

Research Article

High Pressure Processing Treatment of Fresh-Cut Carrots: Effect of Presoaking in Calcium Salts on Quality Parameters

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Effect of high pressure (HP) treatment (200–600 MPa; 0–20 min) on quality of fresh-cut carrot slices was evaluated after presoaking in selected calcium salt solutions (1% calcium chloride, 1% calcium lactate, 1% calcium gluconate, and distilled water as control) for one hour. Results showed that calcium chloride (CaCl₂) solution pretreatment was most effective for preserving the hardness of carrot slices at 400 and 600 MPa and this treatment also resulted in the least amount of color change in carrots, followed by calcium lactate, gluconate, and control pretreatments. The average sensory evaluation scores during 9 days of refrigerated storage at 4°C in control, CaCl₂, calcium lactate, and calcium gluconate presoaking treatments followed by HP treatment were 6.4 ± 0.5, 8.0 ± 0.5, 7.8 ± 0.4, and 7.6 ± 0.3, respectively, on a zero to 9 scale for quality.

1. Introduction

Carrot is a popular vegetable used to make salads and home-cooked meals and ready-to-eat products. Minimally processed carrots are prepared by peeling off the outer layer of the carrots root and cutting to slices or cubes [1] and keeping them refrigerated in some form of containers/packaging. However, such practices usually suffer from browning, excessive microbial load, and tissue softening [2] that limits its market value and shelf life.

High pressure processing (HPP) has attracted considerable attention because of the increasing consumer demand for fresh-like, minimally processed, and microbiologically safe food products [3]. HPP can reduce adverse impact on color, texture, and flavor of food products and possibly inactivate foodborne pathogens and spoilage microorganisms [4–8]. Compared to traditional heating processes, HPP provides several advantages such as uniform pressure treatment, reduced thermal gradient, and short processing times. Therefore, it can be used as a nonthermal alternative over the conventional thermal process for improving the shelf

life and quality of foods [9]. Several studies have explored using HPP for carrots and have demonstrated that treated carrots retained textural characteristics similar to those of fresh control samples [10, 11]. However, HPP also could result in softening of carrot due to destruction of cell membrane and loss of soluble pectin along the cell liquor [12]. Basak and Ramaswamy [13] reported that the extent of the initial loss of texture was more prominent at higher pressures and partial recovery of texture was possible at lower pressures. Subsequently, some pretreatment approaches have been employed in HPP to enhance carrot quality, such as pressure-assisted thermal processing [4], calcium pretreatment [14, 15], or their combinations.

Calcium salts have been traditionally used to extend the shelf life of fresh whole and minimally processed fruits and vegetables [16]. The effect of calcium in tissue firmness is due to its ability to strengthen the cell wall by binding free carboxylic group of the pectin chains forming cross-links or bridges and at the same time preserving the structural and functional integrity of membranes [17]. Different calcium salts, such as calcium lactate, calcium chloride,

calcium phosphate, calcium propionate, and calcium gluconate, have been used in food industry for preserving and/or enhancing of the product firmness [18–20]. Calcium chloride has been widely used as preservative and firming agent in the fruit and vegetable processing industry for whole and fresh-cut commodities, but the use of calcium chloride has also been associated with bitterness and off-flavors [21]. Calcium lactate and calcium gluconate have shown some benefits of calcium chloride, such as product firmness improvement and without the bitterness and residual flavor [22].

Some studies on calcium salt infusion as pretreatment combined with HPP on carrot have also been carried out [4, 14, 23]. These studies confirm that combining HPP with calcium chloride soak could lead to a significant texture improvement of carrots during thermal processing, compared with treating the carrots directly in a calcium solution or only HPP. There is no information regarding quality changes in carrots soaked in different Ca salts solutions followed by or combined with HPP. Tola and Ramaswamy [24] demonstrated that adding of salts to vegetables can bring about advantages in ohmic heating of the products and in some instances especially when using gluconic acid salts for partial acidification of vegetables. They further reported that lowering of the pH of carrots below 4.5 would allow them to be processed as a food belonging to acid group (pH < 4.5) which requires reduced process severity (acidified thermal processing) both by thermal processing and HPP and contribute to better quality and great energy savings.

The objective of this research was therefore to (a) evaluate the effect of presoaking in three calcium salts (calcium lactate, calcium chloride, and calcium gluconate) followed by HP treatment to improve the texture, color, and sensory properties of fresh-cut carrots and (b) assess the stability and sensory quality of the treated product after a short-term refrigerated storage at 4°C.

2. Materials and Methods

2.1. Sample Preparation. Carrots (*Daucus carota*) were purchased from a local market (Gouzhuang town, Hangzhou) and stored at 4°C before processing and analysis. About same size carrots (18–20 cm long, 2.5–3.5 cm in diameter) were cleaned and peeled first. The two ends of the carrots were cut off and then they were sliced to get 1 cm thick cylindrical slices. Enough carrot slices were prepared before treatment.

Five randomly selected carrot slices (1 cm thick, 2.5–3.5 cm in diameter) were vacuum-packed with an immersion treatment solution into a bag. Four solution immersion pretreatment liquids were used: (a) control: soaked in distilled water, (b) calcium chloride (CCL) pretreatment: soaked in 1% (w/v) calcium chloride (CaCl₂) solution, (c) calcium lactate (CLA) pretreatment: soaked in 1% (w/v) calcium lactate solution, and (d) calcium gluconate (CGL) pretreatment: soaked in 1% (w/v) calcium gluconate solution. All samples were soaked for 1 h at 25°C prior to pressure treatment.

2.2. High Pressure Processing Equipment. High pressure treatments were given in laboratory scale high pressure equipment. The system consisted of a HPP unit (UHPPF-750, 5 L, Kefa, Baotou, China) and equipped with K-type thermocouples (Omega Engineering, Stamford, CT, USA) and a data logger (34970A, Agilent Technologies GMBH, Germany) for temperature measurement and a thermostat jacket connected to a water bath (SC-25, Safe, China) for maintaining the processing temperature. The intensifier used for generating the pressure was a batch type unit which built-up the pressure in a stepwise ladder-like process. Water was used as the pressure transmitting medium in this study, and the pressure vessel was maintained at 25°C before pressurization. Normally sample temperature is expected to increase by 3°C every 100 MPa pressure rise due to adiabatic compression. However, to minimize this adiabatic heating, a low rate of pressurization was maintained (~100 MPa/min) so that the sample temperature easily equilibrated to the set point temperature of 25°C. The pressure release time was kept less than 5 s.

2.3. High Pressure Treatment and Storage. High pressure treatments were given to all presoaked solutions at 200, 400, and 600 MPa for 0, 2, 5, 10, 15, and 20 min at 25°C, respectively, using the HPP system. After treatment, the carrot slices with vacuum packaged were stored at 4°C for up to 9 days. Texture analysis was performed immediately after treatment (0 day) and after 3, 6, and 9 days of storage.

2.4. Texture Measurements. Textural properties were evaluated using a Texture Analyzer (CT3, Brookfield, MA, USA) with a 25 kg load cell. The cylindrical carrot slice was compressed with a cylindrical aluminum plate at a constant crosshead speed of 1 mm/s. Texture was expressed as hardness as the maximum force needed to compress the sample to 50% of its original thickness. The mean value of the compression force of 5 carrot slices was considered as texture hardness value measurement. The relative hardness was defined as ratio of sample hardness to control which was the one without any treatment.

2.5. Color Measurement. Color of samples was measured using a portable tristimulus colorimeter (CM-600d, Minolta, Japan) at room temperature. The colorimeter (with illuminant D65, 10° observer) was calibrated with a standard white tile ($L = 99.47$, $a = -0.18$, and $b = -0.13$) before each color measurement. All measurements were carried out in triplicate, with L , a , and b values being recorded, and mean values were taken for calculation. L , a , and b values represent degree of lightness, redness, and yellowness of samples, respectively.

The color degradation of samples was expressed as a comprehensive index in the form of *total color difference* (ΔE value) and *whiteness index* (WI value). ΔE value was calculated by following equation [25]:

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2}, \quad (1)$$

where L_0 , a_0 , and b_0 represent the readings of untreated samples and L , a , and b represent the instantaneous readings after treatments.

The WI value represented the purity of white color. A larger WI denoted better color retention in carrot. The WI value was expressed as the equation

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}. \quad (2)$$

To exclude the influence of measuring background, samples were measured for color by placing them on white paper.

2.6. Sensory Analysis. Ten panelists (six female, four male, aged 19–21) participated in the sensory analysis. All panelists were given preliminary training to recognize and score visual and feel the quality attributes of carrot samples. The carrot attributes included visual/feel texture, color, flavor, and overall appreciation. The score ranged from 1 to 9, where 1 stands for very poor, 3 stands for poor, 5 stands for fair, 7 stands for good, and 9 stands for excellent. To maximize panelist's individual differences, each panelist received different carrot samples several times during the training. Each sample with different treatments was blind-coded and prepared in quintuple before sensory analysis. The samples were not evaluated for taste because they were not fully microbiologically tested for edible safety. None of the samples showed any sign of spoilage or sliminess.

2.7. Statistical Analysis. Each treatment was carried out in triplicate. Experimental results were expressed as mean value. Statistical significance was accepted at a level of <0.05 . A one-way analysis of variance (ANOVA) was performed using SPSS (Version 20, IBM, USA).

3. Results and Discussion

3.1. Effect of Different Soaking and HP Treatment on Carrot Texture. Texture is an important quality parameter of processed fruits and vegetables that dictates their market value and consumer acceptance [24]. According to the US Department of Agriculture (1998), texture accounts for 30% score for the market acceptability of canned carrots.

Texture value as measured by the relative hardness of carrot samples presoaked in different solutions and HP treated are shown in Figure 1. In control samples, with water as presoaking treatment, the hardness values after HP treatment at 200 MPa varied from 95.2% (for 0 min) to 81.9% (for 20 min), significantly higher ($P < 0.05$) than at 400 MPa level (varied from 68.2 to 56.1%) and 600 MPa levels (66.1% to 64.7%). Similar results have been reported in a previous study [12, 13], who pointed out that the pressure level was significant factor reducing the hardness of HP treated vegetables. But in Jung et al. [9] study, which was little different from the present study, the hardness of carrots significantly decreased after treatment at 300 and 500 MPa for 10 min or less while the hardness was reported to recover when the holding time was 15 min or more. The effect of high pressure processing on texture varies with the material as well as the degree of the applied pressure [4].

Presoaking with calcium salt solutions proved to be effective in preserving the hardness of carrot slices at higher pressures (400 MPa and 600 MPa), but not obvious at 200 MPa, except with CGL samples. As shown in Figure 1, in CCL and CLA pretreatment samples, the relative hardness at 200 MPa was higher than other at 400 MPa and 600 MPa. In CCL pretreatment, the carrot hardness was preserved much better at pressure holding time of 10 min. The relative hardness factor was 88.7% at 200 MPa for 10 min, 85.3% at 600 MPa, and 73.2% at 400 MPa, respectively. In CLA, HPP treatments at 200 MPa for 5 min had the highest hardness retention of 85.0% than at 400 and 600 MPa. The tissue hardness was recovered at 400 and 600 MPa for 10 min, and the average retention values were 73.7% and 77.6%, respectively, while in CGL the range of relative hardness by different treatments was 81.3%–65.7%, and they showed no significant difference ($P > 0.05$) at three pressure levels when the holding time was more than 10 min. The results of this work therefore show some synergistic effect of HP treatment and calcium salt pretreatment and holding time on tissue firmness.

Texture of fruits and vegetables changes due to beta-elimination depolymerization of the cell wall pectin polysaccharides [24]. This depolymerization leads to pectin solubilization and, consequently, to decreased intercellular adhesion [26]. While combining HPP with calcium salt presoaking processing, the texture improvement could be due to the occurrence of two effects. First, the HPP lowers the pectin DM by activating endogenous PME and then by adding calcium ions, this low methoxyl pectin can be cross-linked [14, 23].

Moreover, it was also found that effects of texture improvement depended on calcium source. Similar observation has been reported by Silveira et al. [27]. However, Manganaris et al. [19] reported that calcium increased in tissues had no dependence on the source used, when comparing the effect of calcium lactate, chloride, and propionate dipping in peaches. These may be associated with the different treatment methods. Several studies suggested that factors such as solubility, the diffusive capacity of the tissues, and the ability to form bridges with cell wall pectates vary depending on the source used and must be taken into account in their choice [27].

3.2. Carrot Hardness Retention Rate during Storage. Considering the inactivation effect of HPP on food borne pathogens [9], the hardness of carrots during storage was evaluated after treatment at 600 MPa for 10 min which was adequate for killing all vegetative pathogens. As shown in Figure 2, the relative hardness of carrot slices gradually reduced during storage period. Higher level of hardness loss of carrot was found in control, with less than 63.4% retention during the storage time. Samples with presoaking in calcium salt solutions had significantly ($P < 0.05$) higher relative hardness than that in control. The relative hardness in CCL was 85.3% on day 0, 82.5% on day 3, 79.8% on day 6, and 77.4% on day 9, which were higher than that in CLA (77.8%, 78.4%, 73.5%, and 69.6%, resp.) and CGL (75.3%, 74.2%, 71.9%, and 70.6% resp.). The significant differences ($P < 0.05$) are showed clearly

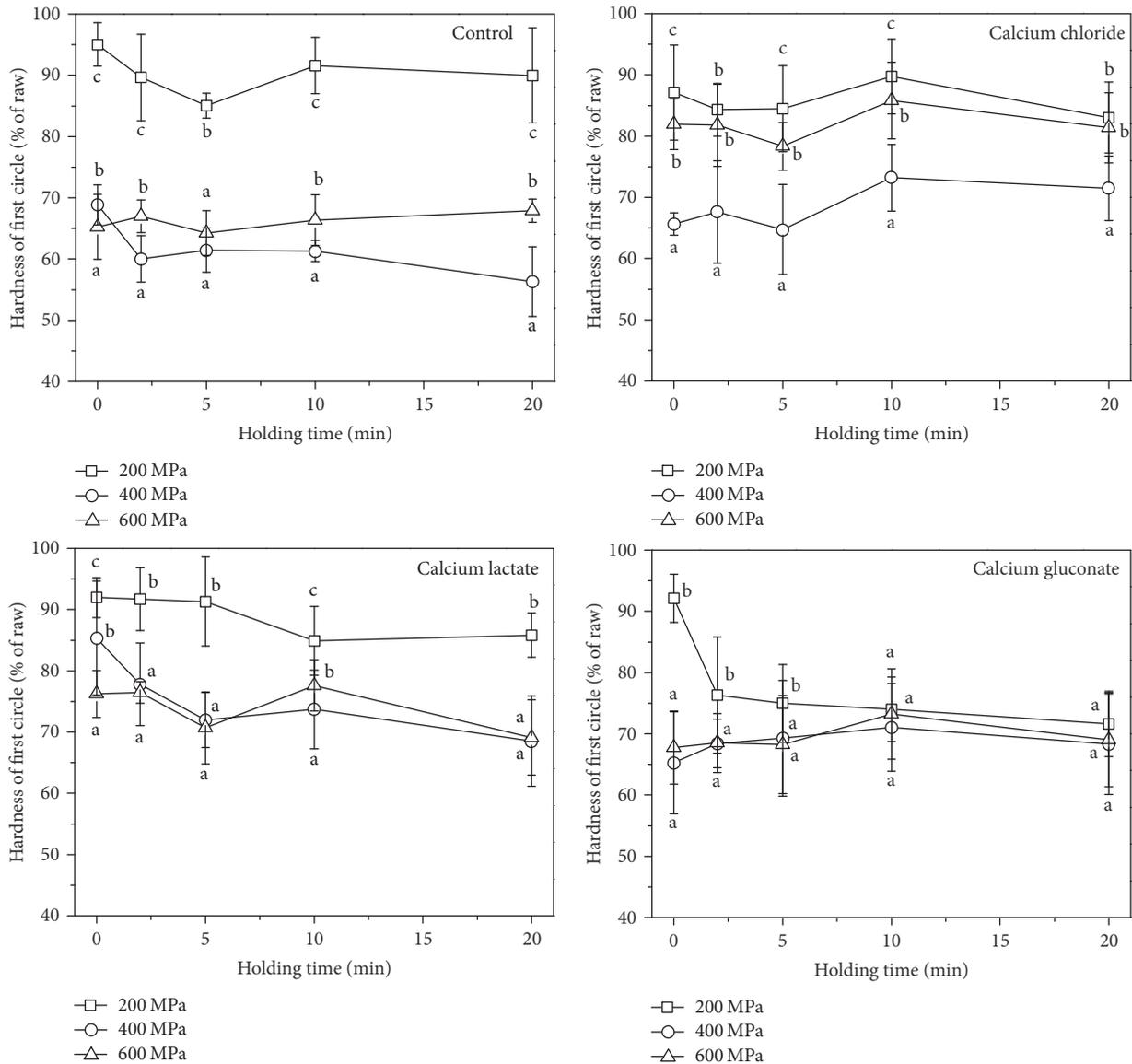


FIGURE 1: Effect of HP treatment after different presoaking: control : distilled water, 1% calcium chloride, 1% calcium lactate, and 1% calcium gluconate on relative hardness of fresh-cut carrots. Results are expressed as mean \pm the standard deviation. ^{a,b,c}Different letters mean significant difference ($P < 0.05$) among the pressure levels for the same holding time.

on day 9. Calcium chloride soaking was the best presoaking treatment for treatment at 600 MPa for 10 min to result in best texture retention following storage for 9 days at 4°C; CLA was next.

Luna-Guzman and Barrett [18] found that calcium lactate treatment for shelf life extension of fresh-cut cantaloupe provided similar or better tissue firming than control. Aguayo et al. [28] reported the effect of calcium on fresh-cut melon quality depended on the type of calcium salts. Calcium chloride and weak organic acid salts like calcium propionate and lactate were very effective in maintaining fruit firmness during 8 days storage at 5°C. These results suggested that the different calcium salts have different effects on texture of different vegetables.

3.3. Color Degradation. Appearance is another one of the most important sensory quality attributes of fresh and processed food products and their marketing [29]. In this study, the color changes in cut carrots predipped in different calcium salt soak solutions followed by HP treatment were evaluated. The mean L , a , b values and the whiteness index (WI values) of untreated carrot samples were 59.5 ± 10.3 , 31.6 ± 9.48 , 35.2 ± 8.29 , and 31.7 ± 8.15 , respectively. For control, the initial total color difference ΔE is zero. The changes in color values are presented in Figure 3 for ΔE value and in Figure 4 for WI value. As shown in Figure 3, in control, 200 MPa treatment had maximum color differences as compared with untreated samples, and significant differences ($P < 0.05$) in ΔE value were found between 200 MPa

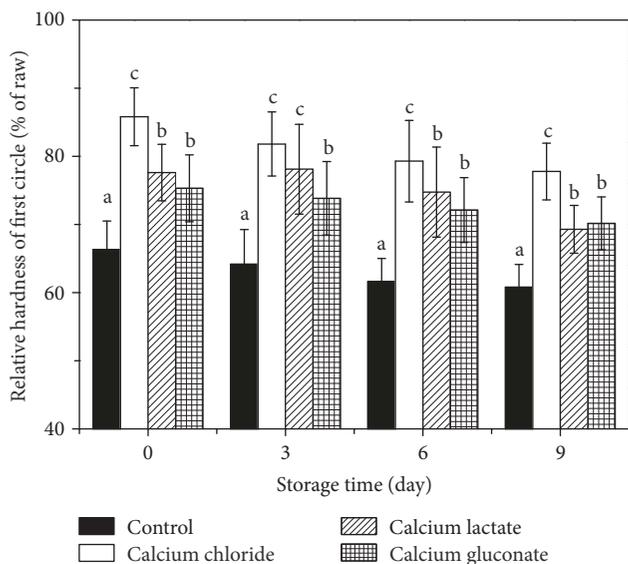


FIGURE 2: Effects of HP treatment (600 MPa, 10 min) after different presoaking: control: distilled water, 1% calcium chloride, 1% calcium lactate, and 1% calcium gluconate on relative hardness of fresh-cut carrots after storage at 4°C for up to 9 days. Results are expressed as mean \pm the standard deviation. ^{a,b,c}Different letters mean significant difference ($P < 0.05$) among the groups for the same day.

and 400/600 MPa for different holding times. Jung et al. [9] reported that the ΔE value of HP treated (100, 300, and 500 for 20 min) carrots was 1.5–8.2, which slightly differs from this study.

Color in carrots is mainly due to the presence of carotenoid pigments. The more intense visual color observed may be contributed by the permeabilization of plant cells caused by HPP, which could leak the carotenoid pigments into the intercellular space. Oey et al. [5] demonstrated that pressure treatment at low and moderate temperatures had only limited effect on color pigments such as chlorophyll, carotenoids, and anthocyanin.

For samples pretreated in CCL and combined with HP treatment at 400 and 600 MPa, the ΔE values ranged between 2 and 6, significantly lower ($P < 0.05$) than in treated control, CLA, and CGL samples. The smallest ΔE value was observed at 400 MPa for 10 min, and no significant difference was observed at 600 MPa for 10 min. In CLA samples, ΔE values varied from 6 to 10, and no significant differences ($P > 0.05$) were observed at 200 MPa, 400 MPa, and 600 MPa for different holding times, while the smallest ΔE value in CGL samples was found at 600 MPa for 10 min (6.68) which was higher than many other treated samples. Moreover, the intensity of redness (a value) and greenness (b value) in CGL pretreatment samples reduced the most in fresh carrot after HPP (data not shown).

Since a white lignin type material is more likely to form on the surface of carrots after processing [30], the whiteness index WI value would be a good color index to evaluate the effect of HP treatment on color change from of fresh-cut carrots. In control at 200 MPa for different holding times, the WI values increased significantly ($P > 0.05$), while a slight

decrease was observed at 400 MPa. Similar results were also observed by Hu et al. [31], who found that the fresh-cut carrot WI value increased at 200 and 600 MPa and decreased at 400 MPa.

In calcium salt pretreated samples, WI values significantly ($P < 0.05$) increased at 400 MPa for different holding times, as compared with the control. In CCL pretreated samples, WI values were significantly ($P < 0.05$) reduced at 600 MPa when the holding time was more than 2 min, as compared with the control. Moreover WI values also reduced at 400 MPa for 10 min, compared to control, and values were not significantly ($P < 0.05$) different from samples treated at 600 MPa for 10 min. In CLA pretreated samples, WI values were at 33–38 and no significant differences were found between different treatments. The maximum WI value of 38 was observed at 600 MPa for 10 min in CGL pretreated samples, and the minimum WI value of 31.6 was associated with at 400 MPa for 5 min in CGL pretreated samples.

In this study, the formation of white lignin type material was not observed. Results indicated that carrot pretreated in CaCl_2 had better effect on color retention, and the optimal treatment condition for better color retention was 400/600 MPa for 10 min.

3.4. Sensory Evaluation. In this study, sensory evaluation scores of samples pretreated in different soaking calcium salt solution followed by HP treatment at 600 MPa for 10 min and then stored for 9 days at 4°C are shown in Table 1. Other treatment conditions were not included due to microbiological concerns. The sensory study was not intended for making recommendation for commercial processing, but rather to gather some scientific data on stability of minimally processed fresh-cut carrots subject to HPP. Commercial adaptation would probably require a more thorough study on destruction kinetics of spoilage and pathogenic bacteria and enzyme inactivation, establishment, verification, and challenge studies to make sure the process is safe. However, the process employed (600 MPa 10 min as used in this sensory evaluation study) is more than adequate for eliminating all common vegetative pathogens of different strains and species of *E. coli*, *Listeria monocytogenes*, and *salmonella* as per published data on HPP of vegetables.

The highest scores of carrot sensory texture score of 7.8 ± 0.1 and 7.8 ± 0.2 were obtained in CaCl_2 presoaked samples stored for 0 and 3 days. Control samples had the lowest texture score, less than 6, when stored more than 3 days. In presoaked calcium salt solutions samples, texture scores were more than 6 during the entire storage, meaning that carrot texture was improved by calcium pretreatment and HPP.

The average flavor scores during storage in CLA and CGL samples were 8.2 and 8.1, slightly higher than in CCL (7.8). This result also is somewhat different from Luna-Guzman and Barrett [18], who used CaCl_2 (~173 mM) dipping for fresh-cut cantaloupe slices finding undesirable bitterness, which was avoided when using calcium lactate at the same concentration. Sensory analyses of minimally processed products treated with calcium salts have reported

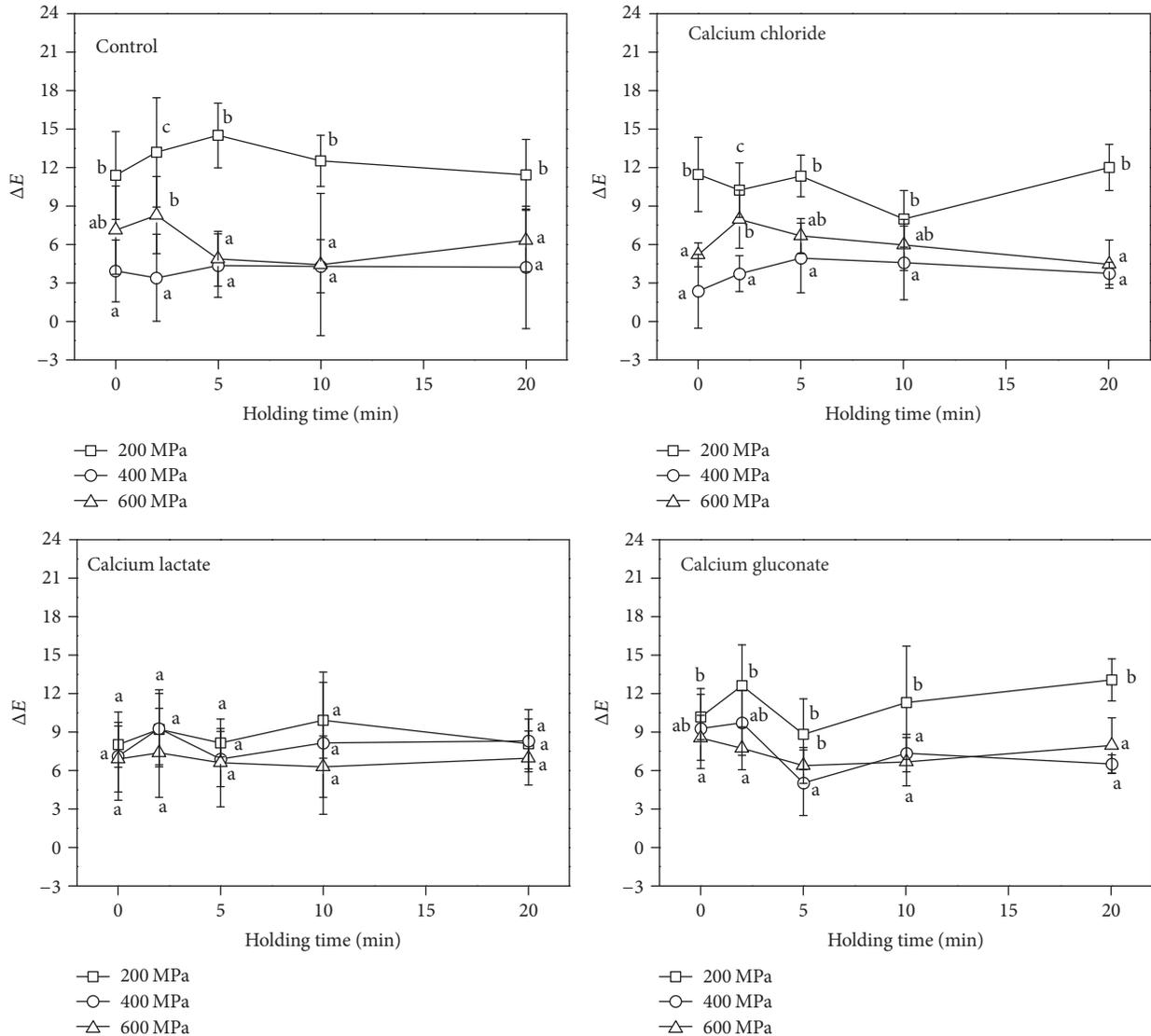


FIGURE 3: Effects of HP treatment after different presoaking: control : distilled water, 1% calcium chloride, 1% calcium lactate, and 1% calcium gluconate on ΔE of fresh-cut carrots. Results are expressed as mean \pm the standard deviation. ^{a,b,c}Different letters mean significant difference ($P < 0.05$) among the pressure levels for the same holding time.

bitterness as a side effect of the treatment, in some cases depending on the source of Ca used [16]. But in this study, the average flavor scores were not significantly different ($P < 0.05$) between the samples pretreated in different calcium salts. These may be associated with the calcium concentrations used. The relatively low concentration calcium salts used in this study had a slightly salty taste and lip feel following the treatment, but panelists did not show any distinct preference for CLA and CGL over CCL pretreated samples.

Considering features of carrot color and flavor, all treated samples obtained scores higher than 6, which indicated that the sensory characteristics met the panelists' acceptance standards. In addition to texture, overall appreciation was another indicator showing the effect of different treatments on carrot quality. Control samples had the lowest average acceptance

score (5.6 ± 0.5) in overall appreciation, considerably lower than the average score of CCL (7.5 ± 0.5), CLA (7.2 ± 0.4), and CGL (7.2 ± 0.3) pretreated samples.

4. Conclusions

This study demonstrated an effective use of different Ca salts for presoaking prior to HPP for enhancing hardness, color retention, and sensory characteristics of fresh-cut carrots. The result showed that calcium chloride presoaking and HP treatment at 400 MPa or 600 MPa for 10 min was an effective method for preserving the hardness and color retention of fresh-cut carrots. The presoaking in other calcium salts had different quality retention effects on carrots after HP treatment.

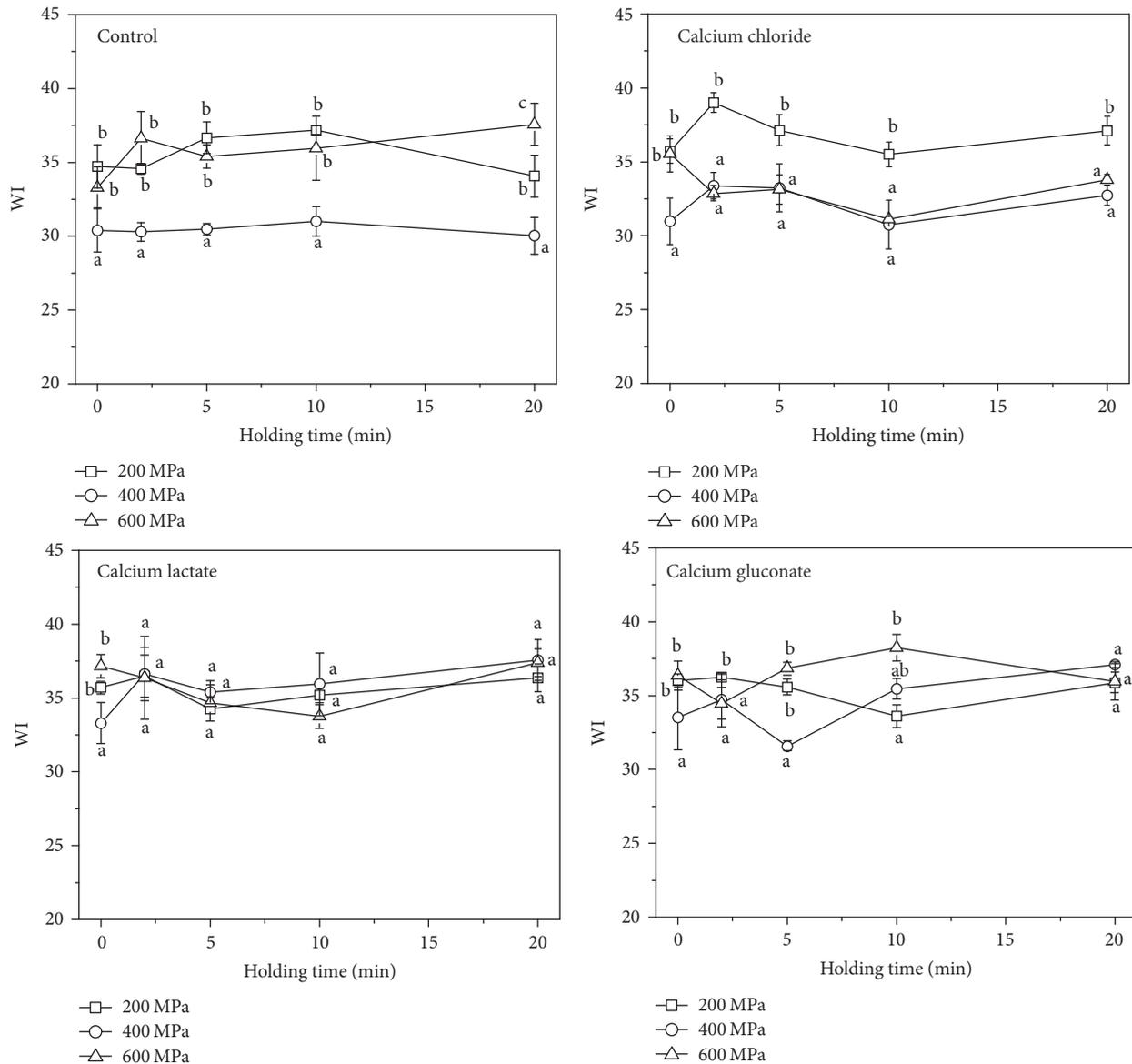


FIGURE 4: Effects of HP treatment after different presoaking: control: distilled water, 1% calcium chloride, 1% calcium lactate, and 1% calcium gluconate on WI of fresh-cut carrots. The WI values of untreated carrot samples were at average of 31.7. Results are expressed as mean \pm the standard deviation. ^{a,b,c}Different letters mean significant difference ($P < 0.05$) among the pressure levels for the same holding time.

The calcium content in the diet is critical in most stages of life [32]. To give consumers the opportunity to increase their calcium intake, the industry has been resorting to fortify food and beverages with calcium [33]. Use of calcium salts as for presoaking treatment prior to HP treatments would naturally help to improve intake of calcium salts in the diet and also facilitate better quality retention in the cold stored product.

Additional Points

Practical Applications. This study was focused on hardness and color improvement in high pressure (HP) treated carrots after presoaking in various calcium salt solutions. The HP

treatment combined with prior CaCl_2 soaking had the most positive effect on preserving the hardness and color of carrots; calcium lactate soaking gave the next best result. High pressure treatment with a calcium salt solution presoaking followed by HP treatment would help to improve the overall acceptability during refrigerated storage and also fortify food with calcium.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

TABLE 1: Sensory evaluation scores of different presoaking treatments and HPP of carrot samples during storage refrigerated storage at 4°C.

Treatments	Storage time (days)	Texture	Color	Flavor	Overall acceptability
Control (distilled water)	0	6.0 ± 0.3 ^a	8.3 ± 0.2 ^a	8.0 ± 0.6 ^a	7.0 ± 0.4 ^a
	3	5.8 ± 0.4 ^{ab}	8.2 ± 0.1 ^{ab}	8.2 ± 0.4 ^a	6.8 ± 0.3 ^a
	6	5.5 ± 0.2 ^{bc}	8.2 ± 0.3 ^{ab}	7.8 ± 0.3 ^b	6.0 ± 0.3 ^b
	9	5.0 ± 0.3 ^c	8.0 ± 0.3 ^b	6.2 ± 0.4 ^c	5.6 ± 0.5 ^c
CaCl ₂ + HPP	0	7.8 ± 0.1 ^a	8.5 ± 0.5 ^a	8.0 ± 0.5 ^a	8.3 ± 0.4 ^a
	3	7.8 ± 0.2 ^a	8.3 ± 0.6 ^a	8.0 ± 0.3 ^a	8.3 ± 0.2 ^a
	6	7.3 ± 0.3 ^b	8.0 ± 0.6 ^b	7.7 ± 0.5 ^b	8.0 ± 0.6 ^a
	9	7.0 ± 0.3 ^b	8.0 ± 0.4 ^b	7.5 ± 0.6 ^b	7.5 ± 0.5 ^b
Ca lactate + HPP	0	7.2 ± 0.4 ^a	8.3 ± 0.2 ^a	8.3 ± 0.2 ^a	8.0 ± 0.3 ^a
	3	7.0 ± 0.2 ^a	8.3 ± 0.7 ^a	8.2 ± 0.4 ^a	8.0 ± 0.5 ^a
	6	7.0 ± 0.1 ^a	7.8 ± 0.4 ^b	8.3 ± 0.5 ^a	7.8 ± 0.2 ^a
	9	6.2 ± 0.1 ^b	8.0 ± 0.4 ^b	8.0 ± 0.3 ^a	7.2 ± 0.4 ^b
Ca gluconate + HPP	0	7.3 ± 0.2 ^a	8.0 ± 0.3 ^a	8.3 ± 0.4 ^a	7.8 ± 0.2 ^a
	3	6.8 ± 0.5 ^b	8.0 ± 0.2 ^a	8.0 ± 0.2 ^a	8.0 ± 0.6 ^a
	6	7.0 ± 0.4 ^b	7.8 ± 0.5 ^a	8.2 ± 0.3 ^a	7.5 ± 0.5 ^b
	9	6.5 ± 0.2 ^c	8.0 ± 0.7 ^a	8.0 ± 0.5 ^a	7.2 ± 0.3 ^b

Results are expressed as mean ± the standard deviation. ^{a,b,c}Different letters mean significant difference ($P < 0.05$) among the storage for same group.

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