure treatment is dependent on the physiological state of the cell, together with the time-dependent effect on enzyme activity.

The objective of the present work was to determine the effects of high-pressure treatments up to 500 MPa combined with mild heat treatments up to 60 °C on peroxidase (POD), polyphenol oxidase (PPO), and pectin methylesterase (PME) activities in tomato puree, because these enzymatic activities are directly related to quality modifications of this fruit-derived product.

MATERIALS AND METHODS

Plant Material. Fully ripe tomatoes (*L. esculentum* var. Pera) from Valencia (Spain) were obtained from commercial sources. Fruits for processing were selected on the basis of their maturity and were disease free. Characteristics of tomato puree are shown in Table 1.

UHP Equipment. A high-pressure unit GEC Alsthom ACB 900 HP, type ACIP No. 665 (Nantes, France), with 2350 mL capacity, was used.

Combined UHP/Temperature Treatments. UHP treatments (50−500 MPa) were employed. Time of the treatments was constant at 15 min, and the temperature of the immersion medium (initial sample at atmospheric pressure: 20 °C) was varied between 20 and 60 °C. Samples were placed in polyethylene bottles (250 mL) and then introduced in the pressure unit filled with pressure medium (water). Pressure was increased and released at 2.5 MPa/s. After pressure treatments, samples were immediately analyzed or stored at −80 °C for enzyme activity determinations.

Quality Determinations. Soluble solids of fresh made tomato puree were determined using a digital refractometer (Atago, Tokyo, Japan). Results were reported as °Brix at 20 °C. For titratable acidity, the puree was macerated and 10 g samples were accurately weighed into beakers. Distilled water (40 mL) was added to each sample. The resulting mixture was

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Table 1. Physicochemical and Biochemical Characteristics of Tomato Puree before Presurization

characteristic[a]	tomato puree
titratable acidity (g of citric acid/100 g of fw)	0.38 ± 0.02
pH	4.08 ± 0.05
soluble solids (°Brix at 20 °C)	5.6 ± 0.1
total solids (mg/100 g of fw)	5.69 ± 0.17
moisture content (%)	94.3 ± 0.18
POD activity [ΔOD min^{-1} (g of fw)]	40.41 ± 1.33
PPO activity [ΔOD min^{-1} (g of fw)]	0.79 ± 0.03
PME activity [ΔOD min^{-1} (g of fw)]	5.32 ± 0.08
Protein content (mg/g f.w.)	0.31 ± 0.05
color	
L (luminosity)	23.42 ± 0.08
a_L	12.39 ± 0.05
b_L	9.95 ± 0.04

[a] Values are average ± standard deviation of three independent determinations.

titrated with 0.1 N NaOH to pH 8.1 monitored with a pH meter (Microph 2000, Crison, Spain). The results were expressed as grams of citric acid per 100 g of sample. The pH of samples was determined before titration. For moisture content, the AOAC (1984) vacuum oven method was modified, using a microwave oven operating at 200 W for 20−25 min, as described in Cano et al. (1990).

Color of tomato puree was measured in a cylindrical sample cup, 5 cm diameter × 2 cm high, filled to the top, using a colorimeter Model 25-9 (Hunter Associates Laboratory, Reston, VA). Standard color plate No. C2-19952 with reflectance values $L = 77.65$, $a_L = -1.51$, and $b_L = 21.41$ was used as reference.

Biochemical Analysis. *Enzyme Extractions.* The enzyme extracts for the determination of PPO and POD were made by homogenization of 10 g of each sample with 10 mL of 0.2 M sodium phosphate buffer (pH 6.5) [containing 1% (w/v) insoluble polyvinylpolypyrrolidone (PVPP)] or for PME determination with 10 mL of 0.2 M sodium phosphate buffer (pH 7.5) in an ultrahomogenizer (Omnimixer, Model ES-207, Omni International, Inc., Gainsville, VA) with external cooling, for 3 min with stop intervals each 30 s.

POD. The peroxidase activity was assayed spectrophotometrically using aliquots (0.025 mL) of extract and a reaction mixture composed of 2.7 mL of 0.05 M sodium phosphate buffer (pH 6.5) with 0.2 mL of 1% (w/v) *p*-phenylenediamine as H-donor and 0.1 mL of 1.5% (w/v) hydrogen peroxide as oxidant. The oxidation of *p*-phenylenediamine was measured using a double-beam spectrophotometer (Perkin-Elmer, Model Lambda 15, Bodenseewerk, Germany) at 485 nm and 25 °C (Cano et al., 1997).

PPO. The PPO activity was assayed using aliquots (0.1 mL) of extract and 3.0 mL of a solution of 0.15 M cathecol in 0.05 M sodium phosphate buffer (pH 6.5). The reaction was measured with the spectrophotometer at 420 nm and 25 °C (Cano et al., 1997).

PME. The PME activity was assayed using aliquots (0.05 mL) of extract, adjusted to pH 7.5 if necessary, and a reaction mixtured composed of 2.8 mL of 0.5% (w/v) citrus pectin (pH 7.5) and 0.2 mL of 0.01% (w/v) bromothymol blue in 0.003 M potassium phosphate buffer (pH 7.5). The reaction was measured spectrophotometrically at 620 nm and 25 °C (Hagerman and Austin, 1986).

All enzyme activities were determined by measuring the slope of reaction. The enzyme activity unit was defined as the change in absorbance per minute per gram of fresh weight of sample.

Protein Determination. Soluble proteins were analyzed according to the Bradford (1976) method, measuring optical density (OD) at 595 nm, with bovine albumin as a standard.

Experimental Design. High-pressure treatments were carried out in triplicate. Values from chemical and biochemical analysis are averages of three independent determinations. A STATGRAPHICS (Statistical Graphics system, ver. 7.0) software program was employed for statistical data analysis and graphic presentation.

Table 2. Levels of Variables in Tomato Puree UHP Processing According to Experimental Design

pressure (MPa)	temp (°C)	pressure (MPa)	temp (°C)
50	20.0	434.1	54.1
115.8	25.0	500.0	60.0
275.0	40.0		

Table 3. Regression Model Fitted for Peroxidase (POD) Inactivation in Tomato Puree[a]

	RC	SE	SL
constant	69.48	2.41	0.00
linear			
P	−1.86	1.91	0.36
T	0.89	1.91	0.65
quadratic			
$P \times P$	−11.46	2.05	0.00
$T \times T$	−9.81	2.05	0.00
interaction			
$P \times T$	6.85	2.70	0.03

[a] *P* = pressure (MPa); *T* = temperature (°C); RC = regression coefficient; SE = standard error; SL = significance level.

Table 4. Regression Model Fitted for Polyphenol Oxidase (PPO) Inactivation in Tomato Puree[a]

	RC	SE	SL
constant	2.010	0.05	0.00
linear			
P	0.012	0.04	0.76
T	0.044	0.04	0.31
quadratic			
$P \times P$	−0.490	0.04	0.00
$T \times T$	−0.470	0.04	0.00
interaction			
$P \times T$	0.050	0.05	0.42

[a] *P* = pressure (MPa); *T* = temperature (°C); RC = regression coefficient; SE = standard error; SL = significance level.

Surface response methodology (SRM) was used to study the simultaneous effect of two processing variables. The experiments were designed according to a central composite rotatable design (Cochran and Cox, 1957). The variables studied were pressure and temperature. Five levels of each variable were chosen in accordance with the principles of central composite design (Table 2). Thirteen combinations of two variables were performed following the designs of Cochran and Cox (1957). Assessment of error was derived from replication of treatment combination as suggested in the design.

For each factor assessed, a second-order polynomial equation was fitted as follows:

$$y = b_0 + \sum b_i x_i + \sum b_{ii} x_i^2 + \sum b_{ij} x_i x_j$$

where y is the estimated response; b_0, b_i, b_{ii}, and b_{ij} are equation parameter estimates (constant, b_0; parameter estimates for linear terms, b_i; for quadratic terms, b_{ii}; for interaction terms, b_{ij}); x_i and x_j are levels of factors; and k is the number of factors. For each factor the variance was partitioned into linear, quadratic, and interaction components to assess the adequacy of the second-order polynomial function and the relative importance of the components (Tables 3−6). The significance of the equation parameters for each response variable was assessed by *t* test.

RESULTS AND DISCUSSION

Initial enzyme activities in freshly prepared tomato puree (Table 1) showed a very high POD activity [40.41 ΔOD min^{-1} (g of fw)$^{-1}$], a relatively low PPO activity [0.79 ΔOD min^{-1} (g of fw)$^{-1}$], a fair PME [5.32 ΔOD

Table 5. Regression Model Fitted for Pectin Methylesterase (PME) Inactivation in Tomato Puree[a]

	RC	SE	SL
constant	7.42	0.20	0.00
linear			
P	0.04	0.15	0.78
T	0.20	0.15	0.23
quadratic			
$P \times P$	−0.95	0.17	0.00
$T \times T$	−1.40	0.17	0.00
interaction			
$P \times T$	−0.58	0.22	0.03

[a] P = pressure (MPa); T = temperature (°C); RC = regression coefficient; SE = standard error; SL = significance level.

Table 6. Regression Model Fitted for Protein Extracted in Tomato Puree[a]

	RC	SE	SL
constant	0.190	0.010	0.000
linear			
P	−0.090	0.008	0.000
T	0.004	0.008	0.590
quadratic			
$P \times P$	0.020	0.008	0.040
$T \times T$	−0.040	0.008	0.002
interaction			
$P \times T$	0.030	0.011	0.017

[a] P = pressure (MPa); T = temperature (°C); RC = regression coefficient; SE = standard error; SL = significance level.

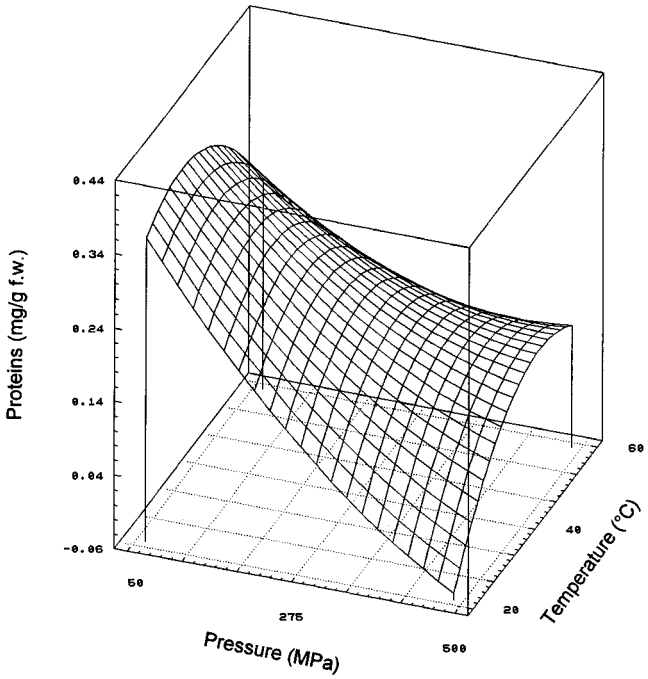

Figure 1. Influence of high-pressure and temperature treatments (15 min) on soluble protein content of tomato puree.

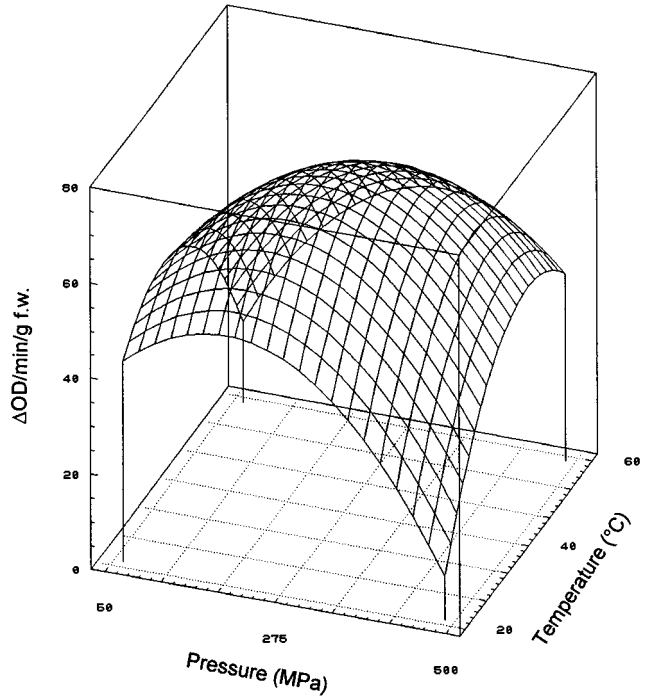

Figure 2. Influence of high-pressure and temperature treatments (15 min) on POD activity of tomato puree.

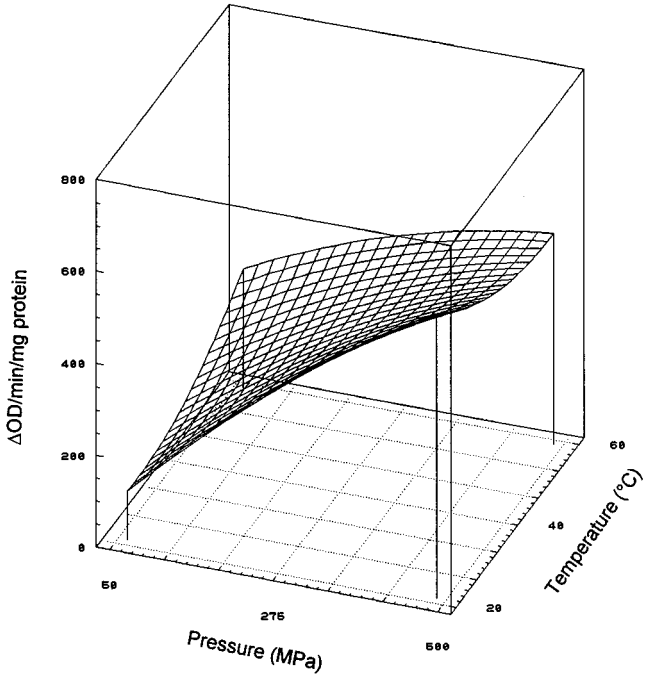

Figure 3. Influence of high-pressure and temperature treatments (15 min) on specific POD activity of tomato puree.

min^{-1} (g of fw)$^{-1}$], and a protein content of 0.31 mg/g of fw. These are the control values employed for UHP/temperature inactivation studies. Tomato puree proteins suffered a continuous denaturation when pressure increased to 500 MPa at room temperature (20 °C) (Figure 1). However, when combined treatments UHP/temperature were employed, the effects on soluble proteins were less important. In general, UHP/mild-temperature treatments caused a continuous denaturation of soluble proteins, but this denaturation increased when pressure was higher. Better results, in terms of protein modification, were obtained at temperatures of 20/30 °C and pressures between 100 and 300 MPa.

POD activity in tomato puree showed an activation when combined treatments were carried out at pressures below 350 MPa at room temperature (20 °C), while a significant inactivation of this enzyme can be obtained using treatments at pressures above 350 MPa (Figure 2). However, combinations of higher pressures (400−500 MPa) and mild temperatures (30−60 °C) produced an increase of this activity. The observed effects of UHP/temperature treatments in tomato POD activity were opposite from those reported by Cano et al. (1996) for POD inactivation in strawberry puree and orange

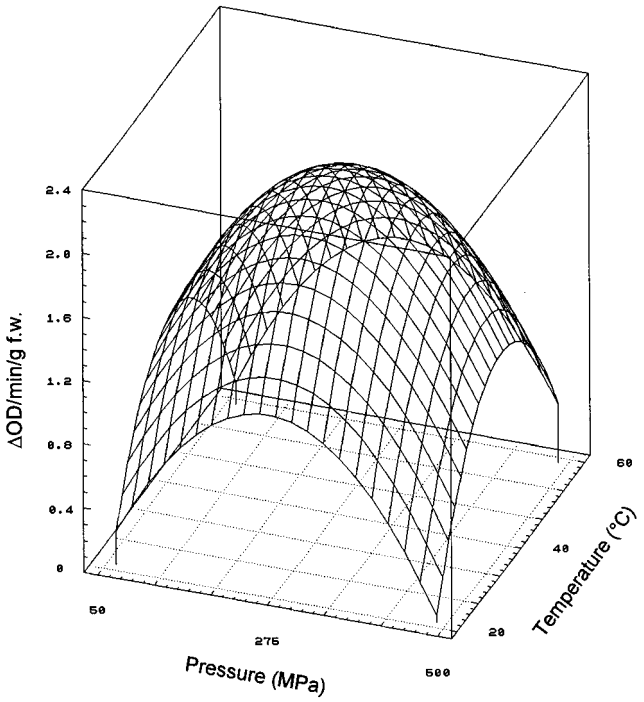

Figure 4. Influence of high-pressure and temperature treatments (15 min) on PPO activity of tomato puree.

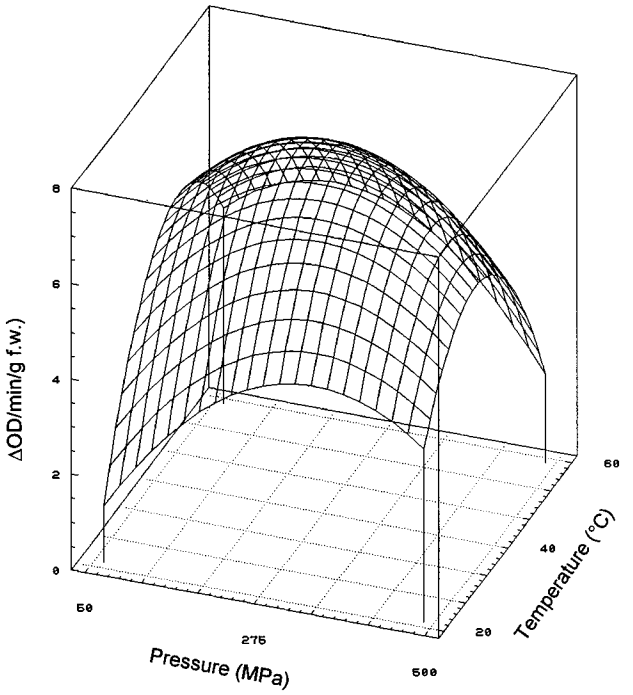

Figure 6. Influence of high-pressure and temperature treatments (15 min) on PME activity of tomato puree.

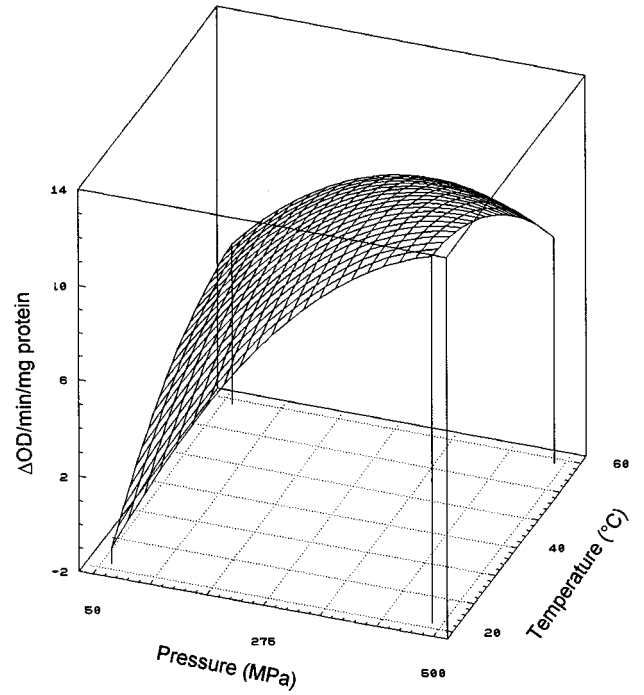

Figure 5. Influence of high-pressure and temperature treatments (15 min) on specific PPO activity of tomato puree.

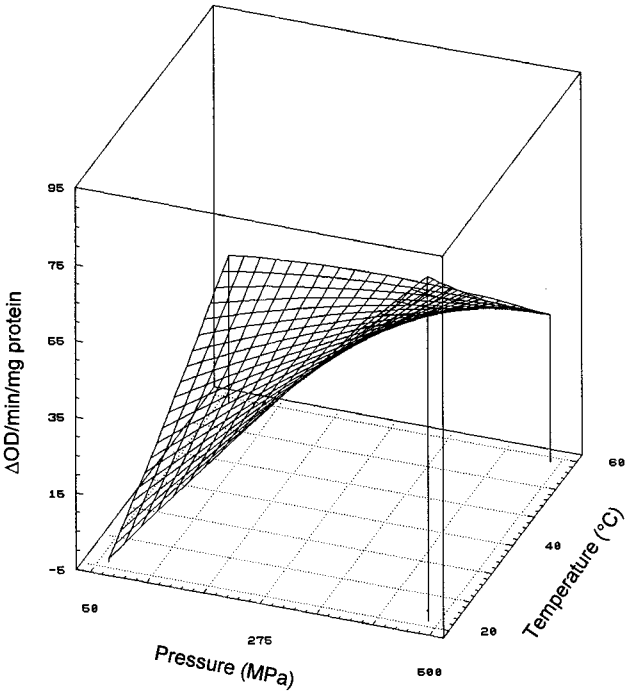

Figure 7. Influence of high-pressure and temperature treatments (15 min) on specific PME activity of tomato puree.

juice. In this work, strawberry POD can be successfully inactivated using combinations of pressures up to 280 MPa and temperatures up to 45 °C. In the same way these authors obtained good POD inactivation (50%) in orange juice employing a combination of 400 MPa and 32 °C. In all studies a constant treatment time (15 min) was employed. If the results are reported as specific activity [ΔOD min^{-1} (mg of protein)$^{-1}$], the effects of combined UHP/temperature treatments seemed to be different (Figure 3). Figure 3 shows an increase in specific POD activity when pressure was increased to 500 MPa (20 °C). This effect could be related to the increase in the solubilization by pressure of POD forms linked to cellular membranes. However, to carry out the estimation studies on pressurized/depressurized tomato puree, the results obtained from the calculation of enzymatic activity per gram of fresh weight could be the better indicator.

Similar results were observed for tomato puree PPO activity (Figure 4). The effect of different pressurization/depressurization treatments on this enzyme showed that tomato puree PPO suffered an increase in activity when pressures below 200 MPa were employed, while a significant activity loss was observed using pressures

from 200 to 500 MPa, working at room temperature (20 °C). The better result in terms of PPO inactivation was obtained using a combination of 200 MPa/20 °C (15 min). If specific PPO activity was plotted, an evident solubilization of this enzyme was obtained with increasing pressure up to 500 MPa (Figure 5). However, combination of 500 MPa treatments at different temperatures could cause a real denaturation of this enzyme.

PME of fresh tomato puree was a 5.32 [ΔOD min^{-1} (g of fw)$^{-1}$]. This initial activity was reduced to 35% using a combined treatment of 150 MPa/30 °C, during 15 min (Figure 6). This combination was the most efficient in terms of PME inactivation. In general, PME is the most affected enzyme in tomato products by UHP treatments. This higher efficiency of low-pressure/mild-temperature treatments on tomato puree PME was also reported in pressurized orange juice attending PME activty (Cano et al., 1996). Moreover, as with the above-mentioned enzymes, POD and PPO, if specific PME activity is plotted, a continuous solubilization of the enzyme could be observed when the pressure was increased, working at room temperature (20 °C) (Figure 7). However, good results in terms of PME denaturation can be obtained using the UHP/temperature combinations (150 MPa/30 °C, 15 min) mentioned.

The activation effects, observed in some cases as a consequence of combined UHP/temperature treatments, could be attributed to reversible configuration and/or conformation changes of the enzyme and/or substrate molecules (Ogawa et al., 1990; Anese et al., 1995). The pH dependence of such activation effects seemed to confirm this hypothesis. Tomato puree showed a relatively high pH value (4.08) and also a fairly low soluble solids content (5.6 °Brix at 20 °C) with regard to other studies of UHP/temperature effects on other fruit-derived products such as strawberry puree or orange juice. Anese et al. (1995) reported that the pH of enzymatic crude extracts from juices seemed to strongly affect the extent of enzyme inactivation, which reached a maximum at pH 6.0. In addition, the soluble solids content of the sample also influences the inactivation of enzymes (Ogawa et al., 1990). Increased soluble solids protect PME against pressure as well as heat inactivation. However, in the present study tomato PME experiments showed a significantly greater inactivation using UHP/temperature combination than orange juice (Cano et al., 1996), in spite of the tomato product having higher pH and a lower soluble solids content than the orange juice. In this way, other factors in addition to pH and soluble solids must contribute to the effectiveness of combined UHP/temperature treatments in enzyme inactivation of fruit-derived products.

In the present study, a combination of UHP/temperature treatment could be selected to obtain a self-stable derived product stored at refrigeration temperatures. However, other factors such as microbiological quality of pressurized/depressurized products must be take into account to make a definitive selection of the optimal parameters of UHP/temperature combination to produce a cold-stored fresh tomato puree without any additives.

LITERATURE CITED

Anese, M.; Nicoli, M. C.; Dall'aglio, G.; Lerici, C. R. Effect of high pressure treatments on peroxidase and polyphenoloxidase activities. *J. Food Biochem.* **1995**, *18*, 285−293.

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Washington, DC, 1984.

Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

Cano, M. P.; Marín, M. A.; Fuster, C. Freezing of banana slices. Influence of maturity level and thermal treatment prior to freezing. *J. Food Sci.* **1990**, *55* (4), 1070−1073.

Cano, M. P.; Hernández, A.; De Ancos, B. High Pressure and Temperature Effects on Enzyme Inactivation in Strawberry and Orange Products. *J. Food Sci.* **1997**, *62* (1), 85−88.

Cheftel, J. C. Applications des hautes pressions en technologie alimentaire (Application of high pressures in food technology). *Act. Ind. Aliment. Agro-aliment.* **1990**, *108* (3), 141−153.

Cheftel, J. C. Effects of high hydrostatic pressure on food constituents: an overview. In *High Pressure and Biotechnology*; Balny, C., Hayashi, R., Heremans, K., Masson, P., Eds.; John Libbey Euro-text: Montrouge, 1992; pp 195−209.

Cochran, W. G.; Cox, G. M. *Experimental Design*, 2nd ed.; Wiley International: New York, 1957.

Dönenburg, H.; Fenselau, K.; Wiesner, P.; Knorr, D. Effect of high pressure treatment on the activity of pectinases, polyphenol oxidase and peroxidase of a plant cell culture from *Lycopersicon esculentum*. Presented at the High Pressure Processing of Foods, FLAIR-Flow Europe Symposium, Cologne, Nov 1996.

Farr, D. High pressure technology in the food industry. *Trends Food Sci. Technol.* **1990**, *1*, 14−16.

Hagerman, A. E.; Austin, P. J. Continuous spectrophotometric assay for plant pectin methylesterase. *J. Agric. Food Chem.* **1986**, *34*, 440−444.

Hoover, D. G.; Metrick, C.; Papineau, A. M.; Farkas, D. F.; Knorr, D. Biological effects of high hydrostatic pressure on food microorganisms. *Food Technol.* **1989**, *43* (3), 99−107.

Knorr, D. High pressure effects on plant derived products. In *High Pressure Processing of Foods*; Ledward, D. A., Johnston, D. E., Earnshaw, R. G., Hasting, A. P. H., Eds.; Nottinghan University Press: Nottingham, U.K., 1995; pp 123−135.

Kunugi, S. Effect of pressure on activity and specificity of some hydrolytic enzymes. In *High Pressure and Biotechnology*; Balny, C., Hayashi, R., Heremans, K., Masson, P., Eds.; John Libbey Euro-text: Montrouge, 1992; pp 129−137.

Mertens, B.; Knorr, D. Development of nonthermal processes for food preservation. *Food Technol.* **1992**, *46* (5), 124−133.

Ogawa, H.; Fukuhisa, K.; Kubo, Y.; Fukumoto, H. Pressure inactivation of yeast, molds and pectinesterase in Satsuma mandarin juice: effect of juice concentration, pH and organic acids and comparison with heat sanitation. *Agric. Biol. Chem.* **1990**, *54* (5), 1219−1225.

Porreta, S.; Birzi, A.; Ghizzoni, C.; Vicini, E. Effects of ultrahigh hydrostatic pressure treatments on the quality of tomato juice. *Food Chem.* **1995**, *52*, 35−41.

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