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The effect of calcium dips combined with mild heating of whole kiwifruit for fruit slices quality maintenance

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Abstract

The effect of moderate heat treatment combined with calcium dips on the quality of minimally processed kiwifruit was studied. Whole fruits were treated for 25 min at 45 °C by dipping in deionised water or $CaCl_2$ solutions (1%, 2% and 3% (w/v)) and cooled to 4 °C. Twenty-four hours later fruits were peeled, sanitized, cut into slices and packed. The firmness of kiwifruit slices' was subsequently evaluated during 8 days of storage. Calcium content, pectinmethylesterase activity and heat shock proteins accumulation were also investigated. Heat treatment conducted in water induced a firming effect and avoid softening of fruit slices while calcium dips had a marginal effect on this parameter. A calcium loss was observed due to dip treatment, but this effect was minimized when treatment was conducted in 3% $CaCl_2$ solution. The firming effect provided is due to the activation of pectinmethylesterase and the presence of calcium in treatment solution reduces or inhibits enzyme activation. Under the tested conditions, no heat shock proteins de novo synthesis was detected.

Keywords: Kiwifruit; Mild heat treatments; Calcium; Pectinmethylesterase; Minimal processing

1. Introduction

Pre and postharvest calcium application has been demonstrated to produce beneficial effects on whole fruit quality, decreasing the incidence of physiological disorders (Gerasopoulos & Drogoudi, 2005; Manganaris, Vasilakakis, Diamantidis, & Mignani, 2007; Serrano, Martínez-Romero, Castillo, Guillén, & Valero, 2004), mould growth (Naradisorn, Klieber, Sedgley, Scott, & Able, 2006) and delaying softening (Antunes, Panagopoulos, Rodrigues, Neves, & Curado, 2005; García, Herrera, & Morilla, 1996; Naradisorn et al., 2006).

Changes in texture occur due to changes in the chemistry of the primary cell wall components cellulose, pectins, and hemicelluloses which occur during growth and development (Sams, 1999). Calcium is directly involved in strengthening plant cell walls through its ability to cross link with carboxyl groups of polyuronide chains of pectins found in the middle lamella (Lara, García, & Vendrell, 2004; Sams, 1999). Calcium ions help in the stabilization of cell membranes (Picchioni, Watada, Conway, Whitaker, & Sams, 1995) and cell turgor pressure can also be affected (Mignani et al., 1995), decreasing fruit softening.

Calcium salts have also been used to preserve the quality of minimally processed commodities. Calcium chloride dips allowed firmness retention of fresh-cut watermelon for 7 days of storage (Mao, Jeong, Que, & Huber, 2006) and improved firmness of fresh-cut strawberries (Aguayo,

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Jansasithorn, & Kader, 2006). If combined with 1-methylcyclopropene under controlled atmosphere it also slowed down the loss of appearance quality, changes in titrable acidity and microbial growth of strawberry wedges (Aguayo et al., 2006). Fresh-cut 'Kensington' mango evidenced a smaller softening rate when treated with 3% $CaCl_2$ however combining calcium application with low oxygen atmosphere was found to be the most effective treatment for extending the shelf-life of mango slices (Souza, O'Hare, Durigan, & Souza, 2006). Quality of fresh-cut cantaloupe (Luna-Guzmán & Barrett, 2000; Luna-Guzmán, Cantwell, & Barrett, 1999) and honeydew (Saftner, Bai, Abbott, & Lee, 2003) melons was improved with calcium chloride, calcium lactate, calcium propionate and calcium amino acid chelate dips. However, post-cutting dips of ascorbic acid and calcium lactate slightly extended the shelf-life of peach and nectarine slices but the overall advantages of such treatments were marginal (Gorny, Hess-Pierce, & Kader, 1999).

Moderate heat treatments, applied to whole fruit, have been demonstrated to alter fruit physiology and biochemistry being helpful in quality maintenance of fresh-cut kiwifruit (Beirão-da-Costa, Steiner, Correia, Empis, & Moldão-Martins, 2006; Beirão-da-Costa et al., 2006), cantaloupe melon (Lamikanra, Bett-Garber, Ingram, & Watson, 2005), apples (Barrancos et al., 2003), pears (Abreu, Beirão-da-Costa, Gonçalves, Beirão-da-Costa, & Moldão-Martins, 2003) and peaches (Steiner et al., 2006). This kind of treatments can lead to changes in respiratory and ethylene production rates, enzymatic activity, and in cuticle structure, together or not with protein synthesis, such as heat shock proteins (HSP) (Paull & Chen, 2000; Pavoncello, Lurie, Droby, & Porat, 2001).

Post-cut calcium treatments, when conducted at higher temperatures, seem to be more effective. Calcium chloride combined with heat treatment was effective in preserving the quality of fresh-cut mango cubes (Trindade et al., 2003). A wash treatment solution of 1.5% calcium lactate at 50 °C for 1 min was the best treatment in inhibiting browning and preserving firmness of minimally processed lettuce, by reducing the loss of turgor pressure and enhancing pectinmethylesterase (PME) activity (Martin-Diana et al., 2005, 2006).The firming effect provided by dipping fresh-cut cantaloupe melons in 2.5% calcium chloride was improved when combined with higher dip temperatures (1 min at 60 °C) but dip temperature did not affect fruit metabolism (Luna-Guzmán et al., 1999). However, other authors refer that at lower temperatures (4 °C during 3 min), calcium treatment appears to be beneficial for the storage of cut cantaloupe melon, reducing respiration rate and moisture loss, compared to treatment at room temperature (25 °C during 3 min) (Lamikanra & Watson, 2004).

The combined effect of moderate heat treatments and calcium dips on the whole fruit is scarcely described. To the best of our knowledge, only one published work considered the biochemical effects of whole cantaloupe melon heat treatment, which was performed during 60 min at 60 °C in a 1% calcium lactate solution on fresh-cut fruit during storage (Lamikanra & Watson, 2007).

The objective of our research was to investigate the effects of moderate heat pre-treatments conducted in calcium solutions, on kiwifruit slices firmness and fruit response mechanisms.

2. Materials and methods

2.1. Fruit preparation

Unripe kiwifruit (*Actinidia deliciosa* (A Chev) Liang et Ferguson var deliciosa cv Hayward), without any wound signals, were purchased at a local market, selected for uniform size, and stored at 4 °C prior pre-treatments and processing. Whole kiwifruits were subjected to a previously optimized heat treatment at 45 °C during 25 min (Beirão-da-Costa et al., 2006) in 1%, 2% and 3% (w/v) calcium chloride solution. Non-heat treated and heat treated in deionised water fruits were used as control samples. Treatment time was recorded since the core of the fruit achieves 45 °C.

After overnight storage, fruits were hand peeled, sanitized in chlorine water with 125 mg/L free Cl^-, supplied as sodium hypochlorite at pH 6, gently dried with blotting paper, cut in ≈1.5 cm slices, packed and stored at 4 °C.

2.2. Firmness analysis

Firmness was evaluated by a puncture test on kiwi slices flesh using a TA-XT Plus texture analyser from Stable Micro Systems with a 5 kg load cell. Firmness measurements were taken as the peak load values obtained from a test in which a 4 mm diameter stainless steel probe penetrates the fruit by 4 mm at a crosshead speed of 1 mm s^{-1}. Mean values were calculated from results of 20 measurements in different slices for each sample.

2.3. Pectinmethylesterase (PME) extraction

Twelve grams of kiwifruit outer pericarp (without seeds) was homogenised with 40 mL of cold 1.5 M NaCl in a T25 basic, IKA LABORTECHNIK homogeniser. The homogenate was incubated with agitation for 30 min at 4 °C and centrifuged at 15 000*g* for 10 min, at 4 °C. The resulting supernatant was used for PME activity assay.

2.4. Pectinmethylesterase assay

PME activity was assayed titrimetrically using a pH electrode measuring the H^+ produced by the carboxyl groups released by the hydrolysis of methyl esters of pectin, using the method described by (Kimball, 1991) modified. One hundred milliliters of substrate solution (0.25% (w/v) citric pectin in 0.2 M NaCl) was mixed with 5 mL of PME extract and the pH adjusted to 7.5 with 1 M and 0.05 M NaOH. After the pH reached 7.5, 0.2 mL of

0.02 M NaOH was added and the time required to reach pH 7.5 again was recorded. PME activity was measured at 25 °C, as a reference temperature and at 45 °C, the temperature used in heat treatment. One unit of PME activity was defined as the amount of enzyme that can cause the released of 1 μmol of COO^- per gram of fresh tissue and per minute (μmol g^{-1} min^{-1}).

Heat stability of PME from extracts of kiwifruits treated at the several conditions was evaluated by monitoring enzyme activity at 45 °C for around 30 min. All values are the mean of three replicates.

2.5. Calcium analysis

Two grams of fruit outer pericarp (without seeds) selected from different slices, were ashed at 550 °C. Ashes were suspended in 10 mL of HCl 3 N for 24 h, deionised water was added to a total volume of 50 mL and the mixture filtered through ashless filter paper. Strontium chloride was added to final solutions to control ionization interferences. Ca^{2+} concentration was determined at 422.7 nm using an atomic absorption spectrophotometer (Pye-Unicam SP9).

2.6. Heat shock proteins (HSP) analysis

Thirty grams of fruit outer pericarp (without seeds) selected from different fruits were homogenised with 100 mL of acetone, in a T25 basic IKA LABORTECHNIK homogeniser, vacuum filtered and the residue was dried at 25 °C. Crude protein extract was obtained using 500 mg of this residue macerated with 5 mL of 100 mM Tris–HCl buffer (pH 7.5), containing 3 mM dithiothreithol (DTT) (Sigma) and 1 mM ethylenediamine-tetracetic acid (EDTA) (Pharmacia). The homogenate was centrifuged at 10000*g* for 20 min. Partial purification of protein fraction was performed using an Amicon centrifugal filter (microcon YM 10, Millipore) and centrifugation at 10000*g* for 30 min. Sample denaturation was performed using 0.05 M Tris–HCl buffer containing 2.5% SDS, 5% 2-mercaptoethanol and 12% glycerol and boiled for 15 min before SDS–PAGE.

SDS–PAGE was performed in a 10% polyacrylamide (Pharmacia) and 2.6% BIS gel slabs with 1% SDS (Pharmacia) in a vertical system (Midget Electrophoresis Unit LKB 2050) with refrigeration at constant 60 mA and maximum 250 V in 100 mM Tris–glycine buffer (pH 8.3) (Martins, Mourato, & Mendonça, 2002). Molecular weight standards from 14.4 to 94 kDa (Pharmacia) were also run. Staining was done using a silver staining kit (Pharmacia).

2.7. Statistics

To evaluate significant differences among samples with time an analysis of variance (factorial ANOVA) was performed together with Fisher's LSD mean comparison test, using the "Statistica" v. 6.1 software from Statsoft, Inc.

3. Results and discussion

3.1. Firmness evaluation and calcium analysis

The effects of applied treatments on firmness are shown in Fig. 1. Heat treatment had a firming effect on slices during 8 days of storage while the presence of calcium ions in the dip treatment solution evidenced only a marginal effect. After minimal processing, no significant differences ($P \geqslant 0.166$) were observed among calcium heat treated fruits (HT1%, HT2% and HT3%) and unheated control (Control). In contrast, heat treatment in water (HT) significantly ($P = 0.004$) increased slice firmness, 24% higher than unheated samples. Increasing calcium concentration in solution minimizes the firming effect of heat treatment, in the beginning of storage.

After 3 days control samples underwent faster softening, attaining a 64% of the initial firmness value, and being significantly different from the heat-treated samples.

Similar results were described by Lamikanra and Watson (2007) for cantaloupe melon. These authors concluded that the presence of calcium in the treatment water did not profoundly alter fresh-cut cantaloupe melon texture relative to treated fruit at 60 °C for 60 min without added calcium, during storage.

García et al. (1996) reported that dip treatments in 4% $CaCl_2$ at 25 °C lead to strawberries with lower firmness than control, for 3 days of shelf-life. Nevertheless, fruits treated in 1% $CaCl_2$ at 45 °C showed significantly higher firmness than fruits treated at the same temperature without calcium, with differences noted after 2 days of storage.

Several published works refer that $CaCl_2$ dip could impart a bitter flavour to the fruit. Performed sensory analysis (data not shown) did not reveal the presence of any strange flavour in fruit slices.

Calcium analysis (Table 1) showed that applied treatments did not allow the diffusion of calcium into the fruit and instead lead to some loss of calcium ions. Control

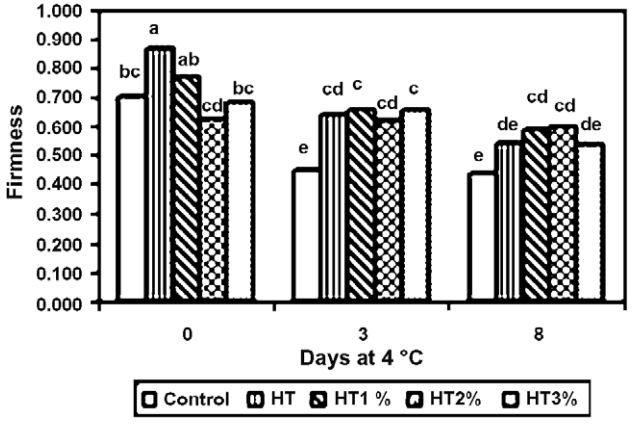

Fig. 1. Firmness evolution of kiwifruit slices during storage. Whole fruits were unheated (control) or heat treated in water (HT), 1% $CaCl_2$ (HT1%), 2% $CaCl_2$ (HT2%) and 3% $CaCl_2$ (HT3%). Above each bar different letters indicate significant differences between samples ($P < 0.05$).

samples had significantly ($P < 0.01$) higher amounts of calcium than heat treated samples, with the exception of fruits previously treated with 3% of $CaCl_2$.

Manganaris et al. (2007) reported that calcium application in peaches by immersion of the whole fruit in calcium chloride, calcium lactate and calcium propionate solutions at two different calcium concentrations, for 5 min, lead to a flesh calcium increase by 50–74% compared to control fruits. $CaCl_2$ (1%) dips of whole strawberries lead to an increase in calcium content but heating at 45 °C enhanced the penetration of ions into the fruit (García et al., 1996).

In our experiments, the initial temperature gradient between the fruit (4 °C) and the water/calcium solutions (45 °C), despite the higher calcium concentrations in the bath, could render the ion exit easier. This process was minimized at 3% of $CaCl_2$ where probably the osmotic pressure of the solution balanced the effect of temperature gradient.

Lurie, Fallik, and Klein (1996) reported that heat treatment fills in cracks present in apple's epicuticular wax decreasing the ability of applied calcium to be transported into fruit tissue. In kiwifruit the thin brown skin covered with small hairs (trichomes) includes a periderm and hypodermal cells, comprising a thick layer of dead, radially compressed cells with suberized cell walls over the hypodermis. Parenchyma cells beneath the hypodermis gradually merge into the fleshy tissue that forms most of the outer pericarp (Crisosto & Kader, 1999; Hallett & Sutherland, 2005). It was this complex and closed structure that probably did not allowed the calcium flow into the fruit.

3.2. Pectinmethylesterase activity

Previous works have justified the firming effect resulting from heat treatments at low temperatures by activation of PME. PME activity of samples obtained in our study is shown in Table 2. It was found that increasing temperature in PME activity assay from 25 °C to 45 °C leads to a significant 2–3 fold increase in measured activity. At treatment temperature (45 °C), the sample treated in water evidenced higher PME activity ($P < 0.03$) than samples treated in $CaCl_2$ solutions at all tested concentrations. Despite the lowest significance level ($P = 0.061$) it can be stated that PME activity is also higher in HT than in control fruits.

Table 1
Calcium content of kiwifruit slices outer pericarp

	Calcium (mg/100 g)
Control	18.43 ± 0.60^{a}
HT	14.48 ± 0.71^{b}
HT 1%	15.46 ± 0.11^{b}
HT 2%	14.13 ± 0.44^{b}
HT 3%	16.31 ± 0.79^{ab}

Results are the mean ± SE of six measurements.
Values followed by the same lower case are not significantly different at 99%.

Table 2
PME activity of kiwifruit slices outer pericarp

	$\mu mol\ COO^{-}\ g^{-1}\ min^{-1}$	
	25 °C	45 °C
Control	2.88 ± 0.07^{ab}	7.35 ± 0.24^{ab}
HT	3.19 ± 0.32^{b}	9.00 ± 0.98^{b}
HT 1%	2.12 ± 0.17^{a}	5.86 ± 0.36^{a}
HT 2%	2.89 ± 0.10^{ab}	6.86 ± 0.41^{a}
HT 3%	3.60 ± 0.48^{b}	6.87 ± 0.47^{a}

Results are the mean ± SE of three measurements. In each column values followed by the same lower case are not significantly different at 95%.

These results may explain why HT samples showed higher firmness values than the other samples. Moreover, in previous works (Beirão-da-Costa et al., 2006) whole kiwifruits subjected to heat treatment at 45 °C during 25 and 75 min showed higher levels of linked calcium than non-heated fruits. Both results evidence that the formation of calcium pectates within the cell wall as consequence of heat treatment was the mechanism involved in firmness increment.

Similar results were obtained for strawberries where fruits heat-treated at 45 °C for 3 h showed higher PME activity than the control, slowing down pectin solubilization by increasing the amount of putative sites for calcium bridge formation within the cell wall, and higher firmness (Vicente, Costa, Martínez, Chaves, & Civello, 2005).

In previous works (Beirão-da-Costa, Steiner, Correia, Empis et al., 2006, Beirão-da-Costa et al., 2006) it was established that, at 45 °C, 25 min was the optimum time for quality preservation of kiwifruit slices, and so PME stability at 45 °C as a function of time was also studied (Fig. 2). Analysis of variance shows the effects of the applied treatments (water, $CaCl_2$ and control) and treatment time, in PME activity of kiwifruits' outer pericarp. A significant effect on enzyme activity was observed by the kind of applied treatment ($P = 0.000$), the duration of

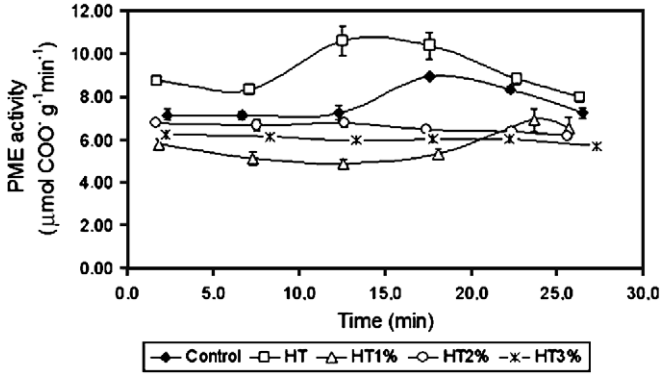

Fig. 2. Effect of treatment time on PME activity of kiwifruit. Whole fruits were unheated (control) or heat treated in water (HT), 1% $CaCl_2$ (HT1%), 2% $CaCl_2$ (HT2%) and 3% $CaCl_2$ (HT3%). At each data point, the bar represents the standard error of the mean. Bars not revealed are hidden with symbols.

treatment ($P = 0.003$) and the interaction of the two effects ($P = 0.000$).

In all samples, there was a measurable PME activity during the whole period analysed. As can be seen in Fig. 2, PME activity on enzyme extract from control fruits was quite stable during the initial period of 10–15 min of the heating process; however after that, a significant ($P = 0.003$) increase in enzyme activity was recorded and maintained for 10 min more. In the authors opinion, despite the fact that in the assay the optimum enzyme activity pH was used, these results might indicate what is occurring during whole fruit heat treatment, and can explain why 25 min was the most suitable treatment time previously established.

When fruits were treated at 45 °C in water, enzyme extract exhibits a significantly ($P \leqslant 0.001$) higher PME activity up to 20 min of heating time but a rapid increase in enzyme activity was observed after 5 min. After 20 min at 45 °C PME activity underwent a decline to values similar to the ones at the beginning of the heating process. These results show that despite the long treatment time, moderate heat treatment applied to whole kiwifruit did not inactivate PME and on the other hand, that an irreversible activation seems to occur.

When samples were previously treated in $CaCl_2$ a decrease of PME activity was observed. These samples showed a lower but quite stable PME activity during the analysed heating period with exception of extracts from fruits that had been heat treated in 1% $CaCl_2$ which showed a small increase in enzyme activity in the last minutes of measurement, although still significantly ($P = 0.008$) lower than control extracts.

Although the role of calcium and temperature as regulators of enzyme activity has been described, the results are still somewhat contradictory. Ni, Lin, and Barrett (2005) evaluated PME activity in leafy, fruit, root and flower vegetables and conclude that, with exception of red bell peppers, all present measurable enzyme activity is enhanced, for 30 min, at temperatures between 50 °C and 70 °C. Anthon, Blot, and Barret (2005) reported that diced tomatoes heated in water at 70 °C showed a rapid methanol production, resulting from PME activity, in the first 10 min but both the initial rate and final extent of methanol production were increased if the tomatoes were heated in 0.5% $CaCl_2$ instead of water. Some authors report that temperature is the most important factor in raising PME activity on lettuce and calcium presence has no significant effect (Martin-Diana et al., 2005, 2006). There was no significant difference in PME activity between carrots treated with and without calcium at 25 °C but carrots dipped in calcium lactate at 50 °C showed higher PME activity (Rico et al., 2007). Whole peaches dipped in calcium salts also showed higher PME activity compared to control fruits but the differences were only detected after 4 weeks of storage (Manganaris et al., 2007).

Our results showed that despite the fact that no diffusion of calcium into whole kiwifruit was observed, heat treatment conducted in those solutions interfered with enzyme activity. It is hypothesized that $CaCl_2$ solutions promote the diffusion of several ions from the fruit to the external solution, changing the ionic balance in fruit cells and leading to internal conditions less favourable to enzyme activity.

3.3. Heat shock proteins analysis

One of the effects of heat treatments is the synthesis of small HSP with low molecular mass (16–20 kDa) able to protect the fruit from later injuries and stress. HSP are involved in heat stress, and might be important in the increase of plasma membrane resistance or in preventing membrane damage, due to their function as a protein protector under severe abiotic stress (Hall, 2002).

To the best of our knowledge, there are no published works reporting heat shock proteins accumulation in kiwifruit as consequence of thermal treatments. Fig. 3 shows the protein analysis of kiwifruit samples by SDS–PAGE. Electrophoresis gels analysis showed that kiwifruit exhibits several protein bands from 68 kDa to 10.5 kDa molecular weight but, despite the different intensity of some bands, no differences were detected in protein profile of samples. It seems therefore that heat treatment at 45 °C for 25 min, either conducted in water or $CaCl_2$ solutions, did not promote HSP synthesis, within this molecular weight range, as it no novel bands were detected in heat-treated samples, compared to control. Another hypothesis is that the synthesis of HSP is in fact occurring but band overlap does not allow the identification of the different proteins with the same molecular weight, but only a two-dimensional electrophoresis could clarify this.

Our results did not agree with others that report the expression of HSP in many horticultural products. For avocado fruit the optimal range for induction of HSP synthesis in mesocarp was found to be between 34 and 38 °C and those proteins are maximally expressed after a heat shock at 38 °C for 4 h (Florissen et al., 1996). Exposure of papaya to 38 °C for 2 h leads to protein synthesis

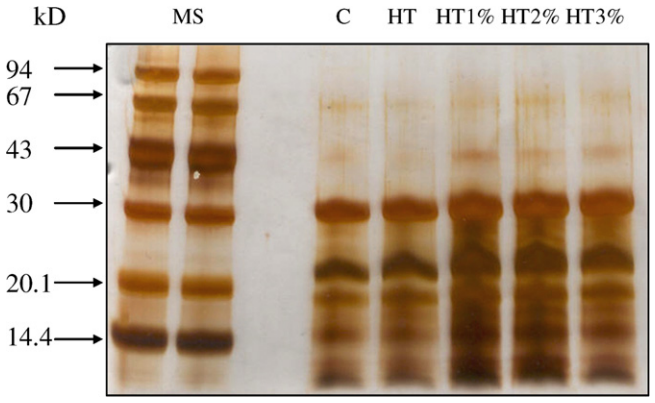

Fig. 3. Protein profile by SDS–PAGE of kiwifruit outer pericarp. Whole fruits were unheated (control) or heat treated in water (HT), 1% $CaCl_2$ (HT1%), 2% $CaCl_2$ (HT2%) and 3% $CaCl_2$ (HT3%). Exemplificative gel of the global protein profile.

changes (Paull & Chen, 1990) occurring the same with apples held 4 days at 38 °C. The new bands in electrophoresis gels correspond to proteins with low (14–22 kDa) and high (68 and 92 kDa) molecular weight (Lurie & Klein, 1990). A hot water brushing treatment (62 °C for 20 s) induced the accumulation of several heat shock proteins probably involved in a complex fruit disease resistance mechanism (Pavoncello et al., 2001). Zhu, Ji, Lu, and Zhang (2003) reported that heat-treated mango at 55 °C during 5 min accumulated three new polypeptides with molecular weights of 13.7, 15.7 and 15.7 kDa involved in chilling tolerance of mangoes. Lamikanra and Watson (2007) reported the development of two proteins bands, in isoelectric focussing, at pI 5.1 and 6.5 in fresh-cut cantaloupe pre-cut heat treated at 60 °C for 60 min.

4. Conclusions

Hot water treatment at 45 °C/25 min of whole kiwifruit is an effective methodology in quality maintenance of fruit slices, increasing fruit firmness and avoiding an excessive softening. The presence of calcium in dip solutions during heat treatment had a marginal effect in fruit firmness. Dip treatment leads to partial calcium loss from the fruit, this effect being minimized when treatment was conducted in 3% $CaCl_2$ solution. PME is activated by heat treatment but in the presence of calcium this effect is reduced or even inhibited. Therefore, it is tentatively concluded that firmness preservation is due to PME activation, though the degree of methylesterification of pectin for the different samples might permit a definite conclusion. No HSP in the range of 94–14.4 kDa were detected as a consequence of the applied treatments which leads us to think that these proteins were not involved in quality maintenance of kiwifruit slices. Although calcium presence did not induce benefits on the quality preservation of fruit slices, it also did not cause negative effects when present in the heat treatment solution.

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