Effects of a Combined Process of High-Pressure Carbon Dioxide and High Hydrostatic Pressure on the Quality of Carrot Juice

S.-J. PARK, J.-I. LEE, AND J. PARK

ABSTRACT: A combined treatment of high-pressure carbon dioxide (HPCD) and high hydrostatic pressure (HHP) was investigated as a non-thermal processing technique to enhance the safety and shelf life of carrot juice. Aerobes were completely inactivated by a combined treatment of 4.90 MPa-HPCD and 300 MPa-HHP. A combined treatment of 4.90 MPa-HPCD and 600 MPa-HHP effectively inactivated enzymes. The residual activities of polyphenoloxidase, lipoxygenase, and pectinmethylesterase were less than 11.3%, 8.8%, and 35.1%, respectively. Cloud and color were considerably affected by HPCD, but not by HHP. Enzyme activities and the total color difference showed a strong correlation with pH, which was dependent on the pressure of carbon dioxide.

Keywords: high-pressure carbon dioxide, high hydrostatic pressure, carrot juice, enzyme inactivation, cloud

Introduction

TRADITIONALLY, HEAT PROCESSES HAVE BEEN USED TO ENSURE THE safety of food against many pathogenic microorganisms. Thermal energy, however, inevitably leads to destruction of heat-sensitive nutrients, texture, color, and flavor (Balny and others 1992). Non-thermal processes, such as high hydrostatic pressure (HHP), are being used as alternatives for food preservation. High-pressure is used commercially in the United States for the production of guacamole, tomato-based salsas, and fresh oysters, and for other products in Europe and Japan (Neil 1999). To secure safety against some pressure-resistant microorganisms, however, HHP should be used in combination with another process. High-pressure carbon dioxide (HPCD) is a candidate for such a combination because of its ability to inactivate microbes and its ease of use.

Carbon dioxide has been used in modified atmosphere packaging (MAP) to improve the shelf life of food by inhibiting bacterial growth. Fraser (1951) first tried gas pressurization with N_2, NO_2, Ar, and CO_2, reporting that CO_2 could inactivate 95% to 99% of *Escherichia coli* at 37 °C and 3.40 MPa. The principle of HPCD treatment is based on gas dissolution in a cell by pressurization that, when rapidly decompressed to atmospheric pressure, causes fatal functional damage to the cell (Balaban and others 1991).

Carrot juice is a natural source of â-carotene (Senti and Rizek 1975). Since canned carrot juice is a low-acid food of approximately pH 6.0, it has a higher risk of bacterial contamination than other acidic foods (orange juice). Hence, it requires severe heat treatment (115 to 121 °C) for protection from spoilage (Desrosier 1976; Kim and Gerber 1988). However, high temperatures (especially retorting temperatures) can destroy carotenes (Khan and others 1975; Kim and Gerber 1988). Hong and others (1999) reported that microbial inactivation by HPCD is governed essentially by penetration of carbon dioxide into cells, the effectiveness of which can be improved by increasing pressure and decreasing the pH of the suspension. Because dissolved carbon dioxide can lower the pH of carrot juice, HHP and HPCD can be combined for a synergistic effect. We, hence, have demonstrated the effectiveness of HPCD as a combined process with HHP in processing carrot juice. The effects of the combined treatment on aerobes, the activities of food quality-related enzymes, and the physical properties of carrot juice were examined and changes in the quality parameters were observed for a 4-wk storage period at 4 °C.

Materials and Methods

Preparation of carrot juice

Carrots purchased in a local market were washed with tap water and peeled. Carrot juice was obtained with a juice extractor (JM-511, Samsung Co., Korea), producing approximately 50% of the weight of the fresh carrots as juice. The juice was filtered through filter paper (No. 4, Whatman™ International Ltd., U.K.) and then used as a sample.

Measurement of concentration of dissolved CO_2

The concentration of dissolved CO_2 was measured using a method described by Ishikawa and others (1995). Ten mL of pressurized distilled-water was spread to an airtight container containing 20 mL of 1 M NaOH. The concentration of dissolved CO_2 was calculated from an amount of acid that was needed to neutralize to pH 7.0 with 0.5 M HCl .

HPCD treatment

Figure 1 shows the HPCD treatment equipment set-up (Kodam Engineering Inc., Seoul, South Korea) using stainless steel tubes, a pressure gauge, and a thermometer connected to a thermocouple (HI 93530, Hanna Instruments (Singapore) Pte Ltd., Singapore). The vessel (inside volume of 500 mL) and the screw type cover were made of stainless steel. To prevent direct contact of the sample with the inside wall of the vessel, a Pyrex® glass vessel was located inside the pressure vessel. A magnetic stirrer was used to mix carbon dioxide gas thoroughly with the sample (100 mL). The inner temperature of the processing chamber was maintained at approximately 5 °C by placing the pressure vessel in an ice jacket. It is well known that the solubility of CO_2 gas into water phase increases with temperature decrease

and that the effect of CO_2 gas treatment depends on contact of the gas with sample. Therefore, we lowered the temperature to increase the effect of CO_2 gas and considered that the simplest way to lower the temperature was using ice bath. A measured temperature of the gas treatment chamber inside was 5 °C.

Treatment was performed at 0.98, 2.94, and, 4.90 MPa for 10 min (holding time). The interior of the pressure chamber and all lines were sanitized with 70% ethanol twice prior to each analysis. Processed carrot juice was aseptically collected in a sterilized glass bottle through the drain out line. We examined the effects of carbon dioxide up to only 4.90 MPa, which we considered a practical value. This is a high-pressure range, but not a super critical state.

HHP treatment

HHP treatment was performed using high-pressure equipment (MFP-7000, Mitsubishi Heavy Industries Co., Yokohama, Japan) with a cylindrical pressure chamber (inside volume of 600 mL). Each 10 mL of carrot juice was packed in a polyethylene terephthalate pouch (14 cm × 10 cm) and heat-sealed with negligible headspace. The inside of the pouch was washed with 2% H_2O_2 and pasteurized at 80 °C for 30 min prior to use. The packed carrot juice (25 °C) was loaded into the pressure vessel and pressurized for 10 min (holding time) up to 600 MPa at 25 °C. The temperature of the processing chamber increases at the beginning of pressurization and decreases with depressurization. However, the increase/decrease of temperature transiently occurs, and the temperature is stabilized to the desired temperature in 1 or 2 min. The delay time inevitably exists because the response of the cooling circulator to a temperature change is slower than the adiabatic heating by pressurization. We, therefore, pre-chilled the chamber before starting the operation to minimize the delay time. The indicated temperature was the operating temperature, and there still existed a transient increase/decrease of temperature for a minute. A compensation for the temperature increment by pressurization was achieved by setting the operating temperature of the water-jacketed pressure chamber as low as the increment, which was approximately 3 °C/ 100 MPa.

The packed juice was immersed in the pressure chamber, and then the chamber was pre-chilled to minimize adiabatic heating due to pressurization. When the temperature of the vessel containing the packed carrot juice reached the desired level, pressurization was started. Distilled water was used as the pressure medium. The pressure build-up velocity was 5 MPa/s. The decompression time was less than 10 s.

Combined treatment of HPCD with HHP

A combined treatment of HPCD with HHP was performed in a stepwise manner. It is well known that the effect of HHP is elevated at higher temperatures and at lower pH. We, therefore, chose the order of treatment as HPCD then HHP to elevate the effect of HHP by lowering the pH of operational environment. Reverse order was not proper to the intention of this study. HPCD could lower the pH of carrot juice from pH 6.5 to pH 4.4 at maximum pressure. Prepared carrot juice (100 mL) was treated with HPCD, and then 10 mL of the processed juice was aseptically packed in a sterilized pouch for HHP treatment. HPCD treatment was performed at pressures of 0.98, 2.94, and, 4.90 MPa for 5 min at 5 °C. HHP was performed at a pressure of 100 MPa to 600 MPa for 5 min at 25 °C. To compare the effects of the combined treatment with individual

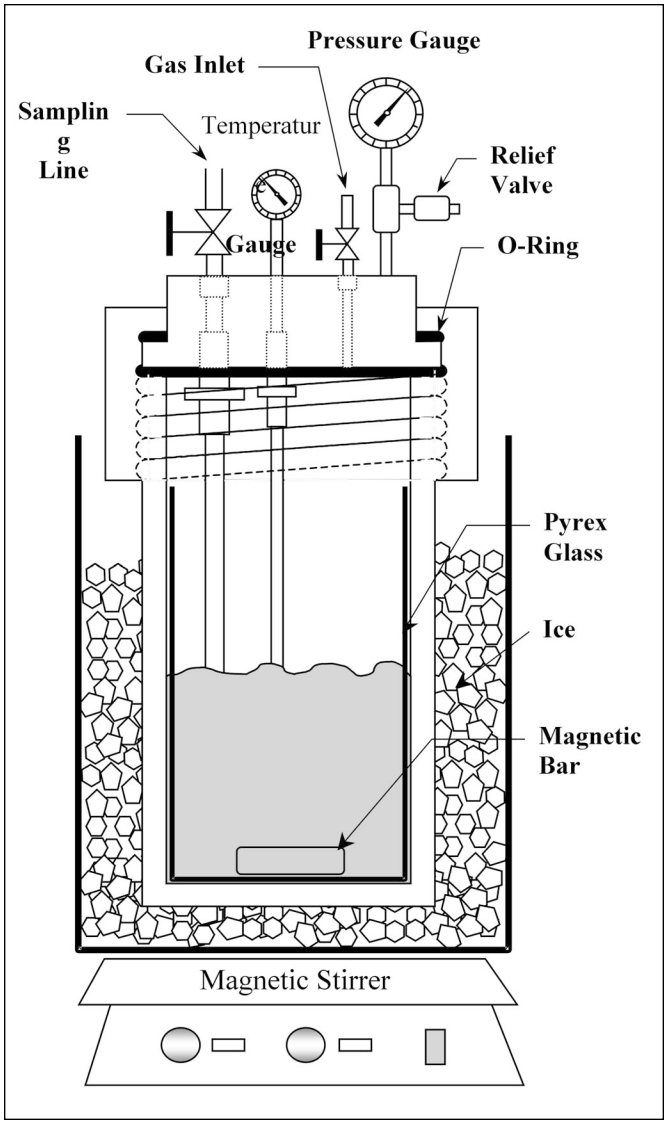

Figure 1—Schematic diagram of high-pressure carbon dioxide processing equipment

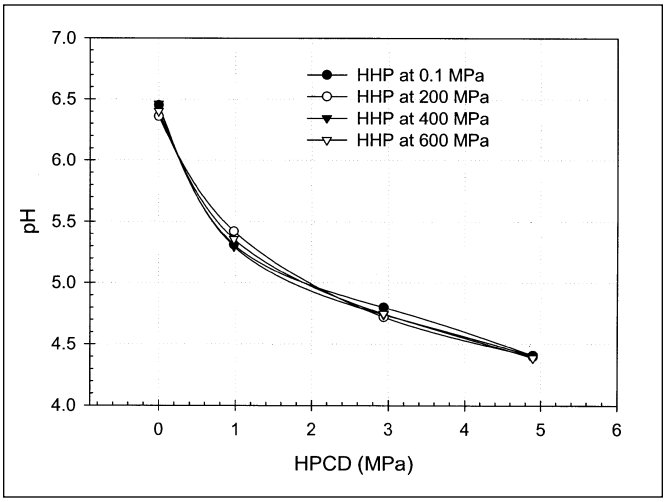

Figure 2—Effect of high-pressure carbon dioxide on the pH of carrot juice

treatments, the total treatment time was set for 10 min.

Enumeration of total aerobes

The pasteurization effects of the treatments were examined following the method of AOAC (1995). Samples were serially diluted with 0.1% peptone water (Difco Laboratories, Detroit, Mich., U.S.A.) and spread on nutrient agar (Difco Laboratories) for enumeration of total aerobes. We observed the recovery of the injured cell for more periods (to a wk), but after 48 h no more recovery was observed. Therefore, the number of colonies formed after incubation at 37 °C for 48 h were counted. All tests were performed in triplicate.

Enzyme assay

Treated carrot juice was immediately filtered through a 4-fold cheesecloth with a suction flask and centrifuged at 10,000 × *g* for 10 min at 4 °C (Unicorn 55R, Hanil Science Industrial Co. Ltd., Seoul, South Korea). The supernatant fluid was used as a crude enzyme. The polyphenoloxidase (PPO) activity was assayed by a modified method of Weemaes and others (1997). A 35-μL sample of crude enzyme was added to a mixture of 1.0 mL of phosphate buffer (0.01 M, pH 6.5) containing 0.1 M catechol. The activity was assayed using a spectrophotometer (DU Series 600, Beckman Instument, Inc., Fullerton, Calif., U.S.A.) at 420 nm and 25 °C. The lipoxygenase (LOX) activity was assayed by the method described by Kim and others (1987). A 100-μL sample of crude enzyme was added to a reaction mixture composed of 40 μL of substrate solution (60% linoleic acid) and 2.4 mL of phosphate buffer (0.1 M, pH 5.7). The oxidation of linoleic acid was measured using a spectrophotometer at 234 nm and 25 °C. The pectinmethylesterase (PME) activity was assayed by a modified method of Hagerman and Austin (1986). The substrate solution was composed of 2.0 mL of 0.5% (w/v) citrus pectin (Sigma Chemical Co., St. Louis, Mo., U.S.A.), 0.15 mL of 0.01% (w/v) bromothymol blue (Sigma Chemical Co.), and 0.85 mL of distilled water. After the substrate solution was adjusted to pH 7.5 with 2 N NaOH, the reaction was started by adding 40 μL of crude enzyme. The activity was assayed using a spectrophotometer at 620 nm and 25 °C. All enzyme activity was calculated from the slope from the linear part of an absorbance against time plot.

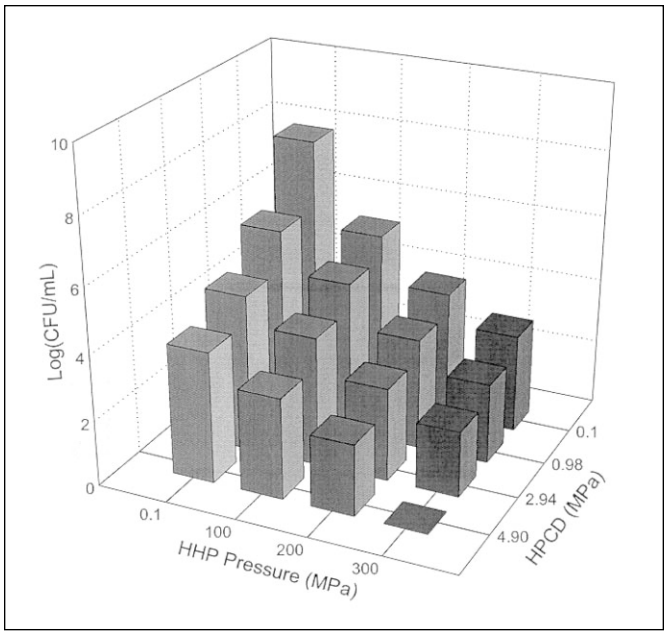

Figure 3—Effect of a combined treatment on the population of total aerobes in carrot juice

Physical properties

The pH of the carrot juice was measured at 25 °C using a pH meter (410A, Orion Co., Boston, Mass., U.S.A.). The cloud of the sample was measured as described by Klavons and others (1991). A 50-mL sample was centrifuged at 360 × *g* for 10 min to remove pulp; then 15 mL of the centrifuged sample was centrifuged again at 30,000 × *g* for 15 min. The collected precipitate was freeze-dried and weighed. Color values were measured using a chronometer (CR 200, Minolta Co., Osaka, Japan) following the Hunter system. L-, a-, and, b-values of the sample were measured at room temperature and the total color difference (DE) was calculated as:

$$DE = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{0.5}$$

where L = lightness of treated sample; L_0 = lightness of control; a = redness of treated sample; a_0 = redness of control; b = yellowness of treated sample; and b_0 = yellowness of control

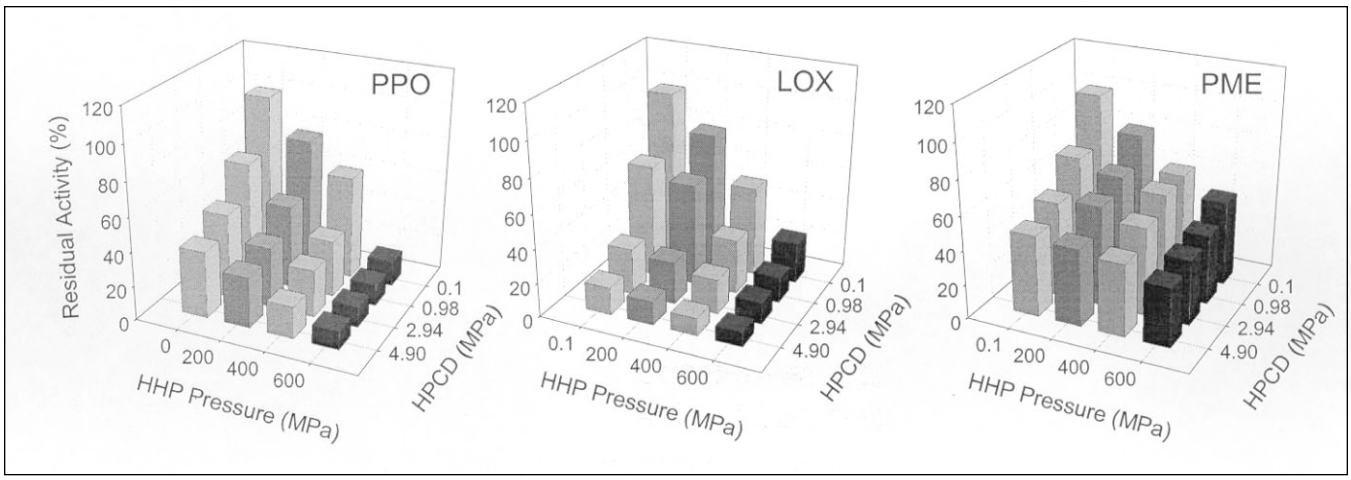

Figure 4—Effects of a combined treatment on the enzymes in carrot juice

Shelf-life study

Processed samples were stored at 4 °C for 4 wk, during which property changes were examined. Changes in the counts of total aerobes, enzyme activities, and the physical properties of the carrot juice were observed every wk during the storage period.

Statistical analysis

All analyses were performed in triplicate. The Student-Newman-Keuls test was used for statistical analysis using Statistical Analysis System software (Version 8.01, SAS Institute Inc., Cary, N.C., U.S.A.). The Student-Newman-Keuls test is one of the analysis of variance procedures. This test controls the experiment error rate under the complete null hypothesis, but not under partial null hypothesis. From the test, we grouped the data to determine whether the data were significantly different or not. Significant differences were defined at $p < 0.05$. A correlation analysis was performed using the data analysis functions of Microsoft Excel 2000™, and all graphs were drawn using SigmaPlot 2001 (SPSS Inc., Chicago, Ill., U.S.A.).

Results and Discussion

Effects on a pH of carrot juice

A pH measurement was performed immediately after opening the pouch of processed juice. The value measured at 25 °C was not significantly different from the value measured at 5 °C. HHP treatment did not significantly affect the pH of the carrot juice, but HPCD decreased the initial pH of 6.5 to pH 4.4 at 4.90 MPa (Figure 2).

Concentration of dissolved CO_2

The concentrations of dissolved CO_2 were approximately 0.3,

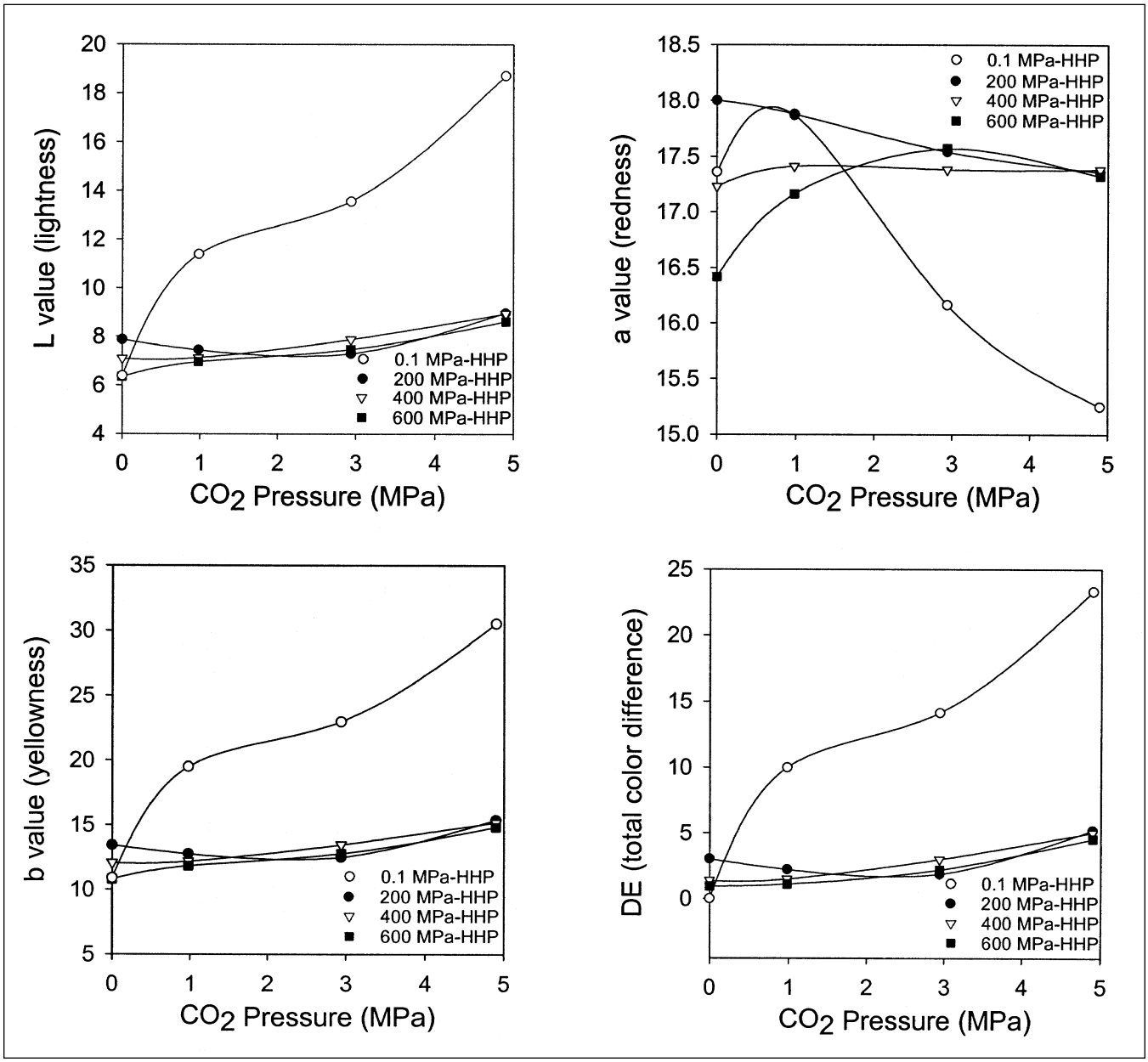

Figure 5—Effects of high-pressure carbon dioxide on the color of carrot juice

0.5, and 0.8 mol/L for 0.98, 2.94, and, 4.90 MPa, respectively, at treatment time of 5 min; 0.4, 0.7, and 1.1 mol/L for 0.98, 2.94, and, 4.90 MPa, respectively, at treatment time of 10 min. The specified concentrations of dissolved CO_2 were measured in ice bath (approximately 5 °C) after opening the pouches, which had been heat-sealed after a pressurization by CO_2 at 5 °C.

Effects on the total aerobes

Figure 3 shows the effects of the processes on the population of total aerobes in carrot juice. The initial count of total aerobes of the control was 8.0 log CFU/mL without any inoculation of specific microorganisms. Generally, a raw carrot juice contains approximately 10^5 to about 10^6 CFU/mL. The reason for unusually high initial count might be explained as follows: We used a carrot that was cultivated by organic farming in Cheju-island (Korea). The organic farming uses no agricultural chemicals or chemical fertilizer. Hence, the carrot used in this experiment may contain more microorganisms than a carrot cultivated by general farming that uses agricultural chemicals and chemical fertilizer. The organic-farmed carrot is distributed in unwashed condition; the surface of the carrot is covered with soil. The soil stuck to surface of carrot was washed with tap water and peeled with a scrubbing brush before juicing. We did not deeply peel the carrot with a knife, but instead just eliminated the skin of carrot with a scrubbing brush. These processes for preparation of carrot juice might cause the high initial counts.

The pasteurization effect of all the treatments increased with increasing pressure. HPCD showed a 4.0-log reduction at a maximum pressure of 4.90 MPa. HPCD exerted a relatively large effect on total aerobes at a low pressure of 0.98 MPa, which gradually increased with pressure. The pasteurization effect of carbon dioxide is probably due to a decrease in pH inside the cells, accompanied by an increased permeation rate of carbon dioxide molecules (Wolfe 1980; Hass and others 1989). Carbon dioxide molecules that permeate into the cell are intermediately converted to bicarbonate or carbamate, leading to malfunction of the cell membrane and cell death (Silliker 1981). Molin (1983) reported that aerobic bacteria are more affected by carbon dioxide treatment than anaerobic bacteria. HHP treatment showed no survivors at pressures at 400 MPa or higher pressure. Sohn and Lee (1998) reported that pressure treatment above 400 MPa inactivates yeasts and molds in carrot juice. The pasteurization effect of the combined treatment increased with pressure. When a pressure of HHP was 400 MPa or higher, HHP treatment alone could inactivate the aerobes, and no survivors were detected. Below the maximum condition of 4.9 MPa-HPCD and 300 MPa-HHP, the effect of the combined treatment was more dependent on HHP than HPCD. Individual treatment results of HPCD at 4.90 MPa for 10 min and HHP at 300 MPa for 10 min were 4.0 log CFU/mL and 3.0 log CFU/mL, respectively. A combined treatment of 4.90 MPa-HPCD with 300 MPa-HHP showed no survivors. These results indicate that a combined treatment for 10 min was more effective than HPCD alone for 10 min or HHP alone for 10 min on inactivation of aerobes.

Effects on enzyme activity

Figure 4 shows the effect of 3 types of treatment on PPO, which is the key enzyme in enzymatic browning of fruits and vegetables. Herein, the activity of PPO decreased with increasing pressure. No treatment, however, could fully inactivate PPO. At 400 MPa and a lower pressure, a combined treatment shown slightly elevated effect on PPO inactivation; a combined treatment of 4.90 MPa-HPCD and 400 MPa-HHP showed a residual activity of 19%; while HPCD alone at 4.90 MPa and HHP alone at 400 MPa showed residual activities of 39% and 61%, respectively.

LOX is a dioxygenase that catalyzes the oxygenation of polyunsaturated fatty acids to corresponding hydroperoxides. In foods, LOX causes destruction of essential fatty acids, producing off-flavor compounds such as hexanal and pentanal. LOX also has a carotene-bleaching effect (Kim and others 1987). Figure 4 shows that a combined treatment effectively inactivates LOX. To lower the residual activity to less than 30%, 600 MPa of HHP or 2.94 MPa of HPCD was required. A combination in the range of 2.94 to 4.90 MPa of HPCD and 200 to 400 MPa of HHP showed a residual LOX activity of less than 25%. Hendrickx and others (1998) indicated that pressure inactivation of soybean LOX can be accurately described by a first order kinetic model and the kinetic parameters seem to be strongly dependent on environ-

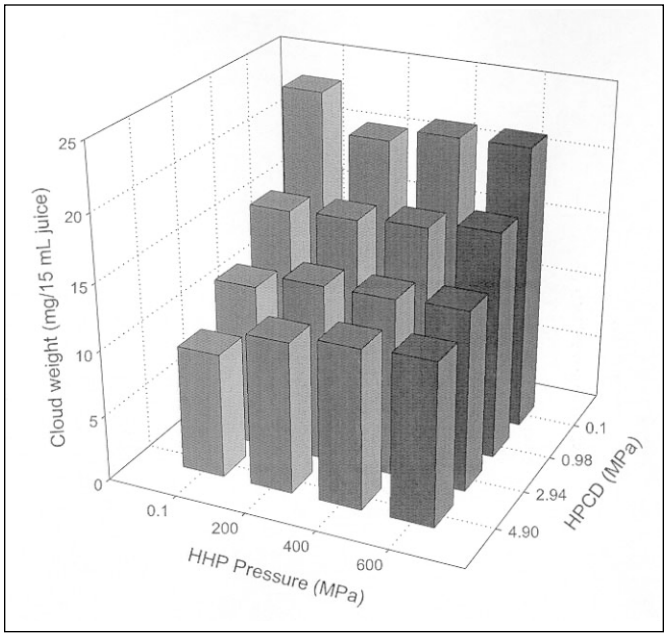

Figure 6—Effect of a combined treatment on the cloud of carrot juice

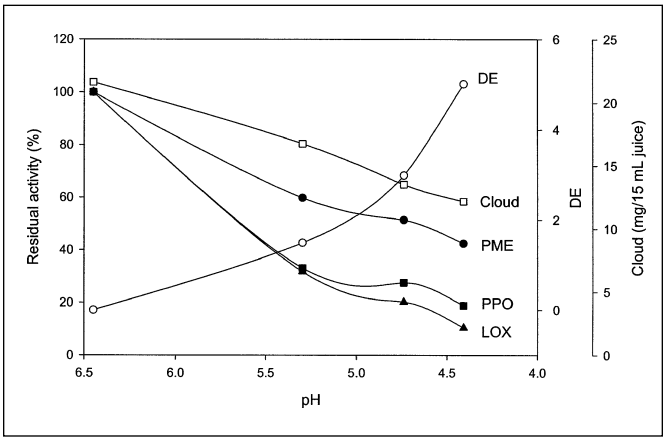

Figure 7—Correlation between pH and PPO, LOX, PE, cloud, and DE. Samples were processed at 400 MPa and 25 °C for 5 min.

mental conditions. Herein, LOX of carrot juice was also inactivated by HHP alone, and inactivation effect was elevated by combined HPCD treatment.

Carrots exhibit PME activity, which can lead to precipitation of pectin in juice with subsequent loss of cloud (Lee and others 1979). Seyderhelm and others (1996) reported that the inactivation rate of tomato PME by HHP was slower at elevated pressure than at atmospheric pressure and that the rate is less-pressure stable in a citric acid buffer (pH 3.5 to 4.5) than in water. Also, the pressure stability decreased with decreasing pH. Nienaber and Shellhammer (2001a), however, reported that orange juice PME was inactivated by HHP with a decreasing pressure at 25 °C. Reports regarding the effects of HHP on the activity of PME have shown different results, perhaps due to the multiform existence of the enzyme and the observation that the same enzyme shows different characteristics according to its origin and environment. Herein, the activity of PME gradually decreased with increasing pressure (see Figure 4).

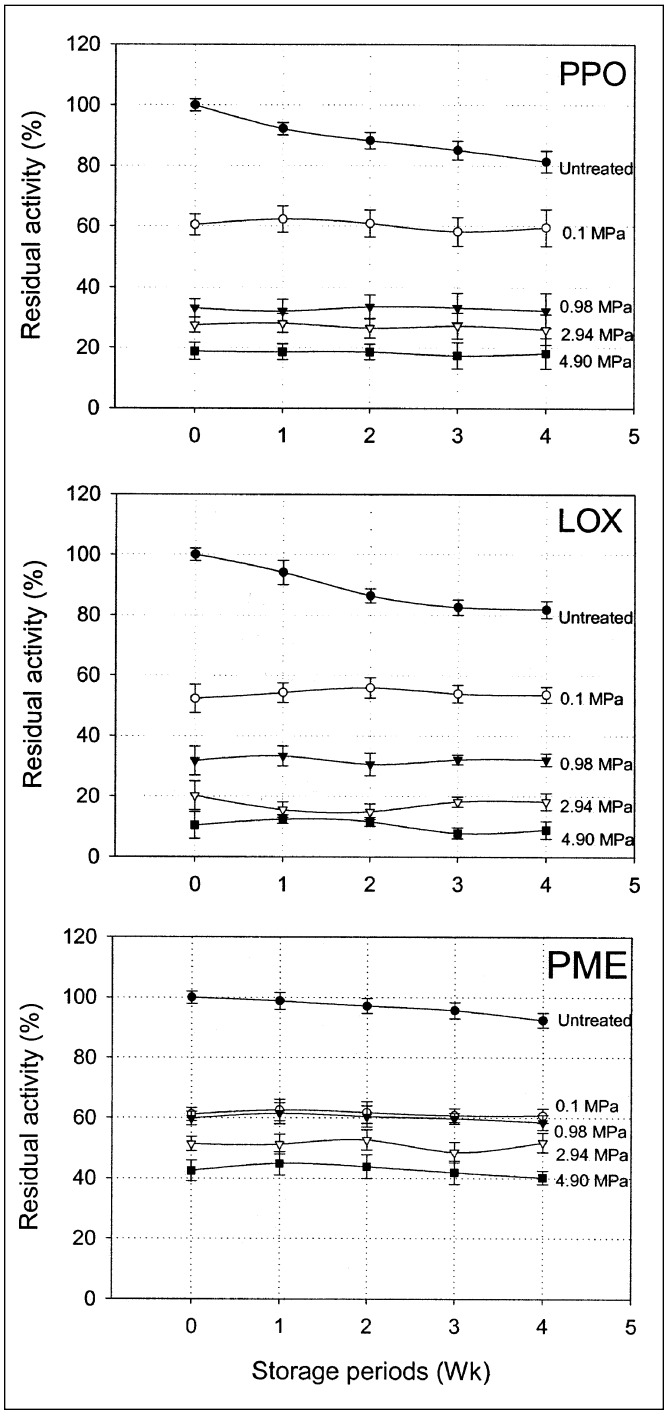

Figure 8—Change of enzyme activities during storage at 4 °C. Samples were combined-treated at a fixed pressure of HHP for 400 MPa.

Many fruit and vegetable juices have an acidic pH, but carrot juice has a relatively high pH of 6.1 to 6.5. PME has an optimal pH near 7.5 (neutral). HPCD lowered the pH of carrot juice to pH 4.4 at 4.9 MPa, while HHP treatment showed no effect on the pH of carrot juice (see Figure 2). The pH of combination-processed carrot juice, therefore, was dependent on the pressure of carbon dioxide. For these reasons, we expected PME to be affected by a lowered pH. However, PME maintained an activity of 53% at pH 4.4 induced by HPCD. The PME of no sample was fully inactivated under maximum pressure conditions. Seyderhelm and others (1996) reported that at least 800 MPa of HHP at 45 °C was needed to inactivate PME and that the inactivation time could be reduced from 5 min to 2 min by increasing the pressure from 800 MPa to 900 MPa.

Color

Jwa and others (1996) reported that supercritical carbon dioxide raised the L-value (lightness) of citrus fruit juice. Herein, HPCD alone showed statistically significant increase of the L-value of carrot juice, while HHP resulted in insignificant change at $p < 0.05$ (Figure 5). A combined treatment caused a slight increase in the L-value with HPCD pressure increase, but the change was not significantly different at $p < 0.05$. Individual treatments of HPCD resulted in a significant decrease in the a-value (redness) of 13%. Though individual treatments of HHP showed fluctuation in the a-value, the difference was not statistically indifferent at $p < 0.05$. Combined treatments maintain the initial a-value. The b-value (yellowness) showed similar tendency to the L-value. HPCD raised the b-value at 4.90 MPa approximately 3 times more than the control. The combined treatments and HHP alone showed stable DE value. HPCD alone, however, raised the value with pressure increase. Consequently, a combined treatment did not significantly affect the color of carrot juice.

Cloud

Cloud stability is an important quality parameter in fresh unpasteurized juice. Goodner and others (1998) showed that HHP treatment of orange juice at 600 MPa for 10 min retains the initial cloud for 90 d. Nienaber and Shellhammer (2001b) reported that fresh unpasteurized orange juice showed an approximate 65% cloud loss within 5 wk under refrigerated storage. Herein, cloud was greatly affected by HPCD, showing a 60% loss at 4.90 MPa (Figure 6). HHP showed little effect on cloud. It is known that the cloud of citrus juice is related to the activity of PME. A combined treatment of 4.90 MPa-HPCD and 600 MPa-HHP showed the lowest PME residual activity of 35% (see Figure 5). However, 47% of cloud was lost under the same treatment condition. As it is difficult to ascertain an exact reason for this with the available data, HPCD seemed to affect the cloud of carrot juice in a non-enzymatic way. A further study may be required to clarify an exact reason. Harshul and others (1999) reported that PME in Australian carrot showed more resistance to a temperature of 80 °C than either peroxidase or catechol oxidase in the carrot varieties studied.

Shelf-life study

As our object was to determine limits for the HPCD and HHP process by combining the individual processes under lower pressure conditions, we fixed the HHP conditions of the combined process at 400 MPa and 25 °C for 5 min, which is considered practical for industrial use. We examined the quality parameters of carrot juice during 4 wk of storage at 4 °C, varying only the HPCD pressure. The pH showed statistically insignificant changes during storage (data not shown). A correlation analysis of the quality parameters during storage at 4 °C showed that the L-, b-values, and DE value have a strong correlation with the HPCD pressure (data not shown). The enzymes showed strong correlations with each other and with pH, probably due to the effects of pH and pressure on proteins. The structural conformation of proteins is subject to the pH of the environment. A decrease of pH proportional to the pressure of carbon dioxide (see Figure 2) was induced by carbon dioxide. Moreover, the effects of pH and pressure were nonspecific for each enzyme. The strong correlations among the enzymes, therefore, are probably due to the nonspecific effects of pH and pressure on proteins. Figure 7 shows correlations between pH and PPO, LOX, PME, DE, and, cloud. Enzyme activities and cloud decreased with a decreasing pH with good linearity. DE followed an equation regressed by the second order and was inversely proportional to the pH. During the storage period, pressure treated sample showed no reactivation of aerobes, and the control showed 8.4 log CFU/mL after 4 wk (data not shown). The PPO and LOX values of the untreated sample showed a statistically significant decrease in activity with storage time. Both showed a residual activity of approximately 80%. The variations of other samples were not significant (Figure 8). Cloud and DE were mildly affected by HPCD at a constant pressure of 400 MPa-HHP, but the values did not change during storage (Figure 9). An untreated sample showed a cloud loss of 20% after 4 wk of storage. Interestingly, cloud was stable during 4 wk of storage, even though PME activity remained between 40% and 60% after treatments during the storage periods (see Figure 8). It seemed that an enzymatic reaction could not progress in packed carrot juice, in which a carbon dioxide dissolved, during storage periods at 4 °C.

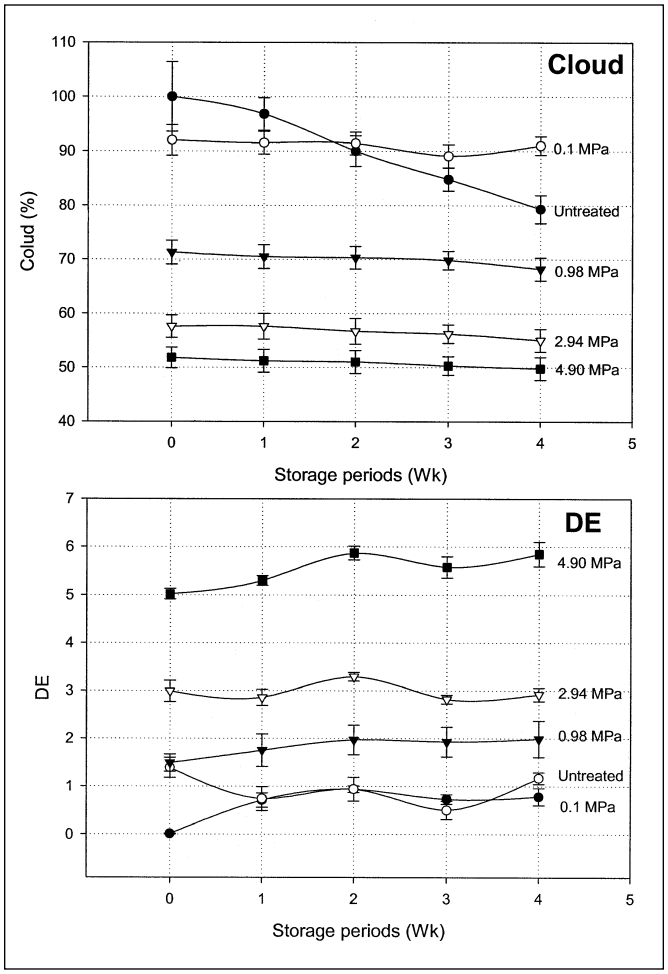

Figure 9—Changes of cloud and DE during storage at 4 °C. Samples were combined-treated at a fixed pressure of HHP for 400 MPa.

Conclusion

PASTEURIZATION OF FRESH CARROT JUICE BY A COMBINED TREATment of HPCD and HHP was possible at HHP pressures above 300 MPa. A combined process showed an elevated effect on the inactivation of quality-related enzymes, probably due to a lowered pH value and high-pressure. HPCD at a pressure of 4.90 MPa, however, showed undesirable effects on the physical properties without regard to the pressure level of HHP. During the storage period there was no reactivation of aerobes and enzymes, and significant changes in the physical properties were not observed. Based on available data, a combined treatment of 2.94 MPa-HPCD and 400 MPa-HHP was matchable to an individual HHP performed at higher pressures in the effects on destruction of microorganisms and the inactivation of enzymes. Considering a dramatic effect on cloud values and simplicity of process, HHP treatment at 600 MPa could be better than a combined treatment in processing of carrot juice. However, an effect of simultaneous treatment of HPCD and HHP, not sequential treatment like this study, is expected to show better results. Consequently, though a combined treatment showed some defects in quality of carrot juice, these results suggest that it is worth to attempt to apply this technique to another object that would have no quality problems.

References

AOAC. 1995. Microbiological methods. In: Cunniff PA, editor. Official Methods of Analysis. 16th ed. Gaithersburg, MD: AOAC international. p 1-111.
Balaban MO, Arreola AG, Marshall M, Peplow AJ, Wei CI, Cornell J. 1991. Inactivation of pectinesterase in orange juice by supercritical carbon dioxide. J Food Sci 56(3):743-746.
Balny EC, Hayashi R, Heremans K, Masson P. 1992. High pressure equipment for food processing. High pressure and Biotechnology 2224:509-514.
Desrosier NW. 1976. Technology of Food Preservation. 4th ed. Westport, Conn.: Avi Publishing Co. 110 p.
Fraser D. 1951. Bursting bacteria by release of gas pressure. Nature 167:33-37.
Goodner JK, Braddock RJ, Parish ME. 1998. Inactivation of pectinesterase in orange and grapefruit juices by high pressure. J Agric Food Chem 46(5):1997-2000.
Hagerman AE, Austin PJ. 1986. Continuous spectrophotometric assay for plant pectin methyl esterase. J Agri Food Chem 34:440-444.
Harshul MV, William SAK, Darryl MS. 1999. Activity, localization and thermal inactivation of deteriorative enzymes in Australian carrot (*Daucus carota* L) varieties. J Sci Food Agric 79:1129-1135.
Hass GJ, Prescott HE, Dudley jrE, Dik R, Hintlian C, Keane L. 1989. Inactivation of microorganisms by carbon dioxide under pressure. J Food Safety 9:253-265.
Hendrickx M, Ludikhuyze L, Ven den Broeck L, Weemaes C. 1998. Effects of high pressure on enzymes related to food quality. Trends in Food Technol 9(5):197-203.
Hong SI, Park WS, Pyun YR. 1999. Non-thermal inactivation of *Lactobacillus plantarum* as influenced by pressure and temperature of pressurized carbon dioxide. Int J Food Sci Technol 34(2):125-130.
Ishikawa H, Shimoda M, Kawano T, Osajima Y. 1995. Inactivation of enzymes in an aqueous solution by micro-bubbles of supercritical carbon dioxide. Biosci Biotech Biochem 59(6):628-632.
Jwa MK, Lim S, Koh JS. 1996. Inactivation of pectinesterase in citrus juice by supercritical carbon dioxide. Korean J Food Sci Technol 28(4):790-795.
Khan NA, Khalil JK, Khan I. 1975. Changes in the carotene content of carrots

during maturity, processing, and storage under different temperature conditions. Pakistan J Sci Res 27:112-114.
Kim H, Gerber LE. 1988. Influence of processing on quality of carrot juice. J Food Sci Technol 20(5):683-690.
Kim YM, Lee CW, Park KH. 1987. Purification and thermal inactivation of 2 lipoxygenase isoenzymes from potato tubers. Korean J Food Sci Tech 19(5):397-402.
Klavons JA, Bennette RD, Vannier SH. 1991. Nature of the protein constituent of commercial orange juice cloud. J Agric Food Chem 39:1545-1548.
Lee CY, Bourne MC, Van Buren JP. 1979. Effect of blanching treatments on the firmness of carrots. J Food Sci 44:615-619.
Molin G. 1983. The resistance to carbon dioxide of some food related bacteria. Eur J Appl Microbiol Biotechnol 18(4):214-217.
Neil HM. 1999. High-pressure pasteurization of juice. Food Technol 53(4):86-90.
Nienaber U, Shellhammer TH. 2001a. High-pressure processing of orange juice: Kinetics of pectinmethylesterase inactivation. J Food Sci 66(2):328-331.
Nienaber U, Shellhammer TH. 2001b. High-pressure processing of orange juice: Combination treatments and a shelf life study. J Food Sci 66(2):332-336.
Senti FR, Rizek RL. 1975. Nutrient levels in horticultural crops. HortScience. 10:243-256.
Seyderhelm I, Boguslawski S, Michaelis G, Knorr D. 1996. Pressure induced inactivation of selected food enzymes. J Food Sci 61(2):308-310.
Silliker JH. 1981. The influence of atmospheres containing elevated levels of carbon dioxide on the growth of psychrotrophic organisms in meat and poultry. In: Robert TA, Hobbs G, Chritian JHB, Skovgaard N, editors. Psychrotrophic microorganisms in spoilage and pathogenicity. London, U.K.: Academic Press. p 369-375.
Sohn KH, Lee HJ. 1998. Effects of high pressure treatment on the quality and storage of kimchi. Int J Food Sci Tech 33:359-365.
Weemaes CA, De Cordt SV, Ludikhuyze LR, Broeck I, Hendrickx ME, Tobback PP. 1997. Influence of pH, benzoic acid, EDTA, and glutathione on the pressure and/or temperature inactivation kinetics of mushroom polyphenoloxidase. Biotechnol Prog 13:25-32.
Wolfe SK. 1980. Use of CO and CO_2 enriched atmospheres for meats, fish and produce. Food Technol 34(3):55-59.
MS 20010548 Submitted 10/2/01, Accepted 1/6/02, Received 1/14/02

This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (HMP-98-F-3-0007).

Authors are with the Dept. of Biotechnology, Yonsei Univ., Seoul 120-749, Korea. Direct inquiries to author Park (E-mail: foodpro@yonsei.ac.kr).