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Effect of Stepwise Blanching and Calcium Chloride Solution on Texture and Structural Properties of Jalapeño Peppers in Brine

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Summary

Jalapeño peppers incur texture changes during thermal processing due to loss of turgor pressure, tissue softening, and pectin solubility in the cell wall, which affects their quality and acceptability as a processed product among consumers. The objective of this work is to evaluate the effect of stepwise blanching with calcium chloride solution on firmness, calcium absorption and microstructural changes of jalapeño peppers. Batches of 1 kg of pepper halves were blanched at 65 °C for 4 min at different concentrations of $CaCl_2$ (0, 0.075, 0.15 and 0.3 mol/L). After blanching, the peppers were removed, placed in plastic bags and submerged in a water bath at 65 °C for different holding times (0, 15, 30 and 45 min). Then the peppers were blanched again in an acidified solution at 96 °C for 3 min to remove enzymatic activity. Afterwards, peppers were packed in glass containers with brine at 95 °C and pasteurized at 85 °C for 10 min. The product was stored for 10 days at room temperature, then analyzed for texture, calcium absorption, pH, acidity, and microstructural changes. Results showed that $CaCl_2$ concentration and blanching improved firmness with a maximum value of 3.68 N, a 4.3-fold increase over control. Calcium absorption increased with $CaCl_2$ concentration reaching a maximum of 0.68 g of Ca^{2+} per kg of samples. Microscopy showed reduced cell wall damage in the samples that were blanched in 0.3 mol/L of $CaCl_2$ at 45 min of holding time. The optimum processing conditions determined in this study can be used for improving the firmness of peppers.

Key words: texture, calcium, microscopy, blanching, brine, jalapeño pepper

Introduction

Consumption of jalapeño peppers has increased mainly due to their crisp texture and taste that make for a desirable pungency when used as ingredients in a great variety of products including, among others, pickled peppers and stuffed pepper halves. However, pepper tissue softens notably when pasteurized in high acid and salt brine (*1*), affecting the quality and acceptability of these products.

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Pasteurization is a process used to preserve pickles and chili peppers in brine at low concentrations of sodium chloride and acetic acid. This is performed by heating the product to an internal temperature of 85 °C with a holding time of around 10 min. During thermal processing, noticeable changes take place in the edible portion of the tissue, damaging one or more parts of the cell structure. This is reflected in a loss of tissue firmness due to plasmalemma disruption, allowing free diffusion of water and low-molecular mass components resulting in the loss of turgency (*2,3*) and solubilization of pectic substances of the cell wall and middle lamella. All this causes separation and cellular collapse (*4*) leading to tissue softening.

During processing, some insoluble pectins are lost, and there is an increase of water soluble pectins which cause changes in physical properties, such as texture, leading to tissue softening (*5*). Excessive softness could be overcome by stepwise blanching, consisting of low--temperature blanching to stimulate the pectin methylesterase (PME) enzyme, followed by a second blanching at a high temperature to inactivate enzyme activity, improving tissue firmness by the action of PME (*6,7*). PME demethoxylates pectic substances involved in cellular adhesion, producing methyl alcohol, demethoxylated polygalacturonic acid chains, and the pH is reduced (*8,9*). The carboxyl groups of the polygalacturonic acid chain react with calcium and magnesium ions at the cell wall level (*10,11*) forming insoluble pectates on the cell wall, thus increasing tissue firmness and resistance to acid hydrolysis (*12*). Because of this, treatment with Ca^{2+} has been used to maintain the texture of peppers undergoing preservation. A combination of low-temperature blanching and calcium addition, followed by blanching at a higher temperature for a short time as pretreatment has been found to be effective in both minimizing texture degradation of vegetables and modifying it in a controlled manner (*12–14*).

Although in some commercial processes calcium chloride ($CaCl_2$) has been added as an ingredient of brine, as well as during the pasteurization, no systematic study of the effect of stepwise blanching and calcium addition on the firmness and structural changes in pickled peppers has been reported. There is only one previous study on the addition of calcium salts to improve the texture of canned jalapeño peppers (*15*). Some studies report a connection between blanching pretreatment and microstructural changes. Fuchigami *et al.* (*16*) used light microscopy to evaluate the effect of low temperature blanching on carrots (2 h at 60 °C) compared to raw or cooked carrots. The results showed less cell damage in carrots subjected to low temperature blanching. Stanley *et al.* (*14*) studied the effects of holding time in stepwise blanching on the structure of canned green beans, and observed that 30 min of holding time at 65 °C resulted in a microstructure composed of cells joined by a well-defined middle lamella, while a shorter holding time resulted in degraded middle lamella with separated cells. Heredia-Léon *et al.* (*17*) used scanning electron microscopy (SEM) and observed that stepwise blanching of chili peppers at 65 °C for 60 min of holding time preserved the integrity of the cell wall with minimal collapse, resulting in greater firmness.

In order to evaluate the changes in texture, the effect of stepwise blanching with calcium chloride solutions on firmness retention, calcium absorption and structural tissue changes in jalapeño peppers in brine have been studied.

Materials and Methods

Materials

The raw materials used in this study were jalapeño peppers (*Capsicum annuum*) of the Mitla variety, purchased at a local market. The peppers were stored for 10 days at a temperature of 9–10 °C and relative humidity (RH) of 90 % until used.

Experimental method

The experimental method developed at the pilot plant is shown in Fig. 1. Batches of 1 kg of jalapeño pepper halves were subjected to the first blanching at 65 °C for 4 min with calcium chloride solutions at different concentrations (0, 0.075, 0.15 and 0.3 mol/L), in order to activate the PME and diffuse Ca^{2+} ions throughout the tissue. Immediately after blanching, the peppers were removed without cooling, placed in plastic bags and submerged in a water bath at a constant temperature of 65 °C for various holding times (0, 15, 30, and 45 min) to foster the action of PME and Ca^{2+} ion bonds. At the end of each holding time, a sample was taken to evaluate the concentration of calcium infused in the tissue. Then the samples were subjected to the second blanching in acidified water with 2 % citric acid at 96 °C for 3 min, to completely stop all enzyme activity and attain a balance between tissue and brine acidity. The samples were packed in brine with 2 % citric acid at 95 °C in glass jars with lids. They were then immediately subjected to thermal treatment by immersing the jars in water at 85 °C for 10 min. The control treatment included low-temperature blanching in distilled water without holding time but pasteurized and followed the same procedure as other samples. The jars were stored for 10 days at room temperature and the texture of the drained samples from each treatment was evaluated by determining maximum puncture force, tissue calcium content, damage or improvement in cell wall integrity and middle lamella through light microscopy and electron transmission microscopy. pH and titrable acidity were determined in the pepper tissue, reaching values of 2.8 and 1.5 %, respectively, in all the treatments.

Analytical determinations

pH was measured as per official AOAC method no. 981.12 (*18*) for acidified foods, using a Corning potentiometer model 425 (Corning Incorporated, Corning, NY, USA), and by performing 3 tests to get a mean value. Titratable acidity was determined as per official method no. 942.15 (*18*), reported as g of citric acid per kg of jalapeño peppers, with all determinations performed three times for each treatment. Ca^{2+} content in jalapeño peppers was determined for each of the samples in duplicate through atomic absorption spectrophotometry (model 3100, Perkin Elmer, Überlingen, Germany) after wet

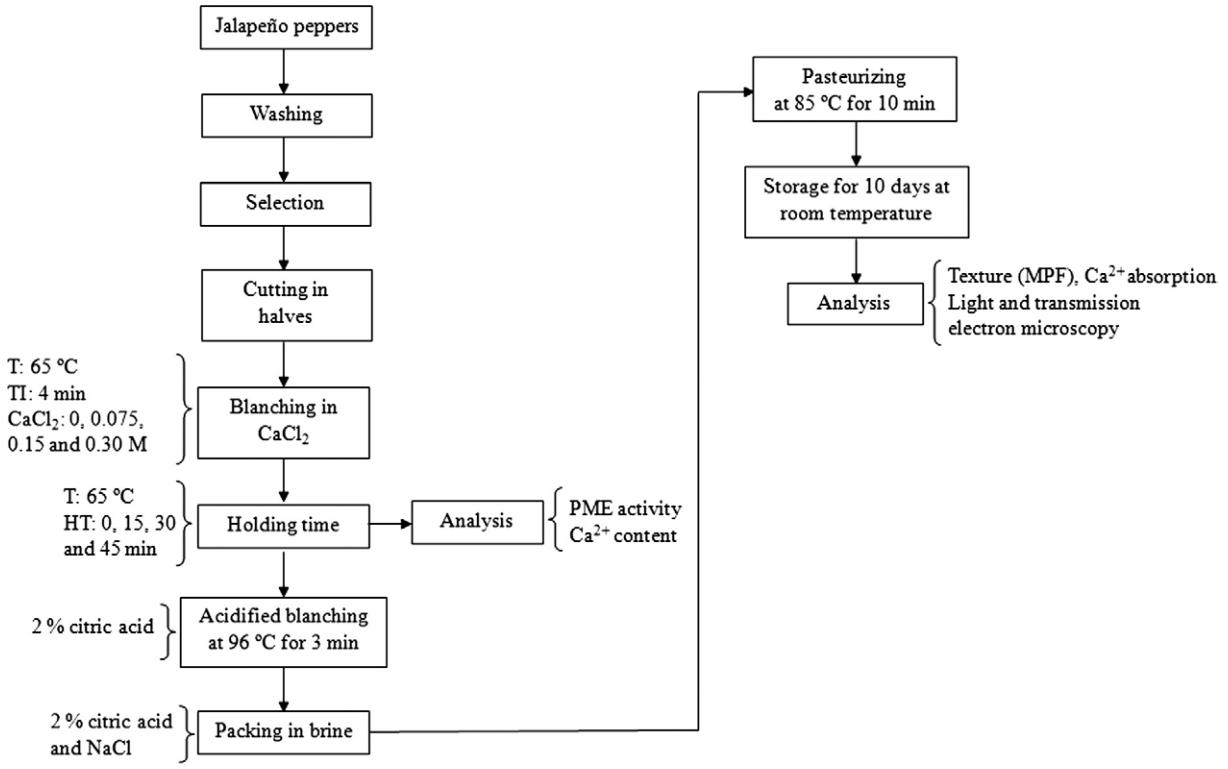

Fig. 1. Experimental procedure for stepwise blanching of jalapeño peppers

ashing with concentrated hydrochloric acid as described in method no. 956.00 (*18*). The reported values are an average of two repetitions and are expressed as g of Ca^{2+} per kg of fresh sample.

Texture measurement

The maximum puncture force (MPF) was evaluated using the simple puncture method in a Texture Analyzer (TAX-T2, Texture Technologies Corp., Scarsdale, NY, USA/ Stable Micro Systems, Haslemere, Surrey, UK) configured to measure puncture force. A load cell of 5 kg was installed in the Texture Analyzer. A cylindrical flat-end punch with 2.38 mm diameter was used with a crosshead speed of 5 mm/s and a travel distance of 12 mm. The maximum force was measured on each jalapeño pepper half by making 5 punctures throughout the placental tissue. Fifteen fresh and processed pepper halves were used for each treatment and an average value of maximum firmness was obtained (*6,19*).

Light and transmission electron microscopy analyses

Jalapeño pepper halves from the treatments that yielded the highest and lowest tissue firmness (control) and fresh tissue were prepared for light and transmission microscopy analyses. The samples were cut into cubes of 1-mm length and fixed in 2.5 % glutaraldehyde, dried with increasing ethanol concentrations, given a final acetone wash, and embedded in resin. Semi-fine cuts were obtained, which were analyzed with an Axioskop 2 plus light microscope (Jena, Germany). Fine cuts were obtained which were analyzed with a JEM-1010 JEOL transmission electron microscope (Akishima, Japan), operated at 60 keV.

Experimental design

A totally randomized design was used with a 4×4 treatment factor array and 2 repetitions per treatment for the study variables described above. Texture and tissue calcium concentration were analyzed by regression analysis. The statistical model that fitted with the experimental data was:

$$Y_i=b_0+b_1X_{1i}+b_2X_{2i}+b_{11}X^2_{1i}+b_{22}X^2_{2i}+b_{12}X_{1i}X_{2i} \qquad /1/$$

where Y_i is the response variable for the i-th experiment, X_{1i} is the holding time for the experiment i, X_{2i} is the concentration of calcium chloride solutions for the experiment i, and b_0, b_1, b_2, b_{11}, b_{22}, b_{12} are regression coefficients.

Statistical analysis

Data were analyzed using SAS v. 8.02 software (*20*), while MINITAB v. 13 (*21*) was used to draw Fig. 2.

Results and Discussion

The main physical and chemical properties of raw jalapeño peppers were determined. The following values were obtained: tissue calcium content of 0.94 g Ca^{2+} per kg of fresh peppers, pH=5.2, titratable acidity of 1.75 g of citric acid per kg of fresh peppers and tissue firmness of 6.3 N. These determinations were used as a reference during the study.

Model fitting

Table 1 shows the firmness and calcium content after low-temperature blanching and storage of jalapeño peppers in brine. The statistical analysis showing the

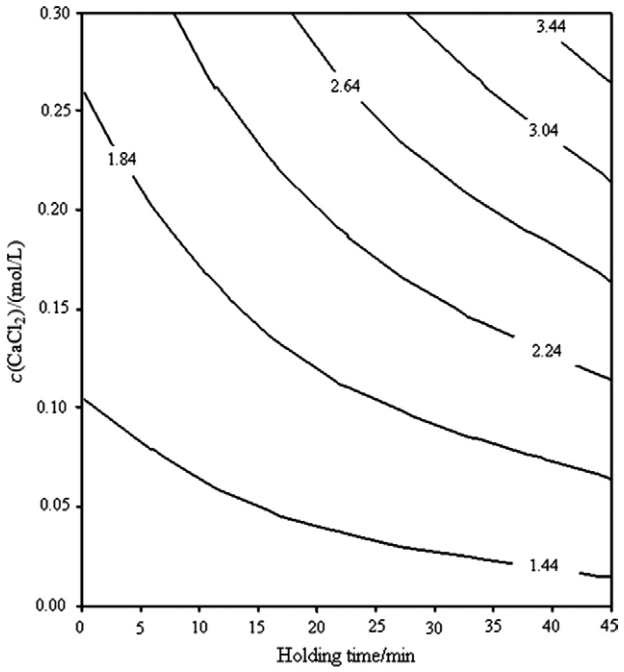

Fig. 2. Effect of holding time and $CaCl_2$ concentration on the texture of jalapeño peppers in brine, blanched at 65 °C. The plot shows firmness values expressed in Newtons

Table 1. Maximum puncture force (MPF) and Ca^{2+} absorption for low-temperature blanched, pasteurized jalapeño peppers in brine

$c(CaCl_2)$	Holding time	MPF[a]	$w(Ca^{2+}$ after blanching)[b]	$w(Ca^{2+}$ after storage)[c]
mol/L	min	N	g/kg	g/kg
0	0	0.9	0.10	0.041
0	15	1.2	0.11	0.103
0	30	1.3	0.08	0.058
0	45	1.5	0.12	0.072
0.075	0	1.6	0.25	0.088
0.075	15	1.7	0.35	0.092
0.075	30	1.9	0.33	0.170
0.075	45	1.7	0.34	0.080
0.15	0	1.7	0.45	0.192
0.15	15	1.6	0.37	0.204
0.15	30	2.4	0.38	0.127
0.15	45	2.3	0.38	0.143
0.3	0	1.6	0.62	0.306
0.3	15	2.9	0.69	0.273
0.3	30	3.2	0.67	0.297
0.3	45	3.7	0.66	0.313

[a]standard error for firmness is 0.05, [b]standard error for Ca^{2+} after blanching is 0.03, [c]standard error for Ca^{2+} after storage is 0.006

significance of each factor on each response variable is given in Table 2. The model developed for maximum puncture force (MPF) fitted adequately (Table 3). Calcium absorption gave a significant lack-of-fit ($p \leq 0.05$) for the full second order model (Eq. 1). A model involving only $CaCl_2$ (X_2) as a predictor fitted the data satisfactorily. The regression coefficients for two regression models are presented in Table 3. R^2 values of 0.90 and 0.95 were obtained with the second order models for texture and calcium absorption, respectively.

Table 2. Analysis of variance for second order models of puncture force and calcium absorption

Source of variation	Degree of freedom	Mean square	
		Puncture force	Calcium absorption
Model	5	3.2401*	0.24862*
Holding time (X_1)	1	0.2589*	0.00061
$CaCl_2$ (X_2)	1	0.2135*	0.14165*
X_1^2	1	0.2166	0.00027
X_2^2	1	0.0020	0.00898*
$X_1 \times X_2$	1	1.6151*	0.00003
Residual	26	0.0612	0.00237
Lack of fit	10	0.1451	0.00445*
Pure error	16	0.0087	0.00107
R^2		0.9105	0.95200

*significant at $p<0.05$

Table 3. Coefficients of the regression models, showing significant predictors for maximum puncture force and calcium absorption by tissue in low-temperature blanched and pasteurized jalapeño peppers in brine

Parameter	Maximum puncture force	Ca^{2+} absorption
	Estimated coefficient±S.E.	Estimated coefficient±S.E.
b_0	(1.2±0.1)*	(0.12±0.02)*
b_1	(0.003±0.004)*	
b_2	(2.6±0.7)*	(2.3±0.3)*
b_{11}		
b_{22}		(–1.7±0.8)*
b_{12}	(0.12±0.02)*	
R^2	0.90	0.95

*significant at $p<0.05$; S.E.=standard error

Absorption of Ca^{2+} ions into pepper tissue

The $CaCl_2$ concentration in the blanching solutions significantly affected the absorption of Ca^{2+} ions. Holding time was not an important factor in the calcium uptake. Fig. 3 shows a plot for predicted and observed values of calcium absorption. As $CaCl_2$ concentration increased, the Ca^{2+} absorbed by the tissue of jalapeño pepper increased linearly. The maximum mass fraction of Ca^{2+} per kg of pepper under these experimental conditions ranged from 0.682 to 0.686 g for samples immersed in 0.3 mol/L of $CaCl_2$ solution. This Ca^{2+} mass fraction was within the suggested limits for these types of products (22). The mass fraction of calcium absorbed under these conditions was 7.7 times the mass fraction of calcium in fresh jalapeño pepper tissue. A similar trend

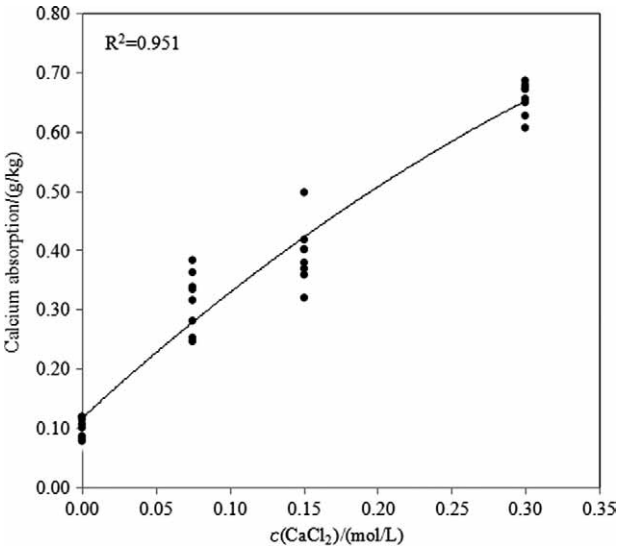

Fig. 3. Predicted and observed values of calcium absorption in the tissue of jalapeño peppers blanched at 65 °C at different $CaCl_2$ concentrations: (●) observed values, (–) predicted values

was observed during the treatment of tomato dice treated with calcium chloride (*23*). This was attributed to the alteration of the cellular wall permeability related to the diffusion of calcium into the tissue and also to the possible reaction of calcium ions with a carboxyl group of de-esterified pectins. Samples stored for 10 days showed that the content of Ca^{2+} ions was below 50 % (0.297 g of Ca^{2+} per kg of sample) with respect to the maximum values obtained after blanching (Table 1). This may be due to Ca^{2+} leaching while immersed in brine solution during storage.

Maximum puncture force

The change in tissue firmness of the jalapeño peppers during different treatments was determined through tissue puncture. Some typical puncture curves are shown in Fig. 4. The analysis of variance of the maximum puncture force shows that $CaCl_2$ concentration, holding time and the interaction of $CaCl_2$ concentration and the holding time had significant effects on tissue firmness ($p \leq 0.05$). Fig. 2 shows that as the $CaCl_2$ concentration and holding time increased, firmness of the peppers increased, reaching a value of 3.7 N, at a 0.3 mol/L concentration of $CaCl_2$, with a holding time of 45 min. The maximum firmness of jalapeño peppers was 4.3 times that of the control sample (0.9 N). The correlation coefficient for calcium absorption and tissue firmness was 0.80 ($p<0.05$), indicating that calcium uptake in tissue influences jalapeño pepper firmness. The calcium ion absorbed in the peppers reacts with pectates forming calcium pectates (*10*). This interaction was probably the main mechanism in the texture improvement. Research has shown that calcium ions have a high affinity for the free carboxyl groups present in the pectins, and can bind calcium ions in the cellular tissue, producing insoluble pectates that prevent cell separation and collapse of the cell structure. This results in an increase in tissue firmness (*8*,*10*,*11*).

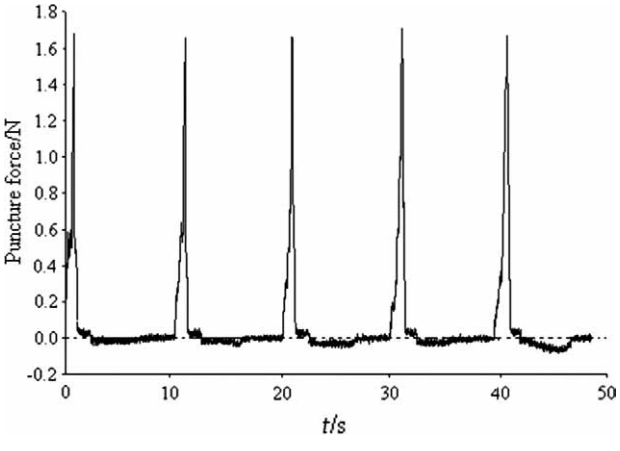

Fig. 4. Typical force *vs.* time curves for the puncture of placental tissue of jalapeño pepper halves in brine, blanched a 65 °C in 0.15 M $CaCl_2$ solution

Optical and transmission electron microscopy

Fresh tissue (firmness of 6.3 N) micrographs revealed that the cells are whole and with normal intracellular spaces (Figs. 5A–D), with an intact and clearly outlined plasma membrane, cell wall and middle lamella (Figs. 5E and F). Microstructural changes associated with the treatment that yielded the greatest firmness (3.7 N) were observed (Fig. 6) as clearly outlined intracellular spaces,

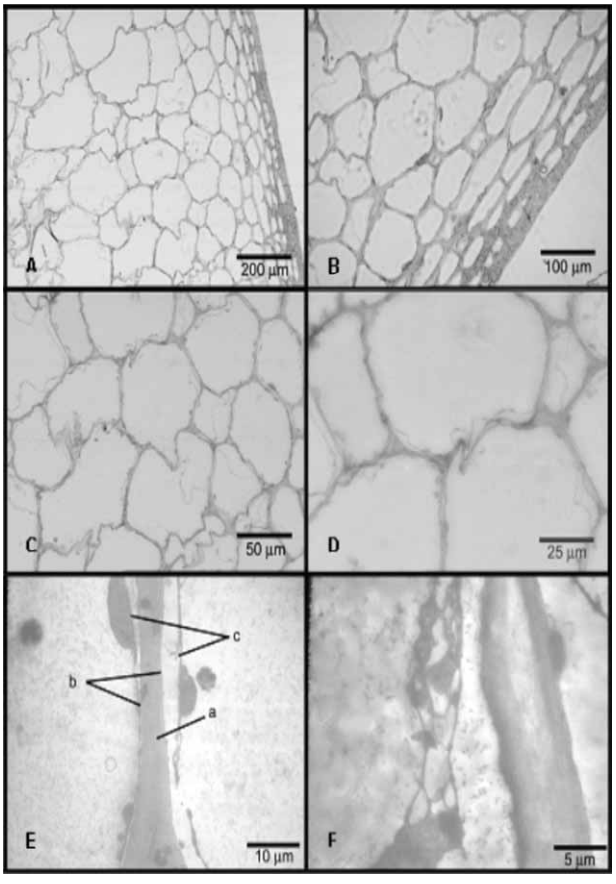

Fig. 5. Optical (A–D) and transmission electron (E and F) micrographs of fresh jalapeño pepper tissue, taken at the following magnifications: A=10×, B=20×, C=20×, D=40×, E=6000×, F=15 000×; a=middle lamella, b=cell wall, c=plasma membrane

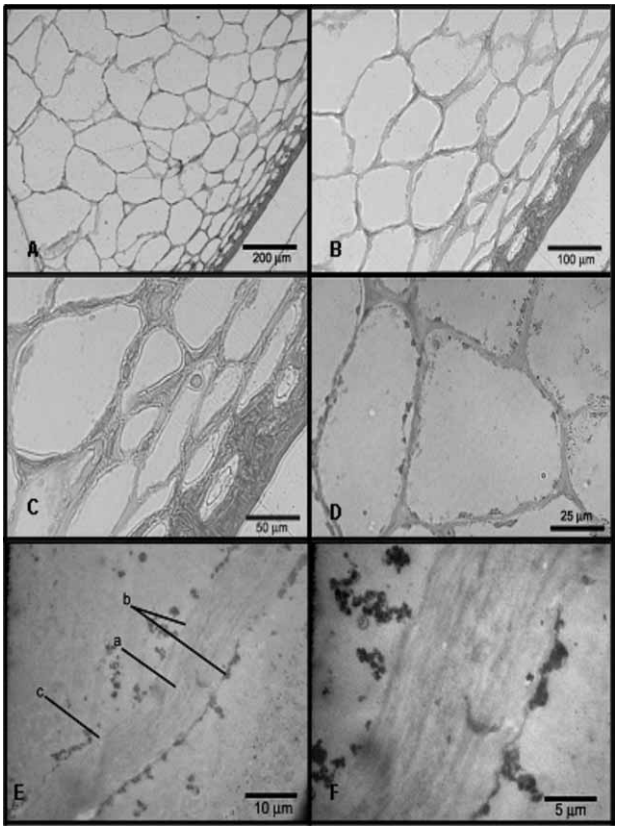

Fig. 6. Optical (A–D) and transmission electron (E and F) micrographs of the tissue from the treatment yielding the highest texture value (3.7 N), taken at the following magnifications: A=10×, B=20×, C=20×, D=40×, E=6000×, F=15 000×; a=middle lamella, b=cell wall, c=plasma membrane

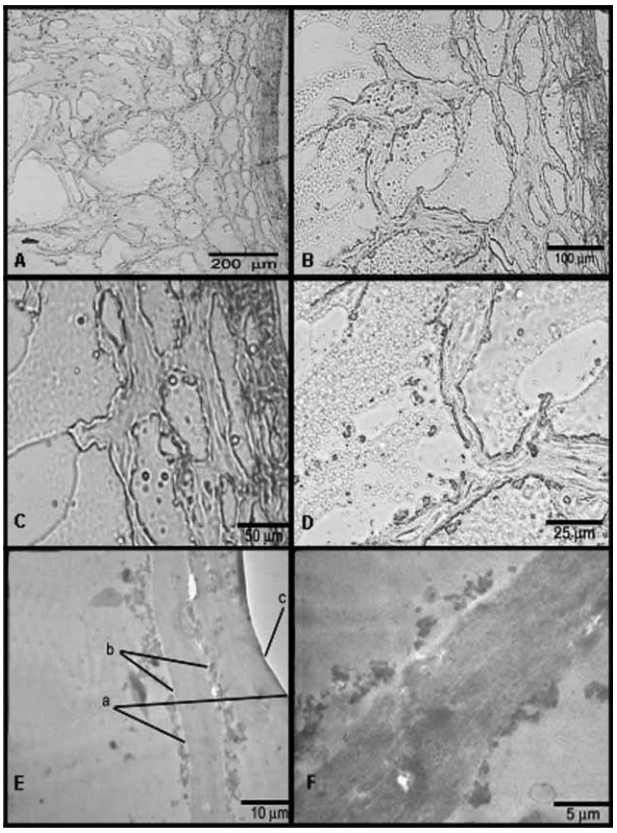

Fig. 7. Optical (A–C) and transmission electron (E and F) micrographs of the tissue from the treatment yielding the lowest texture value (0.86 N), taken at the following magnifications: A=10×, B=20×, C=20×, D=40×, E=6000×, F=15 000×; a=middle lamella, b=cell wall, c=plasma membrane

cell wall, middle lamella and plasma membrane, very similar to the fresh tissue sample, with slight structural changes possibly resulting from tissue damage related to processing. Increased firmness can be attributed to the action of PME, which favours the formation of insoluble pectates in the cell tissue (*8*,*14*,*16*,*17*).

For the treatment where the samples were blanched in water with no holding time, whose maximum firmness was 0.9 N, micrographs revealed cellular disorganization with the loss of intracellular spaces, cellular separation, and cellular structure collapse (Fig. 7), with the resulting damage to the cell wall and middle lamella. This caused detachment of the plasma membrane and a pronounced broadening of the middle lamella due to the thermal effect and acid hydrolysis of the cell wall. The result was cell dehydration, and ultimately a pronounced separation between cell spaces (*24*).

Conclusions

The model adequately described the variation of puncture force in jalapeño pepper tissue. Blanching of jalapeño pepper halves at a temperature of 65 °C for 4 min in a 0.3 mol/L $CaCl_2$ solution with a holding time of 15 to 45 min significantly maintained tissue firmness (3.7 N), attaining 4.3-fold firmness increase when compared to the control sample (0.9 N). The calcium ions absorbed by the tissue (0.686 g of Ca^{2+} per kg of sample) under experimental conditions were 7.7 times of the initial mass fraction. Microscopy showed that the treatments including low-temperature blanching with $CaCl_2$ solutions and holding times resulted in less damage to the cell wall and middle lamella than the control treatment.

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