High Pressure and Temperature Effects on Enzyme Inactivation in Strawberry and Orange Products

M. P. CANO, A. HERNANDEZ, and B. DE ANCOS

ABSTRACT

High hydrostatic pressure treatment (50-400 MPa) combined with heat treatment (20–60°C) effects on peroxidase (POD), polyphenoloxidase (PPO) and pectin methylesterase (PME) activities of fruit-derived products were studied. Assays were carried out on fresh orange juice and strawberry puree. Pressurization/depressurization treatments caused a significant loss of strawberry PPO (60%) up to 250 MPa and POD activity (25%) up to 230 MPa, while some activation was observed for treatments carried out in 250-400 MPa range for both enzymes. Optimal inactivation of POD was using 230 Mpa and 43°C in strawberry puree. Combinations of high pressure and temperature effectively reduced POD activity in orange juice (50%) to 35°C. The effects of high pressure and temperature on PME activity in orange juice were very similar to those for POD.

Key Words: strawberry, orange, hydrostatic pressure, enzymes, heat inactivation

INTRODUCTION

HIGH PRESSURE TREATMENT reduces microbial counts and enzyme activity and affects product functionality (Farr, 1990; Hoover et al., 1989; Cheftel, 1991). 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). Horie et al. (1991) indicated that preservation of jams could be accomplished at pressures of 400–600 MPa and the products retained the original colors and flavors. However, remaining enzyme activities and dissolved oxygen in the pressurized samples were believed to be responsible for color and flavor deterioration during storage; consequently transport and storage under refrigeration was suggested (Horie et al., 1991). Watanabe et al. (1991) reported to the production of non-heated samples using combinations of freeze concentration and high pressure sterilization.

Studies on the effects of pressure on biopolymers such as proteins and enzymes have increased interest in the application of high pressure treatment for food preservation. Effects of high pressure treatments on enzymes may be related to reversible or irreversible changes in protein structure (Cheftel, 1992). However, loss of catalytic activity can differ depending on type of enzyme, the nature of substrates, the temperature and length of processing (Cheftel, 1992; Kunugi, 1992). Ogawa et al. (1990) reported the effect of high pressure treatments on pectinesterase and peroxidase activity in model systems from mandarin juice. Neither enzyme was completely inactivated after pressurization from 100 to 400 MPa, although no recovery of enzyme activity was observed during storage. Similar behavior was observed for polyphenol oxidase activity. In that case the degree of enzyme inactivation varied depending on the type of fruit and vegetable products studied (Knorr, 1995) and strong enzyme activation could be observed in cell-free extracts (Asaka and Hayashi, 1991; Anese et al., 1995).

Our objective was to determine the effects of high pressure treatments up to 400 MPa combined with mild heat treatments up to 60°C on peroxidase (POD; EC 1.11.1.7), polyphenol oxidase (PPO; EC 1.10.3.1) and pectin methylesterase (PME; EC 3.1.1.11) activities in strawberry puree and orange juice. Surface Response Methodology was applied for simultaneous analysis of the effects of variables.

Authors Cano, Hernandez, and DeAncos are with the Plant Foods Science & Technology Dept., Instituto del Frío, Consejo Superior de Investigaciones Científicas, Ciudad Universitaria, 28040-Madrid, Spain. Address inquiries to Dr. M.P. Cano.

MATERIALS & METHODS

High pressure treatments

Strawberries (*Fragaria ananassa*, cultivar Pajaro) and oranges (*Citrus aurantium*, cultivar Salustiana) from Valencia (Spain) were obtained from commercial sources. Fruits for processing were selected to be mature (Table 1) and disease free. Strawberry puree was obtained by homogenization using a blender (Osterizer, Proctor-Sile, Inc., North Carolina, USA). Squeezed orange juice was freshly made by juice extractor (Lomi mod.4, Madrid, Spain). Samples (strawberry puree or orange juice) were placed in polyethylene bottles (250 mL) and then introduced in the pressure unit filled with pressure medium (water).

High pressure treatments were performed in a hydrostatic pressure unit with 2,350 mL capacity, a maximum pressure of 500 MPa and a potential maximum temperature of 95°C (Gec Alsthom ACB 900 HP, Type ACIP no. 665, Nantes, France). Pressure was increased and released at 2.5 MPa/s. Time of pressure treatments was constant at 15 min and temperature of immersion medium (initial sample at atmospheric pressure: 20°C) was varied between 20°C and 60°C. After pressure treatment samples were immediatelly analyzed or stored at −80°C for enzyme activity determinations.

Quality determinations

Soluble solids of strawberry and orange fruits were determined using a digital refractrometer (ATAGO, Tokyo, Japan). Results were reported as °Brix at 20°C. For titratable acidity, the puree or juice of fruits were macerated and 10g samples were accurately weighed into beakers. Distilled water (40 mL) was added to each sample. The resulting mixture was titrated with 0.1N NaOH to pH 8.1 monitored with a pH meter (Microph 2000, Crison, Spain). The results were expressed as g citric acid/100g 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 200W for 20–25 min, as described (Cano et al., 1990).

Color of strawberry puree or orange juice was measured in a cylindrical sample cup, 5 cm diam × 2 cm high, filled to the top, using a colorimeter model 25-9 (Hunter Associates Laboratory mod. 25-9, Reston, VA, USA). As standard color plate No. c2-19952 with reflectance values L = 77.65, a = −1.51, b = 21.41 was used as reference.

Biochemical analysis

The enzyme extracts for determination of peroxidase and polyphenol oxidase were made by homogenization of 10g of each sample with 20–25 mL of 0.2M sodium phosphate buffer (pH 6.5) (containing 4% (w/v) insoluble polyvinylpolypirrolidone (PVPP) and 1% (v/v) Triton X-100 for strawberry samples) in an ultrahomogenizer (Omnimixer, mod. ES-207, Omni International, Inc., Gainsville, VA, USA) with external cool-

Table 1—Physicochemical and biochemical characteristics of fruit products before pressurization

Characteristic[a]	Strawberry puree	Orange juice
Titratable acidity		
(g citric acid/100 g f.w.)	0.47 ± 0.01	0.90 ± 0.04
pH	3.90 ± 0.07	3.69 ± 0.05
Soluble solids		
(° Brix at 20°C)	8.76 ± 0.02	11.33 ± 0.10
Total solids (mg/100 g f.w.)	10.91 ± 0.90	12.69 ± 0.08
Moisture content (%)	89.09 ± 0.24	87.30 ± 0.03
POD activity	1.38 ± 0.08	3.19 ± 0.09
PPO activity	1.48 ± 0.06	—
PME activity	—	6.11 ± 1.34
Color L (luminosity)	20.66 ± 0.06	28.24 ± 0.12
aL	20.74 ± 0.03	−2.97 ± 0.42
bL	8.48 ± 0.04	13.64 ± 0.31

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

Table 2—Levels of variables in fruit-derived products UHP processing according to experimental design

Pressure (MPa)	Temperature (°C)
50	20.0
101.2	25.8
225.0	40.0
348.7	54.1
400.0	60.0

Table 3—Regression model fitted for polyphenol oxidase inactivation in strawberry puree[a]

	RC	SE	SL
Constant	1.51	0.08	0.000
Linear			
P	−0.09	0.06	0.180
T	0.02	0.06	0.730
Quadratic			
P X P	0.03	0.06	0.010
T X T	−0.01	0.06	0.850
Interaction			
P X T	0.09	0.09	0.340

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

Table 4—Regression model fitted for peroxidase inactivation in strawberry puree[a]

	RC	SE	SL
Constant	1.26	0.09	0.000
Linear			
P	−0.04	0.07	0.567
T	−0.15	0.07	0.075
Quadratic			
P X P	0.10	0.07	0.197
T X T	0.18	0.07	0.042
Interaction			
P X T	0.08	0.10	0.414

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

ing, for 3 min with stop intervals each 30 sec. The extract for determination of pectin methylesterase activity in orange juice was made by homogenization of 10g sample with 30 mL of 2M sodium chloride (pH 7.5).

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

Polyphenoloxidase activity was assayed using aliquots (0.075 mL) of extract and 3.0 mL of a solution of 0.07M catechol in 0.05M sodium phosphate buffer (pH 6.5). The reaction was measured with the spectrophotometer at 420 nm at 25°C.

Pectin methylesterase activity was assayed using aliquots (0.1 mL) of extract and a reaction mixture composed of 2.0 mL 0.5% (w/v) citrus pectin (pH 7.5), 0.15 mL 0.01% (w/v) bromothymol blue in 0.003M potassium phosphate buffer (pH 7.5) and 0.75 mL distilled water. The reaction was measured spectrophotometrically at 620 nm at 25°C.

All enzyme activities were determined by measuring the slope of reaction. The enzyme activity unit was defined as the change in absorbance/min/g fresh wt of sample.

Experimental design

High pressure treatments were carried out in triplicate. Values from chemical and biochemical analyses are averages of three determinations. A STATGRAPHICS (Statistical Graphics System, vers. 7.0, USA) software program was employed for statistical data analysis and graphical presentation.

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

Table 5—Regression model fitted for peroxidase inactivation in orange juice

	RC	SE	SL
Constant	2.23	0.22	0.000
Linear			
P	−0.09	0.17	0.614
T	0.02	0.17	0.896
Quadratic			
P X P	−0.21	0.18	0.294
T X T	0.68	0.18	0.008
Interaction			
P X T	0.19	0.24	0.451

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

Table 6—Regression model fitted for pectin methylesterase inactivation in orange juice[a]

	RC	SE	SL
Constant	3.72	0.05	0.000
Linear			
P	0.58	0.043	0.000
T	0.56	0.04	0.000
Quadratic			
P X P	0.80	0.04	0.000
T X T	0.63	0.04	0.000
Interaction			
P X T	0.05	0.06	0.444

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

levels of each variable were chosen in accordance with 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}, 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, x_j are levels of factors and k the number of factors. For each factor the variance was partitioned into linear, quadratic and interaction-components in order to assess the adequacy of the second-order polynomial function and the relative importance of the components. The significance of the equation parameters for each response variable was assessed by t-test.

RESULTS & DISCUSSION

Initial enzyme activities in freshly prepared fruit-derived products (Table 1) showed peroxidase activity was twofold greater in orange juice (3.19 ΔOD/min/g f.w.) than in strawberry puree (1.38 ΔOD/min/g f.w.). Polyphenol oxidase activity in strawberry was 1.48 ΔOD/min/g f.w. and pectin methylesterase activity in control orange juice was 6.11 ΔOD/min/g f.w. These were the control values employed for UHP/temperature inactivation studies. High pressure/temperature treatments of fruit-derived products differed in inactivation of enzyme activities, depending on the plant characteristics, the kind of enzyme and processing conditions (Tables 3 to 6).

Peroxidase activity in strawberry puree was increasingly inactivated up to 300 MPa, for treatments carried out at room temperature (20°C) and 15 min of pressurization (Fig.1). UHP treatments above 300 MPa slightly increased POD activity at this temperature. Results on POD in fresh-made strawberry puree subjected to UHP treatment up to 300 MPa were similar to those of previous studies (Anese et al., 1995; Balny and Masson, 1993). Temperature affected POD activity in a similar way and the POD was inactivated by UHP and temperature treatments up to 45°C and pressures up to 280 Mpa (Fig. 1).

Above that temperature decreased inactivation was observed for all pressures. The best results in terms of POD activity were

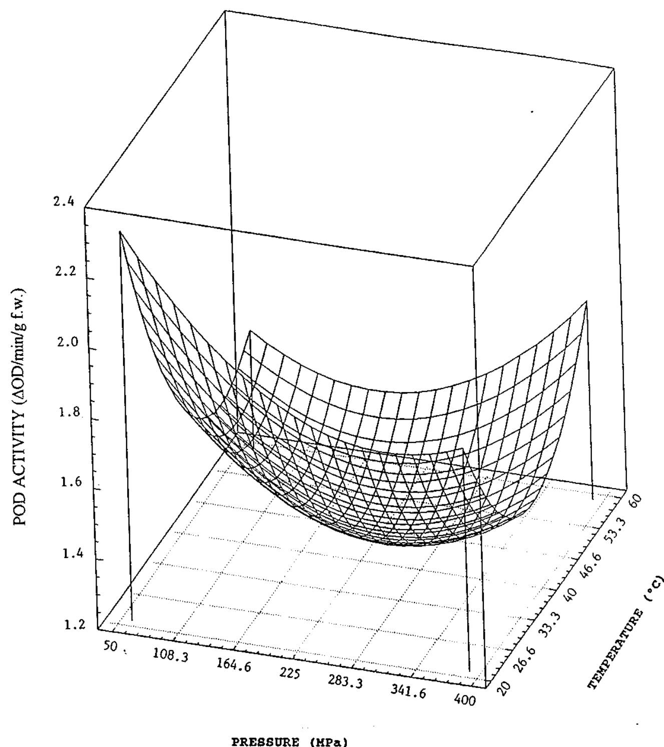

Fig. 1—Influence of high pressure and temperature treatments (15 min) on peroxidase (POD) of strawberry puree.

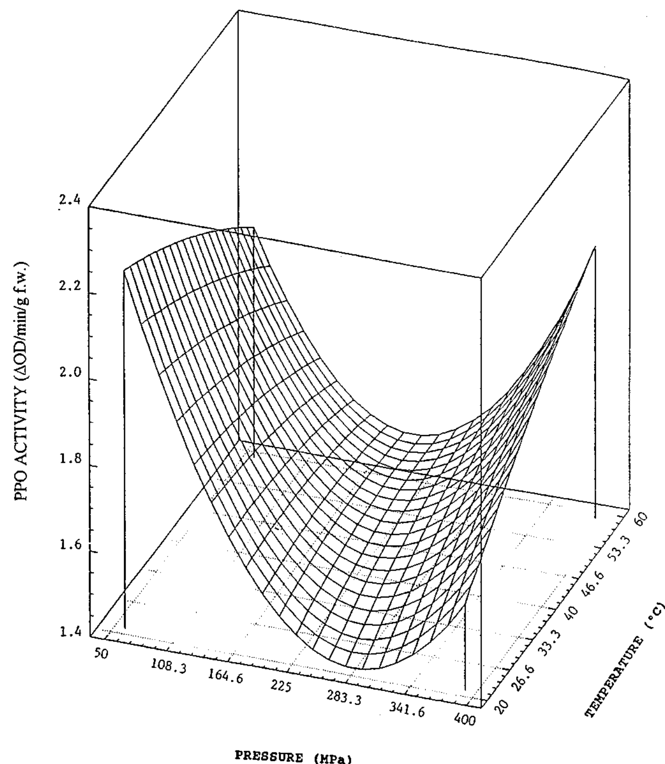

Fig. 2—Influence of high pressure and temperature treatments (15 min) on polyphenoloxidase (PPO) activity of strawberry puree.

obtained using a combination of 230 Mpa and 43°C treatment for 15 min. From these process conditions the strawberry puree had 75% residual POD activity.

Polyphenoloxidase (PPO) activity was affected by UHP and temperature treatments in a different way (Fig. 2). At room temperature and prefixing the pressurization time (15 min), the PPO activity strongly diminished up to 285 MPa. However, UHP treatments in the 285–400 MPa range decreased inactivation. A strawberry puree with 40% residual PPO activity was obtained after a 285 MPa treatment, at room temperature. However, combination of UHP treatment with mild temperature did not give better results (Fig. 2). Strawberry samples treated at different pressures and temperatures in the 20–60°C range showed a significant increase of PPO activity, and a streper slope was seen in samples treated at the highest pressure (400 MPa). Strawberry puree treated at 400 MPa and 60°C (the strongest conditions) showed almost the same PPO activity as the control (unpressurized sample). The effect of the increase in process temperature was not significant at pressures in the 50–200 MPa range.

The effects of UHP and temperature treatments were compared on peroxidase (POD) activity in orange juice (Fig. 3). POD activity underwent a continuous decrease up to 400 MPa at room temperature (process time 15 min). A 25% reduction of initial POD activity was observed in these samples. The combination of high pressure and mild temperature produced results similar to those for strawberry puree. UHP treatment at 400 MPa and 32°C produced the greatest inactivation rate (50%) of POD activity in orange juice. UHP treatments at 32–60°C adversely affected this enzyme activity. POD was strongly activated under these conditions (Fig. 3).

PME activity of fresh squeezed orange juice was a 4.3 ΔO.D./min/g f.w. This initial activity was reduced to 25% by UHP treatment at 200 MPa/30°C. However, at room temperature UHP treatments produced an activation of PME activity in the 200–400 MPa range. Increased processing temperature strongly affected this activation (Fig. 4). Only combinations of low pressures and mild temperatures inactivated PME in orange juice.

Previously published results on the effects of UHP treatments on POD and PPO activities in cell-free extracts showed that

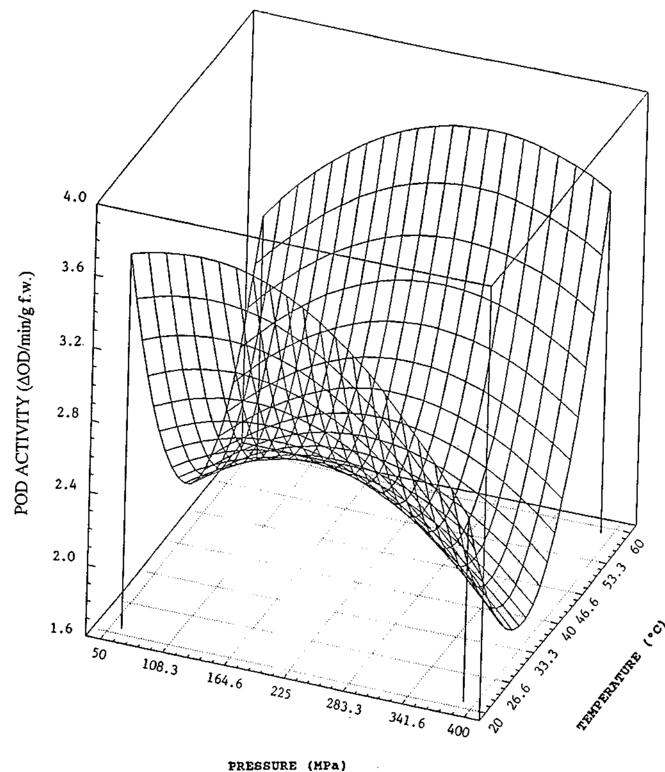

Fig. 3—Influence of high pressure and temperature treatments (15 min) on peroxidase (POD) activity of orange juice.

peroxidase was quite resistant to pressurization below 900 MPa (1 min treatment). A complete loss of enzyme activity was achieved only at 900 MPa, while activation was observed for treatments in the 300–500 MPa range (Anese et al., 1995). Our

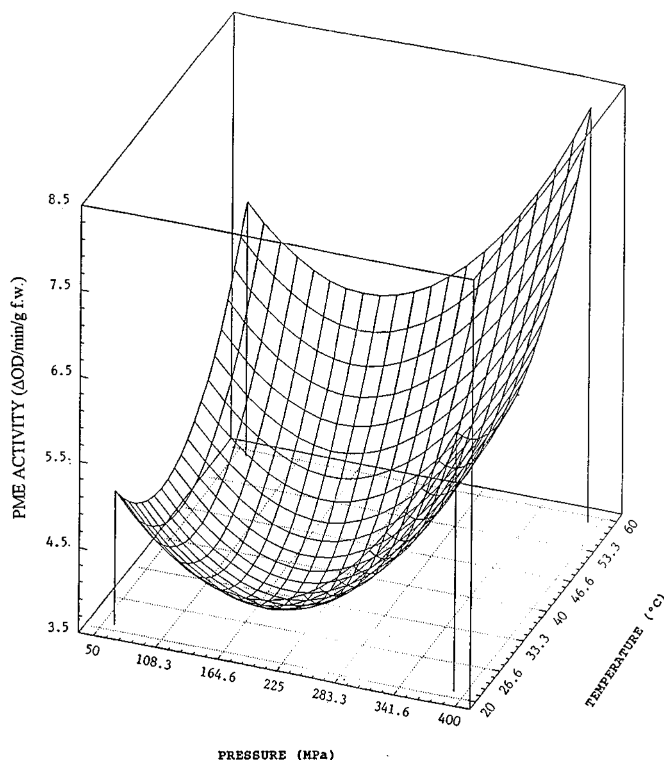

Fig. 4—Influence of high pressure and temperature treatments (15 min) on pectin methylesterase (PME) activity of orange juice.

results confirmed those previous reports in real fruit products. POD activity in strawberry puree exhibited the least pressure dependent activation (Fig. 1). However, strawberry PPO activity exhibited this activation as a consequence of pressurization at 280–400 MPa range (Fig. 2).

Orange POD did not show the same behavior as strawberry POD (Fig. 3). Its activity significantly diminished with UHP treatments from 200 MPa to 400 MPa. However, the increase in process temperature gave different results depending on the range. In general, for each pressure temperatures up to 32°C produced a synergistic effect on POD inactivation. Thus, orange juice samples treated at 400 MPa/32°C had the lowest POD activity, 1.72 ΔO.D./min/g f.w., a 50% reduction of initial fresh orange juice POD activity.

The activation effects, observed for moderate pressure treatments, could be attributed to reversible configuration and/or conformation changes of the enzyme and/or substrate molecules (Ogawa et al., 1990; Balny and Masson, 1993; Anese et al., 1995). The pH dependence of such activation effects seemed to confirm this hypothesis. The differences between compositions of the two fruit-derived products we studied (Table 1) could explain the slight differences observed in the inactivation of POD activity. Orange juice had a lower pH (3.69) and higher soluble solids (11.33 °Brix) than strawberry puree (pH 3.90; soluble solids 8.76 °Brix). Anese et al. (1995) reported that the pH of enzymatic crude extracts from juices seemed to strongly affect the extent of enzyme activation, which reached a maximum at pH 6.0. For the 50–400 MPa range at room temperature, no activation was observed as a consequence of UHP treatment, but the POD inactivation curve was significantly different in the 2 fruit products. The maximum POD inactivation increase in orange juice was observed in 270–400 MPa range (room temperature). For strawberry POD the maximum pressure dependence was between 50–200 MPa at that same temperature (Fig. 1 and 3). Since strawberry pH was higher, enzyme activation may have been more favored than in orange juice (lower pH) (Anese et al., 1995).

PPO activity in strawberry puree showed an activation at 285–400 MPa range and for each pressure a continuous activation related to temperature increase (Fig. 2). Our results confirmed the study reported by Asaka and Hayashi (1991) on PPO from pear extracts at pH 6.5. The enzymes of other commodities such as potatoes and apples also were activated by pressure treatment and, the tissues darkened rapidly after pressurization. They suggested that the PPO activation in pressurized plant products would follow the kind and number of isoenzymes of their PPO enzymatic system. Isoenzymes could show different optimum pH values, and it is not clear whether the activity would be increased by pressurization. In our results, the activation of PPO activity did not produce darkening in fruit puree, but further stability studies will be carried out to establish the possibility of PPO regeneration during chilled storage.

The influence of soluble solids content of samples on inactivation of enzymes has been reported (Ogawa et al., 1990). Increased soluble solids protect PME against pressure as well as heat inactivations. Our results, orange juice had the higher soluble solids content. This was considered a significant factor to contribute to the initial resistance against inactivation of its POD and the PME activation at medium pressures. Also, reported results showed that high pressure treatment could partially and irreversibly inactivate PME, which did not reactivate during storage and transportation (Ogawa et al., 1990). This could be important for achieving commercial stability of fruit-derived products with a nearly fresh quality.

REFERENCES

Anese, M., Nicoli, M.C., Dall Aglio, G., and Lerici, C.R. 1995. Effect of high pressure treatments on peroxidase and polyphenoloxiadse activities. J. Food Biochem. 18: 285–293.
AOAC. 1984. *Official Methods of Analysis*, 14th ed. Association of Official Analytical Chemists, Washington, DC.
Asaka, M. and Hayashi, R. 1991. Activation of polyphenoloxidase in pear fruit by high pressure treatment. Agric. Biol. Chem. 55(9): 2439–2440.
Balny, C. and Masson, P. 1993. Effects of high pressure on proteins. Food Rev. Internat. 9(4): 611–628.
Cano, M.P., Marin, M.A., and Fuster, C. 1990. Freezing of banana slices. Influence of maturity level and thermal treatment prior to freezing. J. Food Sci. 55(4): 1070–1073.
Cheftel, J.C. 1991. Applications des hautes pressions en technologie alimentaire. Actualité des Industries alimentaires et Agro-Alimentaires. 108(3): 141–153.
Cheftel, J.C. 1992. Effects of high hydrostatic pressure on food constitutents: An overview. In *High Pressure and Biotechnology*, C. Balny, R. Hayashi, K. Heremans, and P. Masson (Ed.), p. 195–209. Editions John Libbey Euro-text, Montrouge.
Cochran, W.G. and Cox, G.M. 1957. *Experimental Design*, 2nd ed. Wiley International, New York.
Farr, D. 1990. High pressure technology in the food industry. Trends Food Sci. Technol. 1: 14–16.
Hoover, D.G., Metrick, C., Papineau, A.M., Farkas, D.F., and Knorr, D. 1989. Biological effects of high hydrostatic pressure on food microorganisms. Food Technol. 43(3): 99–107.
Horie, Y., Kimura, K., Ida, M., Yosida, Y., and Ohki, K. 1991. Jam preparation by pasteurization. Nippon Nogeikagaku Kaishi. 65: 975–980.
Knorr, D. 1995. High pressure effects on plant derived products. In *High Pressure Processing of Foods*, D.A. Ledward, D.E. Johnston, R.G. Earnshaw, and A.P.H. Hasting, (Ed.), p. 123–135. Nottinghan University Press.
Kunugi, S. 1992. Effect of pressure on activity and specificity of some hydrolytic enzymes. In *High Pressure and Biotechnology*, C. Balny, R. Hayashi, K. Heremans, and P. Masson, (Ed.), p. 129–137. Editions John Libbey Euro–tex, Montrouge.
Mertens, B. and Knorr, D. 1992. Development of nonthermal processes for food preservation. Food Technol. 46(5): 124–133.
Owaga, H., Fukuhisa, K., Kubo, Y., and Fukumote, H. 1990. 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. 54(5): 1219–1225.
Watanabe, M., Arai, E., Kumeno, K., and Honma, K. 1991. A new method for producing a non–heated jam sample: The use of freeze concentration and high pressure sterilization. Agric. Biol. Chem. 55: 2175–2176.
Ms received 12/9/95; revised 4/2/96; accepted 5/28/96.

This work was supported by Spanish project ALI94-0786-CO2-01, Comision Interministerial de Ciencia y Tecnología. The work was partially presented as a poster to the IX World Congress of Food Science & Technology, Budapest (Hungary), July 31–Aug.4, 1995.