Protective Effect of Ascorbic Acid against the Browning Developed in Apple Fruit Treated with High Hydrostatic Pressure

G. Préstamo*,† and G. Arroyo‡

Instituto del Frío (CSIC), Ciudad Universitaria, 28040-Madrid, Spain, and Departamento de Microbiología III, Facultad de Biología, Universidad Complutense de Madrid, 28040-Madrid, Spain

Apple Reineta variety was used as an apple dessert. The 1−1.5-cm cubes were immersed in a sucrose solution (30% w/v) and subjected to high pressure (HP) of 400 MPa for 30 min at 5 °C. Different ascorbic acid concentrations were used to protect the fruit from the browning developed after the HP treatment. After 2 months of storage at 5 °C, no brown color was observed in the samples treated with 20 mM ascorbic acid, and they were acceptable to consumers. However, untreated samples presented fermentation, and they were not acceptable to consumers. The electric conductivity and potassium content were found to be good indicators of the metabolites released from the fruit to the solution in samples treated with high pressure. HP did not affect the peroxidase activity but eliminated the microbial population.

Keywords: *High pressure; apple fruit; ascorbic acid; metabolites; electric conductivity; peroxidase*

INTRODUCTION

Products treated with high hydrostatic pressure retain their original freshness and vitamin content more than those treated with heat (Smelt, 1998), but the appearance and texture of fruit and vegetables are adversely affected (Tauscher, 1995; Préstamo and Arroyo, 1998). Food structure alterations is an application whereby interesting innovative products will inevitably be the result of this application (Tauscher, 1995; Smelt, 1998). A clear advantage of pressure treatment is its low energy input. Although high hydrostatic pressure is still costly, it is envisaged that with further technological progress it will become less expensive (Smelt, 1998).

Enzymatic browning reactions in some vegetables appear to be activated by pressurization treatment (Shimada et al., 1990; Eshtiaghi and Knorr, 1993). This browning response is a result of oxidative stress (Tauscher, 1995). After pressure treatment, there is an increase in enzymatic activity (Mozhaev et al., 1994), although enzyme inactivation depends on the pressure level used. Some enzymes are relatively easy to destroy, namely, several hydrolases in *Escherichia coli* (100 MPa) and yeast carboxypeptidases (400 MPa). But others such as polyphenoloxidases are more difficult to inactivate. Their activity remained about 40% at 800 MPa for 10 min (Gomes and Ledward, 1996), and pressure of 400 MPa for 30 min at 5 °C was not enough to inactivate the peroxidase (Préstamo and Arroyo, 1998).

Antioxidants are commonly used to protect fruit and vegetables (Eshtiaghi and Knorr, 1993; Préstamo and Manzano, 1993). Ascorbic acid is an active participant in food product browning (Rogacheva, 1995), and it appears to be the most widely studied carbonyl component. Ascorbic acid prevents the oxidation of polyphenoloxidase, which is responsible for enzymatic browning. Préstamo and Manzano (1993) also reported that there is a correlation between peroxidase activity and the browning effect.

Water is one of the most important food ingredients, especially in fruit and vegetable products. Under pressure, the solute concentration in the water diffuses in or out through the ion channels, and the function of the ion channels is to allow specific inorganic ions (mainly Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-) to diffuse. Na^+,K^+-ATPase has a direct role in the fluidity of the cell membrane, regulating the osmotic balance, which makes a cell swell or shrink. It is located in the lipid layer and is involved in active ion transport across the cell membrane (Chong et al., 1985). High pressure inactivates Na^+,K^+-ATPase activity (Chong et al., 1985) and also Ca^{2+}- and Mg^{2+}-ATPases (MacLennan et al., 1985). As a result of all this, the cell membrane permeability changes. Yeast cell ultrastructure and cytoskeleton were investigated by Osumi (1996), and with more than 200 MPa, the cell wall was damaged and the subcellular structure was altered. One of the metabolites that is released in high concentration was potassium and in many instances has been used as a leaching indicator (Eshtiagi and Knorr, 1993).

Certain fruit and vegetables that contain gas vacuoles are severely and irreversibly compressed as a result of high pressure. Consequently, they alter morphologically, the tissue is disrupted, nutrients are lost, and very often there are changes in enzymatic activity leading to adverse effects, such as browning (Dörnenburg and Knorr, 1997). Color, structure, and firmness are affected, especially in leafy vegetables. A decrease in the permeability of the cell wall is a possible explanation for the transparency effect produced after pressure treatment (Shimada et al., 1990; Préstamo and Arroyo, 1998).

This study was designed to assess the effects of ascorbic acid on protecting fruit products from the

* Corresponding author (fax, 91 549 36 27; e-mail, lupep@if.csic.es).

† Ciudad Universitaria.

‡ Universidad Complutense de Madrid.

10.1021/jf9901688 CCC: $18.00
Published on Web 08/27/1999

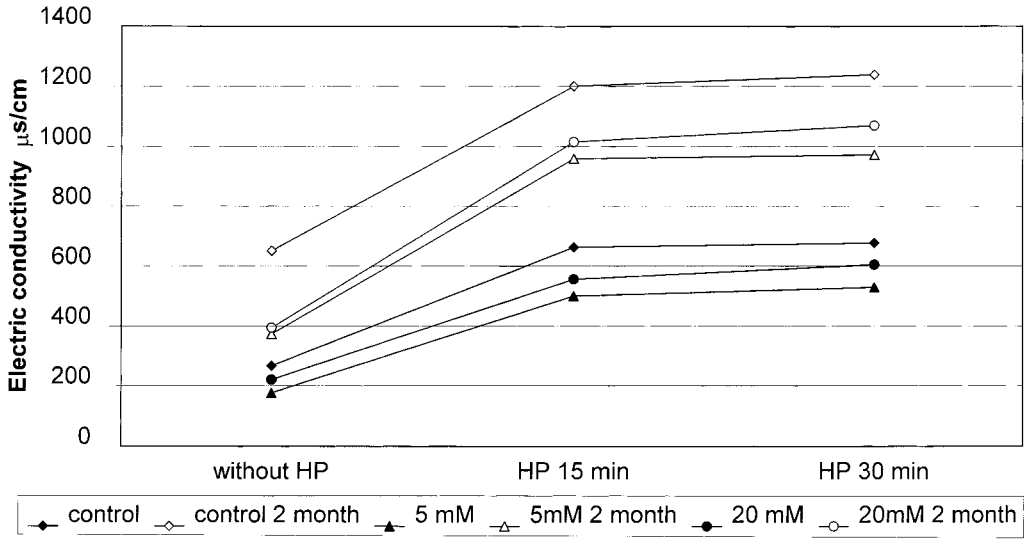

Figure 1. Electric conductivity of the control samples, samples treated with 5 and 20 mM ascorbic acid, and samples treated with HP of 400 MPa at 5 °C for 15 and 30 min. The same samples after 2 months of storage.

browning effect produced by high-pressure treatment. This information will enable better understanding of how fruits response to high pressure.

MATERIALS AND METHODS

Fruit Sample. Apples (Reineta variety) were purchased from the local market. They were washed and cut in 1.5-cm cubes. The samples (about 40 g of fruit) were immersed in a sucrose solution (about 75 mL of 30% w/v, sucrose:water) with different ascorbic acid concentrations (5, 20, and 50 mM). Plastic containers filled with the fruit in sucrose solution were sealed in polyethylene bags under vacuum to prevent leaking into the HP apparatus vessel.

High-Pressure Treatment. The samples were subjected to high pressure of 400 MPa for 30 min at 5 °C, these conditions were chosen from previous works as being the most appropiate. High-pressure levels were generated in a high-pressure apparatus (ACB GEC Alsthom, Nantes, France) using a hydrostatic pump and a fluid of low compressibility (water) sealed in a vessel (steel container of 100 mm diameter, 300 mm height, and 2.35 volume). Water acted as the pressurizing medium, and a thermocouple submerged in the pressure fluid measured the temperature during treatment. The apparatus is capable of achieving a maximum pressure of 500 MPa. Temperature was held constant using a water bath. Temperature and pressure were recorded in a Lab Tech notebook program (Laboratory Technologies Corporation, Wilmington, MA). The samples were pressurized in completely filled, closed 130-mL polyethylene containers. In each experiment, the indicated pressure was achieved within 2−3 min, held for 30 min, and then released to atmospheric pressure within 5−8 min. After the pressure was released, the pressurized samples were analyzed at room temperature. Duplicate samples were used.

Electric Conductivity. The electric conductivity was measured in a conductimeter 522 apparatus (Crison, Giralt, Barcelona, Spain). The values were expressed in μS/cm.

Peroxidase Activity. Peroxidase activity (POD) was determined following the method described by Préstamo and Manzano (1993) using *o*-dianisidine as a chromogenic indicator. Each sample (powdered by liquid nitrogen) of 2.5 g was added to 2 mL of 100 mM Tris-HCl and 150 mM NaCl. A total reaction volume was 1.5 mL, containing 1.250 mL of 50 mM sodium acetate, pH 6 buffer; 0.1 mL of 0.5% hydrogen peroxide; 0.05 mL of enzyme extract; and 0.1 mL of 0.25% *o*-dianisidine (w/v). The reaction was measured at room temperature by spectrophotometry at 460 nm. POD activity was expressed in units of POD mL^{-1}. A unit of POD was defined to be an increase of 0.001 unit of absorbance min^{-1}.

Atomic Absorption. Analytical assays of mineral content were determined with the atomic absorption spectrophotometer apparatus (Perkin-Elmer, 5100 PC model), using an air−acetylene flame, and operating in the emission mode without lamp for K and Na measures. A multielement hollow cathodic lamp was used for Ca^{2+} and Mg^{2+}. The determinations were done in the liquid solution (sucrose, water, ascorbic acid, and the leakage of minerals from the fruit to the solution) while the fruit remained floating in the liquid solution. Calcium, potassium, magnesium, and sodium were analyzed before and after the HP treatment. The values were expressed in mg/L.

Sensory Evaluation. Changes in color, flavor, and texture were evaluated subjectively (Dethmers, 1981). The two-step rating scale used consisted of two categories, the same as or different from the untreated product. Evaluations were carried out before and after pressurization treatment.

Statistical Analysis. The results obtained from the apples treated with ascorbic acid, the effect of the HP and the storage (2 months) between the initial values and after 2 months of storage at 5 °C, were statistically studied with the Anova one-way (SPSS program) using the Bonferroni test Post-Hoc.

RESULTS

In this work, we have chosen the apple Reineta variety to study the browning effect after high-pressure treatment. Apples are easily oxidized when they are cut. To protect the fruit from going brown, an antioxidant treatment was applied before high-pressure treatment. Different ascorbic acid concentrations (5, 20, and 50 mM) were used. But 50 mM ascorbic acid was only used as a preliminary assay to determine the metabolites (Ca^{2+}, K^{+}, Na^{+}, and Mg^{2+}) and microbial content. The pressure treatment of 400 MPa at 5 °C for 30 min was chosen from previous work (Préstamo and Arroyo, 1998).

The electric conductivity results are shown in Figure 1. The values for both treatments (ascorbic acid and HP) as well as the control are given. The metabolite leakage to solution was higher in the control samples than in the samples treated with ascorbic acid and HP (15 and 30 min). The 5 mM ascorbic acid concentration had a protective effect on the samples, and higher ascorbic acid concentration (20 mM) had values closer to the control samples. HP treatment increases in electric conductivity results.

After 2 months of storage at 4 °C, the conductivity increased more in all the samples. The samples treated

Table 1. Electric Conductivity Statistical Results of Control Samples, Samples Treated with 5 and 20 mM Ascorbic Acid and HP of 400 MPa at 5 °C (15 and 30 min)[a]

	ascorbic acid treatment		
HP treatment	control	5 mM ascorbic acid	20 mM ascorbic acid
control	267* ± 1.63	177* ± 1.41	221* ± 2.38
control HP, 15 min	664* ± 7.41	505* ± 8.48	554 ± 4.78
control HP, 30 min	678* ± 6.50	530* ± 2.50	606* ± 4.65

[a] Mean ± SD; $n = 4$; $P = 0.05$.

with 5 mM ascorbic acid concentration had the lowest conductivity. The protective effect is clearly shown in Figure 1. After 2 months of storage, significant differences were observed in the samples not subjected to HP and in the samples treated with HP as mentioned above.

The statistical results showed significant differences among all the samples (Table 1). But for the values after 2 months of storage and the initial ones, no significant differences were observed between samples treated under HP for 15 and 30 min at 400 MPa and 5 °C temperature, as observed in the Figure 2. This means that the same effect is already reached after 15 min of treatment. However, significant differences were observed in the control samples and the samples treated under HP for 15 and 30 min.

The mineral content analyzed in the preliminary data by atomic absorption were potassium, sodium, calcium, and magnesium. The results are given in Table 2. Sodium metabolite did not change after HP treatment, but it did increase with ascorbic treatment. Potassium metabolite increased in both treatments (ascorbic acid and HP). Calcium and magnesium were extracted from the cell in both treatments. From this result, we have chosen potassium as a good indicator of leaking, and Figure 3 depicts the behavior of potassium content analyzed in the control samples, samples treated with ascorbic acid, and samples treated with HP. The results obtained for potassium content had the same pattern as the electric conductivity values. Significant differences were observed in the amount of potassium when HP was applied in comparison to the samples were HP treatment was not applied. Less potassium amount was leached at 5 mM ascorbic acid concentration than at 20 mM ascorbic acid concentration and without ascorbic acid. After 2 months of storage, the amount of potassium increased in all the samples and had the same pattern as electric conductivity.

Both determinations of electric conductivity and potassium content are good indicators of the metabolites leaking and a simple parameter for quality control.

The results of the sensory evaluation for color showed that 5 mM ascorbic acid was not sufficient to stop the oxidative stress produced by HP, and some brown spots were observed. However, with 20 mM ascorbic acid concentration no brown color was detected after HP treatment (15 and 30 min). The 50 mM ascorbic acid concentration was very high. A transparent appearance was observed in all the samples treated with HP. The texture was good (firm), and they had a slightly cooked flavor after HP treatment. The final appearance of the product was good, and the product was acceptable to consumers. After 2 months of storage, the results were pretty similar in the samples treated with HP as the initial ones. Yet, the samples not subjected to HP fermented after 2 months of storage and were not acceptable to consumers.

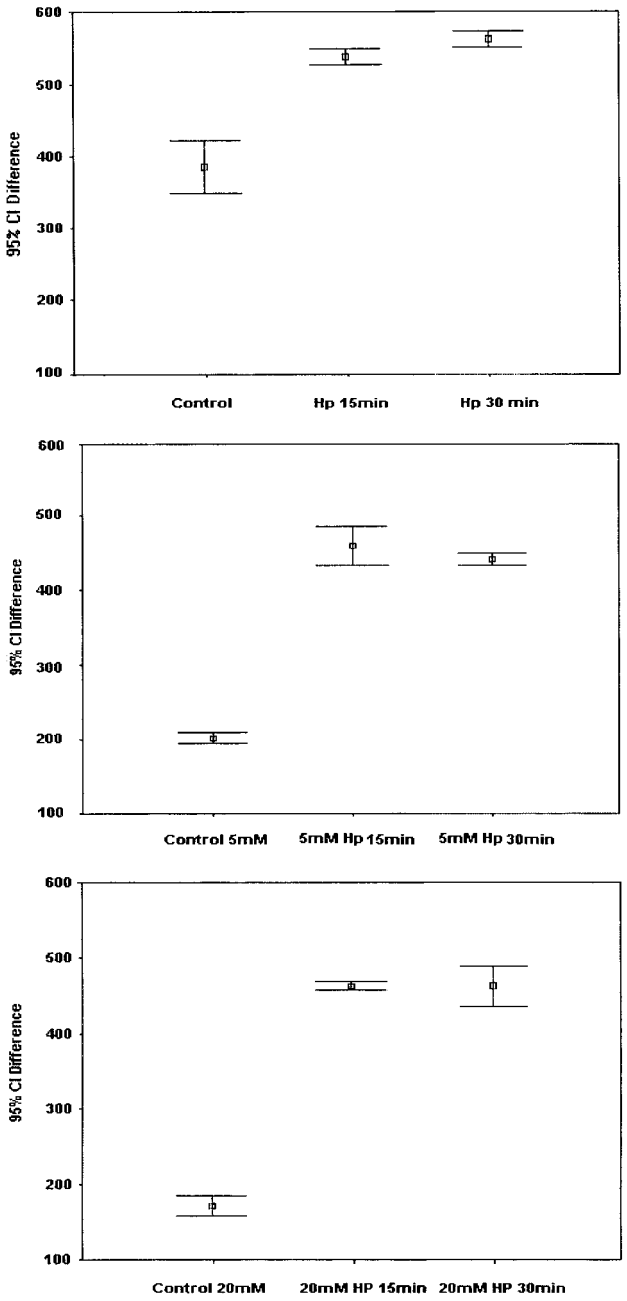

Figure 2. Electric conductivity statistical results for the control, 5 and 20 mM ascorbic acid values, and the samples treated with HP (15 and 30 min) after 2 months of storage and the initial ones.

Table 2. Mineral Content (Potassium, Sodium, Calcium, and Magnesium) Results of Samples Treated with Ascorbic Acid and HP of 400 MPa at 5 °C and 30 min

	mineral content (mg/L)			
treatment	K	Na	Mg	Ca
5 mM ascorbic acid	102.8	5.8	7.4	9.81
50 mM ascorbic acid	194	8	20.4	14.36
5 mM ascorbic acid HP	499	5.4	16.6	13.64
50 mM ascorbic acid HP	579	6.4	22.8	20.08
control	48	10	3.1	13.9

The peroxidase activity in the samples treated with 5 mM ascorbic acid was 2.87 units of peroxidase mL/min. When HP was applied, this activity increased to values of 8.07 units of peroxidase mL/min. In the samples treated with 20 mM ascorbic acid, no peroxidase activity was detected before and after HP treat-

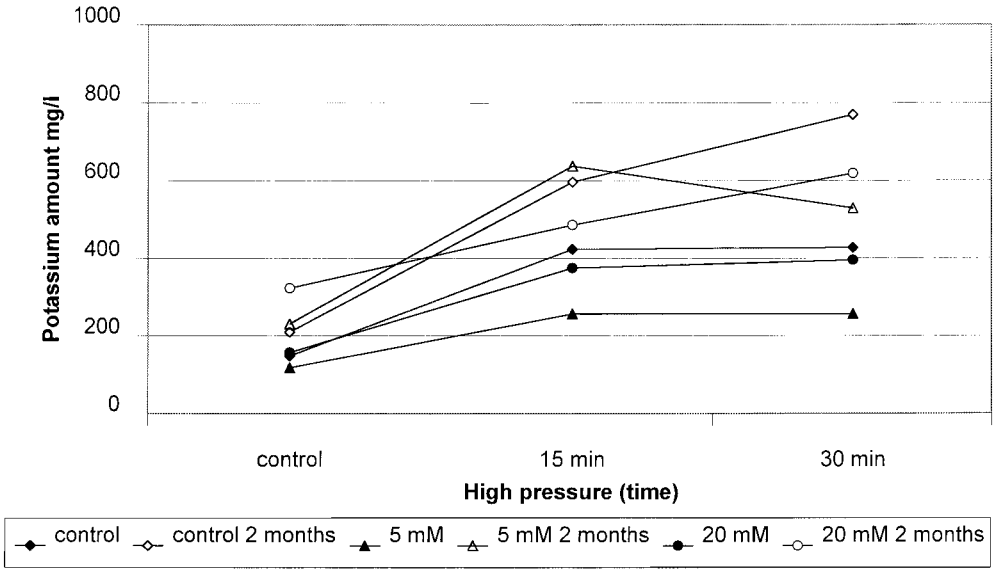

Figure 3. Potassium amount of control samples, samples treated with 5 and 20 mM ascorbic acid, and samples treated with HP of 400 MPa at 5 °C for 15 and 30 min. The same samples after 2 months of storage.

ment. This amount of ascorbic acid inhibited the peroxidase activity.

The initial population of viable mesophiles in apple fruit was low about 1.2×10^3 cfu/mL. The ascorbic acid treatment reduced the viable cells 8×10^2 cfu/mL in 5 mM ascorbic acid and 3×10^2 cfu/mL in 50 mM ascorbic acid. After pressurization, the microorganisms lost their ability to grow on nutrient agar.

DISCUSSION

Most of the data found in the literature have dealt with the pressure resistance of microorganisms in meat (Donnelly, 1994; Ryser et al., 1996), cheese (Szczawinski et al., 1994), and poultry (Ryser et al., 1996) among others. Yet only a few of them have been concerned with fruits, particularly changes that they undergo with HP. In recent years, the inactivation of microorganisms has been the primary goal of pressure treatment. Now attention is shifting to other parameters (enzymes, proteins, and metabolites).

From previous work (Arroyo et al., 1997; Préstamo and Arroyo, 1998), we have noticed that the brown color of vegetable products after high-pressure treatment made them unacceptable to consumers. This effect could be explained partly because some enzymes are not inactivated by high pressure such as peroxidase, 400 MPa was not enough to inactivate the peroxidase (Préstamo and Arroyo, 1998), pressures higher than 800 MPa were necessary to inactivate polyphenoloxidase (Gomes and Ledward, 1996). The HP treatment makes the substrates interact with the enzymes and sometimes produces increases of enzyme activity. Dörnenburg and Knorr (1997) have observed that pressures higher than 150 MPa resulted in irreversible permeabilization of cell membrane and in loss of compartmentalization in the cells.

This study shows that ascorbic acid enhances the effectiveness of pressure treatment and reduces the browning effect. Ascorbic acid interacts with the cell membrane and prevents the cell from browning. The 5 mM ascorbic acid was not enough to protect the cells from browning, but this concentration protected the cell from leaching. The 20 mM ascorbic acid concentration had a lower protection effect in comparison to leaching, but no brown color was detected at this concentration. Ascorbic acid also inactivates some enzymes such as peroxidase (Préstamo and Manzano, 1993). Our results are the same, and 20 mM ascorbic acid was enough to inactivate the peroxidase. The HP did not destroy the ascorbic acid as Horie et al. (1991) reported that strawberry jam prepared by HP retained 95% of the ascorbic acid concentration, and no brown color was detected after 2 months of storage because the ascorbic acid remains in the product.

Ascorbic acid also affects the microbial membrane and is more active in combination with pressure (Mackey, 1995). In the case of the microbes, they are particularly sensitive to ascorbic acid during or after treatment (Horie et al., 1991). After 2 months of storage, the samples subjected to HP were acceptable to consumers. The ones not subjected to HP treatment fermented and were unacceptable to consumers. As a result of HP treatment, the shelf-life of the product is longer. It is well-known that sugar has a protective effect on food products, but in this case it was not enough to prevent browning, and it was necessary to add an antioxidant (ascorbic acid).

In the potassium release, no significant differences were observed between 15 and 30 min. Although 30 min should be chosen to make the product safer in terms of pathogenic microbial contamination, such as *Listeria monocytogenes*, which was inactivated at 400 MPa for 30 min (Préstamo et al., 1999).

The samples treated with HP presented higher values in all the assayed measurements than the samples not subjected to HP, and after 2 months of storage the amount of released potassium increased with 20 mM ascorbic acid and with HP treatment (15 and 30 min). The 5 mM ascorbic acid has a protective effect from leaking, but an increase in ascorbic acid alters the cell permeability (Dörnenburg and Knorr, 1993). Particularly in this work, apple dessert is good because the increase of metabolites in the solution makes the solution tastier.

Electric conductivity increases with ascorbic acid and HP treatment. Both measurements (electric conductivity and potassium content) are good tools for determin-

ing the solutes lost during the HP process. The HP treatment induces mass transport between food and the immersion solution.

Porins are pores forming transmembrane proteins that cross the lipid bilayer as a β-barrel. The porins are found in the outer membrane in many bacteria. The outer membrane is penetrated by various pores forming porin proteins, which allow selected hydrophilic solutes to diffuse across the outer lipid bilayer channels in the outer membrane of Gram-negative bacteria, and their physiological role is to provide a permeability path. Porins are trimer membrane proteins whose structure is well-known and whose response to HP bursts the channel openings (MacDonal and Martinac, 1998) and inactivates the bacteria.

In vegetable products, ion channels are dynamic structures, and they do not remain continuously open. They flip between open and closed states, regulating the cell permeability and maintaining the cell turgor. The ion channels in vegetables act as a porins in bacteria. The HP produces alterations in the ion channels, and the cell loses permeability.

The treatment with ascorbic acid eliminates the browning effects. It is thought that this treatment will be more acceptable to the food industry than ones that involve the use of chemical preservatives.

CONCLUSIONS

The combination of HP and ascorbic acid has been found to reduce the browning effect completely in the treated samples. Ascorbic acid treatment (20 mM) prevents the samples from browning unlike the untreated samples and samples subjected to HP. HP extends the shelf-life of the product, eliminating the microbial population after pressurization. Electric conductivity as well as potassium content are both good tools for determining the solutes lost in the samples treated with HP.

ACKNOWLEDGMENT

We would like to thank the research assistant Gonzalo Guerrero for all his help with the atomic absorption apparatus.

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Received for review February 18, 1999. Revised manuscript received June 23, 1999. Accepted July 7, 1999. This work was funded by the CSIC, ALI-97, 0759 Project.

JF9901688