Effect of Calcium Pretreatments on the Texture of Frozen Cherries. Role of Pectinesterase in the Changes in the Pectic Materials

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Pretreatments by immersion in 10 and 100 mM CaCl$_2$ prevent the loss in firmness of sweet frozen cherries. This effect can even be detected in fruits preserved for 6 months in a frozen state. The increase in firmness, through the treatments, is mostly apparent after freezing and is attributed to the formation of calcium bridges in the pectic material, caused by a decrease in the degree of esterification of pectins and an increase in the pectic fraction soluble in EDTA and the Ca^{2+} cation content in the cell wall. These changes in the pectic materials are of an enzymatic origin, since we observed an increase in the pectinesterase activity of the fruits after treatments with increasing calcium concentrations.

INTRODUCTION

Cell lysis due to the formation of ice crystals during freezing results, among other effects, in an irreversible loss of turgor in vegetable structures during thawing (Reid, 1993). In fruits with a delicate texture such as cherries, this manifests itself in softness of the fruit and changes in their texture.

Many fruit and vegetable transformation processes involve treatments with calcium to preserve their firmness (LaBelle, 1971; Lin Tang and McFeeters, 1983; Howard and Buescher, 1990; Floros *et al.*, 1992). Calcium in plants is normally found in the cell wall-forming calcium bridges between residues of galacturonic acid belonging to adjacent pectic chains. The calcium–pectin complex formed acts as an intracellular cement to give firmness to vegetable tissues. The presence of calcium, in addition to favoring the insolubility of the pectic material, inhibits its degradation by polygalacturonase (Burns and Pressey, 1987). During the normal fruit maturation process, the calcium cations are translocated to the growing zones in the plant (Marchiner, 1986). This has been linked to the solubilization and degradation of the pectic material of the middle lamella, causing the softening of the fruits (Lidster *et al.*, 1978; Fils-Lycaon and Buret, 1990).

The pectic material incorporated *de novo* in the cell wall is highly methylated (Moustacas *et al.*, 1991), and its de-esterification, necessary for the formation of the calcium bridges, is catalyzed by pectinesterase. The action of pectinesterase requires certain concentrations of metallic ions, which together with the electrostatic potential of the cell wall appear to regulate its activity. The optimum concentrations of calcium for different pectinesterases of vegetable origin are between 5 and 25 mM, higher calcium concentrations having an inhibitory effect (Rexova-Benkova and Markovic, 1976).

For cherries, postharvest treatments with calcium have been carried out to attenuate the damage produced during mechanical harvesting and manipulation or to increase their storage times under refrigeration (Lidster *et al.*, 1978, 1979a,b; Facteau, 1982; Facteau *et al.*, 1987). The levels of calcium in cherries have been related with their firmness (Lidster *et al.*, 1979a; Facteau *et al.*, 1987), with the level of soluble pectic material, and with the formation of calcium pectate (van Buren, 1967; Glenn and Poovaiah, 1989).

The aim of this work is to determine the effect of the pretreatment with calcium on the texture of frozen cherries and to analyze the pectinesterase activity and the modifications in pectic material as a result of treatments with calcium, freezing, and their storage in a frozen state.

MATERIALS AND METHODS

Preparation of the Product and Freezing. Cherry fruits (*Prunus avium* L. cv. Pico Colorado) were collected at an optimum stage of ripeness and development (M.A.P.A., 1987), in the Jerte Valley (Spain), where they were selected as commercial grade I. In order to survey the homogeneity of the population used, cherries were selected, sized ($22 < \phi < 25$ mm) and measured for °Brix, color (HunterLab), and weight. Coefficients of variation under 10% were attained and confidence intervals established for the samples used. After selection, the samples were divided into 10 groups: nine for calcium treatment (1, 10, and 100 mM for 1, 5, and 10 min) and one as a control. Eight kilos of cherries were used for each treatment. Treatments were carried out by immersing cherries in solutions 1:10 w/v containing 0.5% citric acid and 0.03% ascorbic acid pH 3, as color protectants, and the different CaCl$_2$ concentrations were tested. No other protectants (such as sugars and pectins) were used to avoid possible interferences in the determination of uronic acids and the degree of esterification in the pectic fractions. Samples were air-dried, and freezing was achieved by forced convection of liquid nitrogen vapor in a time of 33 min at a freezing rate of 49.3 °C/h. The initial temperature was 3.1 °C and a temperature of −70 °C was maintained in the medium until the thermal center of the fruits reached −24 °C. Once frozen, cherries were packed in stratified polyethylene bags (800 g/bag) and sealed with a low vacuum of −0.5 bar to prevent oxidation and damage from surface dehydration. Samples of cherry fruits were taken before freezing and at 0, 3, and 6 month's storage at −24 °C. Fruits were slowly thawed in the sealed bags, at 5 °C for 14 h, then tempered up to 22 °C and subjected to the various assays.

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Measurement of Texture. For the objective assessment of texture, empirical mechanical tests of penetration and shear were performed using an Instron Food Testing Instrument mod. 4501. Penetration tests were performed on 20 cherries using a flat, 3.2 mm diameter cylinder which penetrated the fruit at its equator perpendicular to the suture, at a deformation velocity of 400 mm/min. The parameter provided by the force-deformation curves was the slope of the curve in the linear zone prior to rupture point (N/mm); this parameter reflects the variations occurring in the turgidity of the fruit as a result of freezing (Alonso *et al.,* 1994). The shear test was performed on 20 half cherries, stoned and split at the suture scar, by means of a cell consisting of two smooth plates with concentric perforations (diameter 9.525 mm) (Canet, 1980). The fruit was cut by shearing with a flat cylinder of 9.450 mm diameter at a deformation velocity of 400 mm/min. The parameter provided by the force-deformation curves were the maximum shearing force (N), and this is an expressive parameter with respect to the firmness of the product (Alonso *et al.,* 1994).

Extraction of Pectinesterase. A slightly modified version of the extraction method proposed by Baldwin and Pressey (1988) was used, obtaining soluble and cell wall-bound enzyme extracts as described previously (Alonso *et al.,* 1993). Briefly, 40 g of sample was homogenized with 40 mL of 0.1 M NaCl containing 10 mM DTT and 1% (w/v) Polyclar AT. The homogenate was then adjusted to pH 3 with 1 N HCl and stirred for 30 min. The extracted was then centrifuged for 30 min at 9000g, the supernatant was collected (soluble enzyme extract), and the precipitate was resuspended in 50 mL of 1 M NaCl. The pH was adjusted to 6 with 1 N NaOH, and the suspension was stirred for 60 min before centrifuging at 9000g for 30 min. The supernatant was collected (cell wall-bound enzyme extract) and the precipitate discarded.

Assay of Pectinesterase. The method used was a titration assay with 0.01 N NaOH pH 7 static at 25 °C using a reactive mixture containing 6 mL of enzyme extract (soluble or cell wall-bound) and 6 mL of 0.4% apple pectin (70–75% esterified, Fluka) in 0.1 M NaCl for 5 min of reaction. Controls were performed with heat-denatured enzyme. The unit of activity was defined as microequivalent of carboxyl groups released per minute. The values shown are averages of three repetitions.

Protein concentration was determined by using a modified Bradford (1976) assay (Sedmak and Gossberg, 1977).

Extraction of Alcohol Insoluble Solids (AIS). Fractionation and Determination of the Degree of Esterification of the Pectic Material. For this purpose the method previously described by Facteau (1982) was used. The alcohol insoluble solids (AIS) were obtained from 80 g of destoned cherries per sample. To obtain the different pectin fractions, 100 mg of AIS were used. Samples were shaken in 100 mL of distilled water for 12 h and centrifuged at 3.500g for 5 min. Water soluble pectins were contained in the supernatant, and the sedimented material was resuspended in 100 mL of EDTA (50 mM) and shaken for 12 h. After another centrifugation, the EDTA soluble pectins were separated, and the precipitated materials were resuspended in 100 mL of fungal pectinase, 0.05% at pH 5, shaking it for 1 h at room temperature. The supernatant resulting from the centrifugation of the digest containing pectins solubilized by pectinase.

The process was carried out in duplicate, and each fraction was assayed three times for its content in uronic acids using the method previously described by Scott (1979). The whole process was carried out at 0 °C to avoid carbonization of the samples.

The degree of esterification was measured by assaying for the methanol liberated from the different fractions by basic de-esterification. Methanol was assayed by gas chromatography, following the method described by McFeeters and Armstrong (1984), using a stainless steel Carbowax (15%) 1500 (4 m × 1/8 in.) column over a Chrom W, 80/100 mesh, using 1-propanol as an internal standard. The degree of esterification of the pectic material was expressed as (moles of methanol/ moles of galacturonic acid) × 100%.
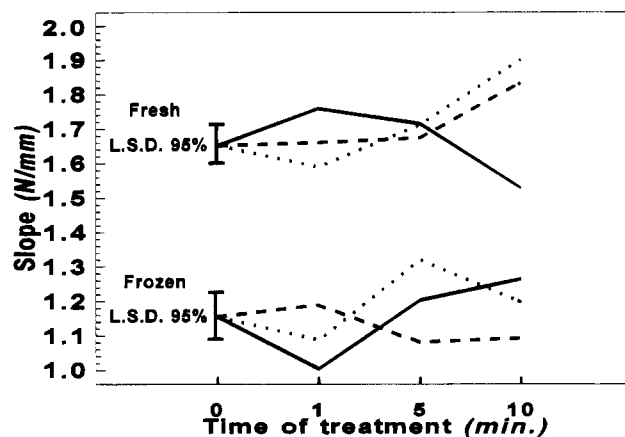
Figure 1. Effect of calcium pretreatments (1 mM (···), 10 mM (- - -), and 100 mM (—)) on the turgidity of fresh and frozen cherries. Turgidity was measured as the slope (N/mm) of the force-deformation curve in the mechanical penetration assay.

Analysis of Calcium and Magnesium in the Cell Wall. The determination of calcium and magnesium in the AIS was carried out by atomic absorption. The AIS dry samples (5 to 10 mg) were ashed at 500 °C for 17 h, dissolved in 0.5 mL of HNO$_3$ and 0.25 mL of LaCl$_3$ (5%), brought to 25 mL with 1 N HNO$_3$, and analyzed for calcium and magnesium using a multielement lamp (Intersitron, Perkin-Elmer) in a Perkin-Elmer 5,100 PC atomic absorption spectrophotometer. An air–acetylene flame was used for the detection of both elements, measuring at 422.7 nm for calcium and at 285.2 nm for magnesium.

Statistical Analysis. Results were analyzed by means of a one-way and multifactor analysis of variance, using the LSD test with a 95% confidence interval for the comparison of the test means. Also, the possible relationships between variables were studied by means of a correlation analysis.

RESULTS AND DISCUSSION

Texture. In fresh fruits treated with low and medium concentrations of CaCl$_2$ (1 and 10 mM), an increase in turgidity, determined from the slope of the force-deformation curve in the penetration assay, was observed as the duration of the treatments increased. Treatment with 100 mM CaCl$_2$, though, resulted in the opposite, turgidity of the cherries decreasing with longer treatment times (Figure 1). The increase in turgidity in cherries treated with low and medium concentrations of CaCl$_2$(1 and 10 mM) could be due to the protective role of calcium on membrane integrity and the restoration of the membrane's selective permeability lost during the maturation process (Marchiner, 1986). In cherries treated with high CaCl$_2$ concentrations (100 mM), the iso- or hypertonic conditions will lead to a loss in turgidity, greater as the immersion treatment times are increased and therefore with the concentration of the cation in the fruit.

Firmness of the fresh fruits, determined from the maximum force required in the shear assay, increased with higher CaCl$_2$ concentration and the inmersion times of the treatments, but only samples treated with 100 mM CaCl$_2$ for 10 min showed firmness values significantly higher to those obtained by the control (Figure 2).

The greatest changes in texture were observed after freezing. In all the treatments, a decrease in turgidity was observed; this was more evident in those fruits which were more turgid when fresh (Figure 1). For frozen cherries pretreated with calcium, the decrease in firmness was inversely proportional to the concentra-

Texture of Frozen Cherries

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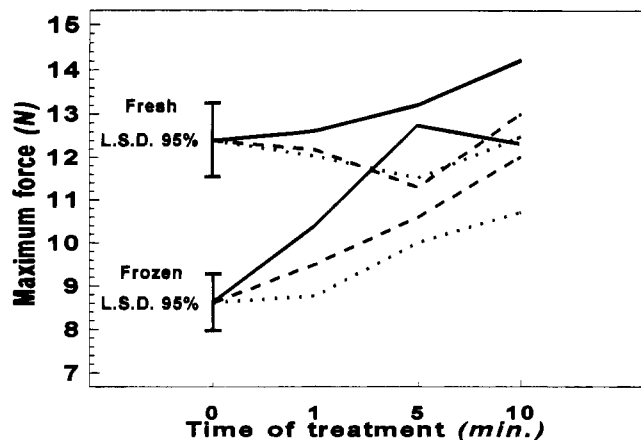
Figure 2. Effect of calcium pretreatments (1 mM (· · ·), 10 mM (- - -), and 100 mM (—)) on the firmness of fresh and frozen cherries. Firmness was measured as the maximum force (N) of the force-deformation curve in the mechanical shear assay.

tion and the immersion time of the treatments, achieving with the longest immersion times firmness values similar to those of the fresh fruits (Figure 2). The freezing results in an irreversible loss of turgor in all treatments during thawing, but the treatments with calcium prevent the softness of the fruit produced by their freezing and thawing (Figure 2) and may be of great importance from the technological point of view.

During frozen storage, turgidity remains within the same values as those obtained after freezing (data not shown). With respect to firmness, cherries treated with 100 mM CaCl$_2$ exhibit a similar firmness to those obtained immediately after freezing, while cherries treated with 1 mM CaCl$_2$ increased in firmness during frozen storage (Figure 3).

Pectic Material. Regarding the total amount of pectins in the cell wall, the total levels of uronic acids obtained were approximately of 300 μg/mg of AIS. These are similar to those obtained by van Buren (1967) in a study of cherries preserved in brine. The concentration of uronic acids in the water-soluble pectic fraction, though, was lower than 15 μg/mg (ca. 6% of the pectic material), lower than the sensitivity of the assay for uronic acids used, possibly as a result of their being in the initial stages of maturation (Fils-Lycaon and Buret, 1990). In fresh fruits, the highest modifications in the pectic materials occurred in the EDTA-soluble fraction (Figure 4A, B), with changes in their concentration and degree of esterification. Concentrations of these uronic acids were found to increase with increased concentrations of CaCl$_2$ and immersion time during treatments (Figure 4A), and a decrease in their degree of esterification was observed with increased treatment times at the different CaCl$_2$ concentrations used (Figure 4B). For fresh cherries, the concentration of uronic acid solubilized by pectinase showed no trend, while for frozen cherries there was a decrease in those treated with 100 mM CaCl$_2$ (Table 1).

Significant correlations (+0.77, $p \leq 0.05$) were found between the concentration of the EDTA-soluble pectic material in the cell wall and the firmness of the fruits. Their increase, as the calcium concentration and the treatment time increase, must originate from the formation of new calcium bridges in the initial pectic material, increasing the intercellular adhesion and the firmness of the tissues.

In frozen cherries, a marked increase in the EDTA-soluble pectic fraction was observed in samples treated

for 5 and 10 min with the three CaCl$_2$ concentrations (Figure 5A). Significant correlation (+0.88, $p \leq 0.05$) were found between these results and the firmness of the frozen fruits.

Calcium and Magnesium in the AIS. The content of divalent cations (Ca^{2+} and Mg^{2+}) in the AIS is shown in Table 2. In fresh cherries, the AIS calcium content did not increase as the concentration of CaCl$_2$ and the immersion times during treatment were increased, although most of the treated cherries showed higher calcium concentrations than the control. After freezing, the levels of calcium in the AIS wall were directly proportional to the concentration of calcium used in the treatments and statistically independent from the immersion times (Figure 6). After being kept frozen for 3 months, samples pretreated with 1 mM CaCl$_2$ showed calcium levels similar to those of samples treated with higher concentrations. This is probably a consequence of the increase in size of ice crystals during preservation in the frozen state. This would result in damage to cell membranes and reduced cellular compartmentalization, thus allowing for a better distribution of calcium in the fruits and an increase in firmness (Figure 3).

The magnesium content in the AIS was lower than that of calcium, and was found, as with calcium, to increase as a result of freezing and during preservation in the frozen state (Table 2).

PE Activity. In fresh fruits, the levels of soluble and cell wall-bound pectinesterase activity were similar for those cherries treated with calcium and the controls (Table 3). Despite this, the changes that occurred in the pectic material were probably of enzymatic origin, with a reduction in the degree of esterification of the EDTA-soluble pectic materials by approximately 50% in those samples treated for 10 min (Figure 4B). Thus it appears that the increase in the speed at which de-esterification occurs *in vivo*, in response to the treatments with calcium, was not preserved in the enzymatic extracts evaluated *in vitro*.

After freezing, an increase in the pectinesterase activity, as a result of the treatments with calcium, was observed, achieving increases of activity over 100% in cherries pretreated with CaCl$_2$ 100 mM (Table 3). This increase of the pectinesterase activity by freezing is probably due to the increase in the concentration of cations in the cell wall (Figure 6) as a consequence of the loss of membrane integrity due to the formation of ice crystals and a better distribution of calcium within the fruit. The formation of small cracks in the cuticula would favor the penetration of the cation from the epidermis, where it normally accumulates as a result of the immersion treatments (Glenn and Poovaiah, 1989).

The mechanism by which pectinesterase is activated by calcium has not yet been satisfactorily explained (Rexova and Markovic, 1976). It has been proposed that calcium binds to free carboxyl groups, reversing the inhibition of the enzyme produced by the presence of free carboxyl groups in pectin (Moustacas *et al.*, 1991; Nari *et al.*, 1991). However there is evidence against this mechanism (Marcus and Schejter, 1983). As the activation by calcium is maintained in the enzymatic extract, our results suggest that activation by calcium could be due to binding of the ion to the enzyme itself rather than a direct effect on the reaction product. Maintenance of the active state in the crude extract could also be attributed to the presence of calcium in the extract, but the direct relationship of the enzyme

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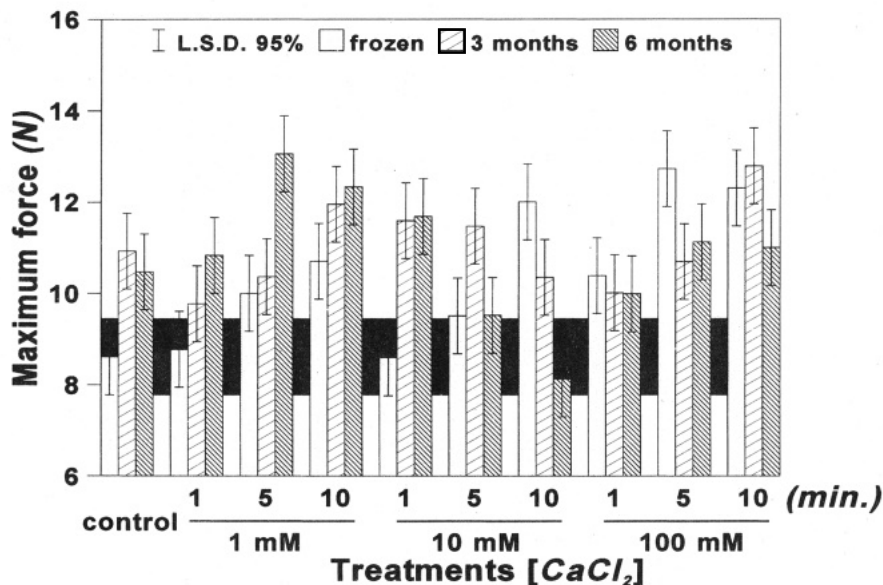
Figure 3. Effect of freezing and their preservation in a frozen state (3 and 6 months) on the firmness of cherries treated with different calcium concentrations. Firmness was measured as the maximum force (N) of the force-deformation curve in the mechanical shear assay. The shaded area shows LSD 95% range of the frozen control.
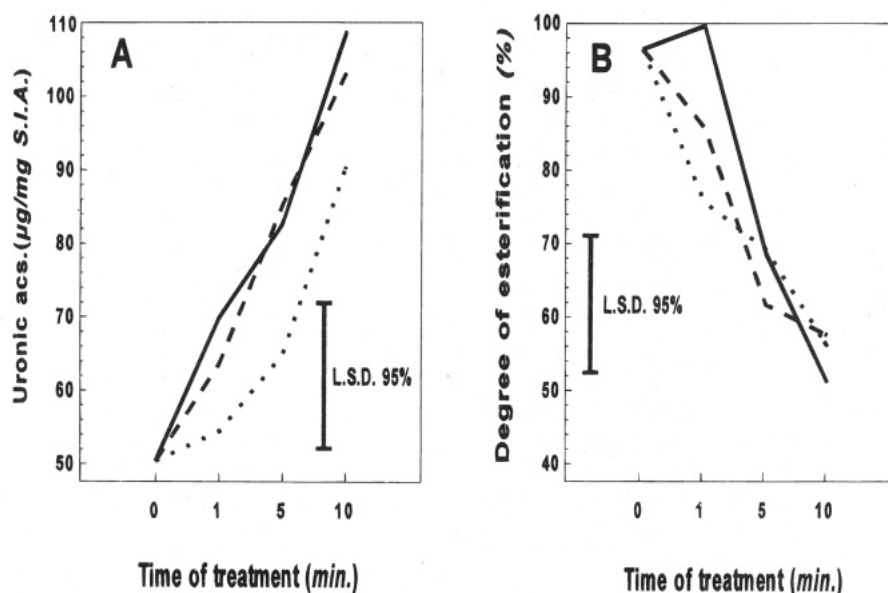
Figure 4. Effect of calcium treatments on the EDTA-soluble pectin fraction of fresh cherries. Uronic acids (A) and degree of esterification (B).
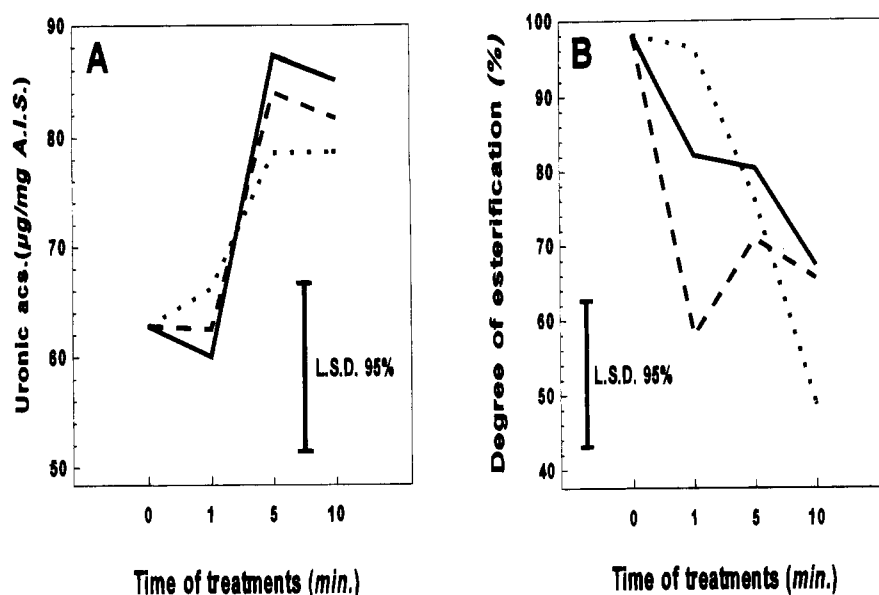
Table 1. Effect of Calcium on the Pectin Fraction Solubilized by Pectinase in Fresh and Frozen Cherries, Uronic Acids, and Degree of Esterification

treatment [CaCl$_2$], time	fresh cherries		frozen cherries	
	uronic acids (μg/mg AIS)	degree of esterification (%)	uronic acids (μg/mg AIS)	degree of esterification (%)
control	194.89^{a,b}	42.45	199.72^c	61.94^{b,c}
1 mM, 1 min	283.36^c	28.60^{a,b}	181.34^{b,c}	61.49^{b,c}
1 mM, 5 min	265.28^{b,c}	34.37^{a,b}	201.70^c	43.70^{a,b}
1 mM, 10 min	210.72^{a-c}	37.35^{a,b}	190.31^{b,c}	29.61^a
10 mM, 1 min	233.11^{a-c}	46.67^{a,b}	192.64^c	52.21^{b,c}
10 mM, 5 min	238.05^{a-c}	47.90^b	191.23^{b,c}	41.64^{a,b}
10 mM, 10 min	161.53^a	33.86^{a,b}	153.31^{a-c}	27.89^a
100 mM, 1 min	246.88^{b,c}	30.55^{a,b}	199.10^c	72.69^c
100 mM, 5 min	223.09^{a-c}	37.86^{a,b}	137.39^{a,b}	61.68^{b,c}
100 mM, 10 min	230.95^{a-c}	26.86^a	118.46^a	55.19^{b,c}
LSD, 95%	79.20	19.85	55.01	20.78

$^{a-c}$ Different letters in the same column indicate significant differences ($p \leq 0.05$).

activity with the calcium concentration and the treatment times indicate that this is unlikely. Calcium, like other metallic ions, can bind with enzymes to cause

changes to a more active conformation (Mildran, 1970). The calcium concentrations that produce an activation of the enzyme are high (Table 2), much higher than the

Figure 5. Effect of calcium treatments on the EDTA-soluble pectin fraction of frozen cherries. Uronic acids (A) and degree of esterification (B).

Table 2. Calcium and Magnesium Content in the Cell Wall (μg/mg AIS) of Cherries Treated with CaCl$_2$, Frozen and Preserved for a Period of 3 Months[a]

| treatment [CaCl$_2$], time | calcium and magnesium content (μg/mg AIS) | | | | | |
| | fresh cherries | | frozen cherries | | frozen storage (3 months) | |
	Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}	Ca2+	Mg^{2+}
control	4.17 ± 0.04	1.16 ± 0.04	3.84 ± 0.78	1.18 ± 0.24	3.98 ± 0.19	1.63 ± 0.12
1 mM, 1 min	5.92 ± 0.37	1.92 ± 0.19	3.74 ± 0.37	0.97 ± 0.04	6.01 ± 0.74	2.22 ± 0.12
1 mM, 5 min	5.69 ± 0.14	2.68 ± 0.53	3.50 ± 0.16	1.04 ± 0.01	7.43 ± 0.14	3.38 ± 0.06
1 mM, 10 min	3.15 ± 0.21	0.97 ± 0.04	4.22 ± 0.50	2.72 ± 1.32	6.55 ± 0.43	2.66 ± 0.23
10 mM, 1 min	3.07 ± 0.02	0.84 ± 0.01	5.82 ± 0.31	2.20 ± 0.06	4.40 ± 0.09	1.73 ± 0.08
10 mM, 5 min	6.35 ± 0.82	2.82 ± 0.36	4.37 ± 0.19	1.67 ± 0.04	3.81 ± 0.59	1.34 ± 0.10
10 mM, 10 min	6.28 ± 0.25	2.80 ± 0.15	4.66 ± 0.27	1.49 ± 0.00	5.71 ± 0.08	2.77 ± 0.29
100 mM, 1 min	6.16 ± 0.32	2.48 ± 0.42	7.07 ± 0.79	3.45 ± 0.52	7.08 ± 0.83	3.44 ± 0.27
100 mM, 5 min	5.49 ± 0.69	1.52 ± 0.03	6.53 ± 0.56	2.29 ± 0.23	5.44 ± 0.50	1.97 ± 0.22
100 mM, 10 min	4.02 ± 0.08	0.89 ± 0.03	6.72 ± 0.01	3.28 ± 0.11	5.78 ± 0.99	2.22 ± 0.14

[a] Average ± standard deviation.

**Table 3. Effect of CaCl$_2$, Freezing, and Frozen Storage on the Soluble and Cell Wall-Bound Pectinesterase**

| treatment [CaCl$_2$], time | pectinesterase activities (units/mg of protein), soluble/bound to cell wall | | | |
	fresh cherries	frozen cherries	3 months	6 months
control	34.13/46.66	32.18/26.62	23.17/20.47	23.48/32.41
1 mM, 1 min	38.47/21.56	22.77/29.64	19.89/15.05	22.73/11.71
1 mM, 5 min	29.38/40.16	24.70/19.71	13.69/15.23	18.66/18.32
1 mM, 10 min	27.69/39.61	55.62/30.40	17.54/19.39	16.74/25.27
10 mM, 1 min	26.93/41.27	39.04/23.96	9.04/16.95	7.91/17.30
10 mM, 5 min	35.84/26.99	43.29/32.46	14.25/26.49	11.50/16.77
10 mM, 10 min	20.44/40.53	72.43/45.78	20.82/17.81	19.25/11.79
100 mM, 1 min	37.61/59.98	33.95/48.97	17.35/28.82	14.24/14.22
100 mM, 5 min	20.57/30.79	73.95/60.44	16.83/18.94	15.35/28.05
100 mM, 10 min	29.13/28.24	61.71/61.01	18.07/13.39	17.15/21.70

optimum concentrations found for pectinesterases (Rexova and Markovic, 1976), but it must be considered that due to the poor penetration of calcium in the fruit from an external calcium solution (Glenn and Poovaiah, 1989), the concentration of calcium inside the fruit must be much lower than that applied.

The increase in pectinesterase activity, observed after freezing, decreases during frozen storage for 3 and 6 months (Table 3), obtaining for the different treatments pectinesterase activity levels oscillating between 25 and 50% of the fresh controls. This decrease in pectinesterase activity could be due to inactivation of the enzyme produced by changes in the ionic environment of the cell wall due to the increase in size of ice crystals during frozen storage (Reid, 1993).

As described previously, the effect of calcium on the firmness of cherries is manifested mostly after freezing and is accompanied by a parallel increase in the activity of pectinesterase.

The increase in pectinesterase activity would lead to a reduction in the degree of esterification of pectins, favoring the formation of new calcium bridges, as shown by the increase in the EDTA-soluble pectic fraction and the level of Ca^{2+} in the cell wall. Thus, after freezing, a significant correlation was found between the level of soluble and cell wall-bound pectinesterase and the concentration of the EDTA-soluble pectic fraction (+0.92 and +0.85, $p \leq 0.05$) and also with the concentration of divalent cations in the cell wall (+0.69 and +0.85, $p \leq 0.05$). The increase in calcium bridges will cause an

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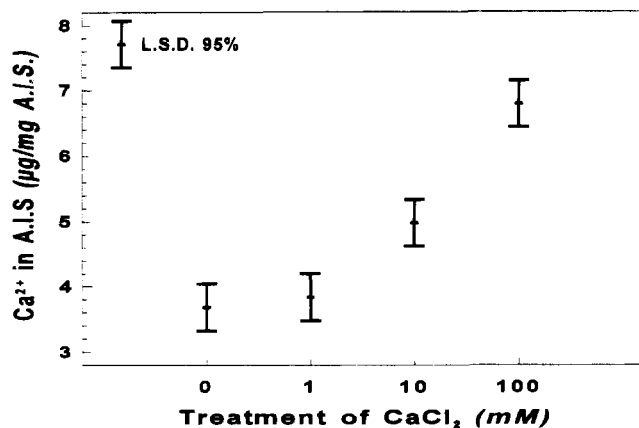
Figure 6. Effect of the CaCl₂ treatments on the calcium content of the cell wall in frozen cherries.

increase in intercellular adhesiveness and strengthening of the tissues which can prevent the loss of firmness in the fruits caused by freezing. In this sense it is interesting to notice the strong correlation ($p \leq 0.05$) between the firmness of the frozen fruit with the soluble ($+0.92$) and cell wall bound ($+0.85$) pectinesterase activity, indicating that the enzymatic origin of the changes occurred in the pectic material, which would be responsible for the increase in firmness of cherries treated with calcium.

LITERATURE CITED

Alonso, J.; Canet, W.; Rodríguez, M. T. Effect of Various Thermal Pre-treatment on the Texture of Frozen Cherries (*Prunus avium* L.). Related Enzyme Activities. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 214–218.

Alonso, J.; Canet, W.; Rodríguez, M. T. Mechanical Assessment of Texture of Sweet Cherries. Effects of Freezing. *J. Sci. Food Agric.* **1994**, *66*, 1–7.

Baldwin, E. A.; Pressey, R. Tomato Polygalacturonase Elicits Ethylene. Production in Tomato Fruit. *J. Am. Soc. Hortic. Sci.* **1988**, *113* (1), 92–95.

Bradford, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254.

Burns, J. K.; Pressey, R. Ca²⁺ in Cell Walls of Ripening Tomato and Peach. *J. Am. Soc. Hortic. Sci.* **1987**, *112(5)*, 783–787.

Canet, W. Estudio de la Influencia de los Tratamientos Térmicos de Escaldado, Congelación y Descongelación en la Textura y Estructura de Patata (*Solanum tuberosum* L.). Ph.D. Dissertation, Universidad Politécnica de Madrid, 1980.

Facteau, T. J. Relationship of Soluble Solids, Alcohol-Insoluble Solids, Fruit Calcium, and Pectin Levels to Firmness and Surface Pitting in Lambert and Bing Sweet Cherry Fruit". *J. Am. Soc. Hortic. Sci.* **1982**, *107* (1), 151–154.

Facteau, T. J.; Rowe, K. E.; Chestnut, N. E. Response of 'Bing' and 'Lambert' Sweet Cherry Fruit to Preharvest Calcium Chloride Applications. *Hortscience* **1987**, *22* (2), 271–273.

Fils-Lycaon, B.; Buret, M. Loss of Firmness and Changes in Pectic Fractions During Ripening and Overripening of Sweet Cherry. *Hortscience* **1990**, *25* (7), 777–778.

Floros, J. D.; Ekanayake, A.; Abide, G. P.; Nelson P. E. Optimization of a Diced Tomato Calcification Process. *J. Food Sci.* **1992**, *57* (5), 1.144–1.148.

Glenn, G. M.; Poovaiah, B. W. Cuticular Properties and Postharvest Calcium Applications Influence Cracking of Sweet Cherries. *J. Am. Soc. Hortic. Sci.* **1989**, *114* (5), 781–788.

Howard, L. R.; Buescher, R. W. Cell Wall Characteristics and Firmness of Fresh Pack Cucumber Pickles Affected by Pasteurization and Calcium Chloride. *J. Food Biochem.* **1990**, *14*, 31–43.

Labelle, R. L. Heat and Calcium Treatments for Firming Red Tart Cherries in Hot-Fill Process. *J. Food Sci.* **1971**, *36* (2), 323–326.

Lidster, P. D.; Porritt, S. W.; Tung; M. A. Texture Modification of 'Van' Sweet Cherries by Postharvest Calcium Treatments. *J. Am. Soc. Hortic. Sci.* **1978**, *103* (4), 527–530.

Lidster, P. D.; Porrit, S. W.; Tung, M. A. Effects of a Delay in Storage and Calcium Chloride Dip on Surface Disorder Incidence in 'Van' Cherry. *J. Am. Soc. Hortic. Sci.* **1979a**, *104* (3), 298–300.

Lidster, P. D.; Tung, M. A.; Yada, R. G. Effects of Preharvest and Postharvest Calcium Treatments on Fruit Calcium Content and the Susceptibility of 'Van' Cherry to Impact Damage. *J. Am. Soc. Hortic. Sci.* **1979b**, *104* (6), 298–300.

Lin Tang, H-C.; McFeeters, R. F. Relationships Among Cell Wall Constituents, Calcium and Texture During Cucumber Fermentation and Storage. *J. Food Sci.* **1983**, *48*, 66–70.

M.A.P.A. *Norma de Calidad para Cerezas.* Edited by Secretaría General Técnica del Ministerio de Agricultura, Pesca y Alimentación, Madrid (Spain), 1987.

Marchiner, H. *Mineral Nutrition of Higher Plants*; Javanovich, H. B., Ed.; Academic Press: London, U.K., 1986.

Marcus, L.; Schejter, A. Single Step Chromatographia Purification and Characterization of the Endopolygalacturonases and Pectinesterases of the Fungus, *Botrytis Cinerea* Pers. *Physiol. Plant Pathol.* **1983**, *22*, 1–13.

McFeeters, R. F.; Armstrong, S. A. Measurement of Pectin Methylation in Plant Cell Walls. *Anal. Biochem.* **1984**, *139*, 212–217.

Mildram, A. S. Metals in Enzyme Catalisis. In *The Enzymes*; Boyer, P. D., Ed.; Academic Press: London, U.K., 1970; Vol. 2, pp 445–536.

Moustacas, A.; Nari, J.; Borel, M.; Noat, G.; Ricard, J. Pectin Methylesterase, Metal Ions in Plant Cell-Wall Extension. *Biochem. J.* **1991**, *279*, 351–354.

Nari, J.; Noat, G.; Ricard, J. Pectin Methylesterase, Metal Ions and Plant Cell-Wall Extension. *Biochem. J.* **1991**, *279*, 343–350.

Reid, D. S. Basic Physical Phenomena in the Freezing and Thawing of Plant and Animal Tissues. In *Frozen Food Technology*; Mallett, C. p., Ed.; Blackie Academic & Professional: Glasgow, U.K., 1993; Chapter 1, pp 1–19.

Rexova-benkova, L.; Markovic, O. Pectic Enzymes. *Adv. Carbohydr. Chem. Biochem.* **1976**, *33*, 323–385.

Scott, R. W. Colorimetric Determination of Hexuronic Acids in Plant Materials. *Anal. Chem.* **1979**, *51* (7), 936–941.

Sedmak, J. J.; Gossberg, S. A Rapid, Sensitive, and Versatile Assay for Protein Using Coomassie Brillant Blue G-250. *Anal. Biochem.* **1977**, *79*, 544–552.

Van Buren, J. P. Pectic Substances of Sweet Cherries and their Alteration During SO₂ Brining. *J. Food Sci.* **1967**, *32*, 435–436.