J Sci Food Agric 85:1773–1778 (2005)
DOI: 10.1002/jsfa.2182

Effect of calcium additives on physicochemical aspects of cell wall pectin and sensory attributes of canned peach (*Prunus persica* (L) Batsch cv Andross)

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Abstract: Quality evaluation of calcium-treated processed peaches was determined by physical, chemical and sensory analyses. Textural and structural properties of canned peaches indicated that calcium-treated peaches gained better firmness retention and greater proportion of uronic acids between the insoluble and water-soluble pectin fraction than untreated peaches, probably because of higher cell wall calcium content. However, a direct relationship cannot be established between calcium and uronic acid content in pectin fractions. A range of sensory descriptors assessed by a trained panel provided results that challenge accepted interpretations in the field of quality evaluation. The results indicated the possibility of processing high quality calcium-treated canned peaches with acceptable qualitative features. Calcium lactate is suggested as a potential calcium source in the peach canning industry, since it provided both better textural features and sensory attributes. The interactions among calcium, the physicochemical properties of the cell wall framework and sensory attributes are discussed.

Keywords: calcium; peach; pectin; cell wall; firmness; sensory analysis; canning process

Abbreviations

CWM	cell wall material
SSC	soluble solids content
TA	titratable acidity
GLC	gas-liquid chromatography
TFA	trifluroacetic acid
Ara	arabinose
Fuc	fucose
Gal	galactose
Glc	glucose
Man	mannose
Rha	rhamnose
Xyl	xylose

INTRODUCTION

Calcium salts of natural products have been used extensively on whole fresh or processed fruits, as well as in the fresh-cut industry, in order to maintain firmness retention and to extend storage life. Calcium chloride has been widely used to improve or maintain textural attributes of either whole fresh fruits, including peach,[1] or sliced fruits,[2] and it is commonly used on an industrial scale as a firming agent during the canning process of many products.[3,4] Although beneficial for product texture, calcium chloride has been found to impart flavour differences.[5] Calcium lactate and calcium propionate are food additives which offer alternative calcium sources. Calcium lactate as a post-cutting dip prevented cut surface browning and tissue softening of fresh-cut[6,7] and processed[8] fruits, whereas calcium propionate extended significantly the postharvest life of fresh-cut products.[9] Although the effect of calcium to other processed fruits is well known,[10] to the best of our knowledge few data are available regarding the effect of calcium sources in canned peaches.[11]

The important role of calcium in fruit physiology[12,13] and its structural role in the cell wall matrix[14] have led to further study about its function, particularly in relation to pectins. Calcium exerts a profound influence on tissue integrity and its role, as a firming agent, has been well documented and briefly consists in the complexing of calcium ions with cell wall and middle lamella pectin,[14] its influence in cell wall strength[13] and its effect on cell turgor pressure.[15]

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(*Received 9 October 2003; revised version received 19 August 2004; accepted 19 January 2005*)
Published online 4 May 2005

The primary cell wall is largely composed of polysaccharides (pectins, cellulose and hemicelluloces). Pectins are a highly heterogeneous group of polymers, accounting for a substantial proportion of the cell wall dry weight.[16] Softening involves cell wall degradation, primarily of pectic constituents, although other types of polymers may be involved,[17] and heat treatment of fruits during canning process can result in texture losses and tissue softening.[18] During processing, cell walls undergo modifications in terms of their physical state and composition, as well as structure-dependent changes in their functional and material properties,[19] through which the mechanism of canning-induced fruit softening was clarified in canned products.[20]

The widespread clingstone peach cultivar 'Andross' was selected for the present work. The benefits of adding calcium were assessed by examining different calcium sources in physicochemical aspects of cell wall metabolism as well as in the field of sensory evaluation.

EXPERIMENTAL

Plant material

Peaches (*Prunus persica* (L) Batsch, cv Andross) were harvested from 10-year-old trees, managed under standard cultural practices, at a commercial ripe stage according to size and colour and cold stored, without causing any chilling injury symptoms, for 1 week (0 °C, 95% RH). After removal from cold storage, peaches were shelled, chopped in half and placed in metal boxes (0.5 kg). Each can contained five or six peach halves which were covered with deionized water enriched with 20% (w/v) sugar, 0.036% (w/v) citric acid and the calcium sources before pasteurization. Calcium chloride, calcium lactate and calcium propionate were used as calcium sources. All chemicals were of analytical grade. In each can the final calcium concentration was adjusted to 360 mg l^{-1} for each treatment. Cans were sealed using vacuum (560 mm Hg) and pasteurized for 25 min at 88–90 °C. After processing, 30 cans from each treatment were stored at room temperature for 6 months until evaluation.

Tissue firmness

Tissue firmness was measured using an Instron Universal Testing Instrument (Instron Model 1122, Instron Corp, Canton, MA, USA) fitted with a 5-kg load cell. A 2.3-mm-diameter, flat-tipped probe was driven 1.1 mm into the cut surface of the disc with a cross-head speed of 50 mm min^{-1}, and the peak force was recorded in newtons (N).

Soluble solids content (SSC), titratable acidity (TA)

The peach halves of each individual can were juiced together to obtain a composite sample and analyzed for SSC using a digital refractometer (Atago Model PR-1, Tokyo). The TA was assessed by neutralization with 0.1 M NaOH using 1% phenolphthalein as an indicator and expressed as g l^{-1} malic acid. All treatments were run with six replicates.

Preparation and fractionation of cell wall material

Cell wall material (CWM) was prepared from samples (40 g) of halved peaches from each can, using procedures described in Reference 21. Samples of CWM (5 mg) were suspended under constant stirring in distilled water for 1 h at room temperature and centrifuged at 10 500 × *g* for 25 min. The supernatant was collected and the previous step was repeated once. The two supernatants were combined to represent the water-soluble pectin fraction. The pellet was dissolved in 2 ml of concentrated H_2SO_4, corresponding to the insoluble pectin fraction.[22] All treatments were run with six replicates.

Determination of uronic acids, neutral sugars and cellulose

Aliquots of water-soluble and insoluble pectin fractions were used for uronic acid determination by the *m*-hydroxydiphenyl method.[23]

Non-cellulosic neutral sugars were derivatized to alditol acetates by hydrolysis of 4 mg of CWM in 2 M TFA, reduction and acetylation.[24,25] The derivatives were identified by gas chromatography on a Dani Chromatograph 1000 (Dani Instruments SpA, Cologno Monzese, Milan, Italy) fitted with a 30-m fused silica capillary column (DB-225, J&W Scientific, Folsom, CA, USA). The temperature of the chromatograph oven was held at 210 °C and hydrogen was used as carrier gas. Quantitation was based on integration of the peaks from the flame ionization detector with a Shimadzu C-R3A (Shimadzu, Kyoto, Japan) chromatography data system.

Cellulose content was determined in the TFA-insoluble cell wall material by the anthrone colorimetric assay.[26]

Calcium determination

Cell wall material and aliquots of water-soluble and insoluble pectin fractions were wet-digested in a triacid solution (HNO_3:H_2SO_4:$HClO_4$, 5:1:1, v/v/v) at 80 °C until a red vapour was detected, then the temperature raised to 150 °C until a clear residue was produced, according to Failla *et al*.[27] Calcium content was determined by atomic absorption using a Perkin-Elmer 403 AA spectrophotometer at 422.7 nm and expressed as μg mg^{-1} CWM. Samples of canned peaches, after washing with deionized water, were used for total calcium content determination, as described above and expressed as μg g^{-1} fresh weight (FW). All treatments were run with six replicates.

Sensory evaluation

A descriptive analysis was performed for sensory assessment, on the basis of the perceived evaluation of canned peaches from the different calcium treatments, using a modified method of Romeih *et al*.[28] A range of

sensory descriptors (appearance, crispiness, aroma and flavour) was assessed by a trained panel (14 persons, consumers of canned peaches) in three sessions. The panellists were asked to evaluate coded samples of the four different calcium treatments. A 10-cm non-anchored scale was used for each attribute with each panellist marking the corresponding intensity of the attribute for all samples. For all attributes, the left side of the scale represented low intensity while the right side of the scale represented high intensity. Appearance was based on visual characteristics of the canned peaches, crispiness was based on the resistance manifested when the sample is pressed lightly by teeth, aroma was based on the intensity of the stimulation perceived when the sample approached the nose, and flavour was based on the intensity of the stimulation perceived when the sample is chewed for some seconds and then breathing out. Preliminary sessions were carried out to assure that the panellists were accustomed with the definitions, listed above.

Statistical analysis

Data were treated for multiple comparisons by analysis of variance (ANOVA), followed by the Duncan's Multiple Range Test at $p = 0.05$. Data in percentages were subjected to arcsine transformation prior to statistical analysis. Intensity scores from the descriptive analysis were assigned a numerical value and analyzed by ANOVA. ANOVA was performed using SPSS statistical software (SPSS Inc, Chicago, USA).

RESULTS AND DISCUSSION

Uronic acids, neutral sugars and cellulose content

The combined total uronic acids, neutral sugars and cellulosic contents of each sample accounted for 65–70% of the CWM. No statistical differences were found both for total uronic acids, and for total neutral sugars and cellulose content for all the treatments (Fig 1). Total uronide content was not significantly different among treatments, averaging approximately 20–21% of the CWM, whereas significant differences were detected in the amount of uronic acids in the water-soluble pectin fraction and the ratio of uronic acids between the insoluble and water-soluble pectin fraction (Table 1). Calcium propionate exhibited the highest ratio of uronic acids between the insoluble and water-soluble pectin fraction (69% more than the control), followed by calcium lactate and calcium chloride. Thus, the ratio of uronic acids between the soluble and insoluble pectin fraction seems to be an appropriate indicator of peach firmness. The same observation has been documented for fresh peaches as well.[29]

Pectin molecules are considered to be complex polysaccharides having side-chains of neutral sugars along the main chain of uronic acid.[30] In the present work no statistical differences were found in the neutral sugar composition among different calcium treatments. The GLC analysis of cell wall material of different treatments indicated the similarity of the neutral sugar composition. Arabinose was present in highest percentage, followed by galactose, glucose and xylose; meanwhile rhamnose, fucose, and xylose were present in smaller amounts (Table 2).

Cellulose has not been reported to be lost from the cell wall material as softening progresses.[31] In our study the cellulose content did not differ among the treatments and accounted for 23–27% of CWM and seemed to be unaffected from calcium treatment.

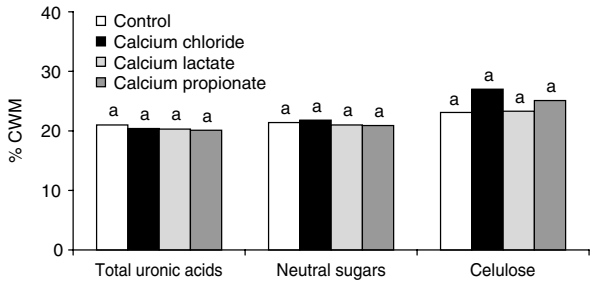

Figure 1. Percentages (% CWM) of total uronic acids, neutral sugars and cellulose of canned fruits treated with different calcium sources.

Table 1. Uronic acid content in the insoluble and soluble pectin fraction and their ratio of canned fruits treated with different calcium sources

	Uronic acid (μg mg^{-1} CWM)		
Treatment	Insoluble pectin	Soluble pectin	Insoluble/ soluble pectin
No calcium	160.0a	50.0a	3.2c
Calcium chloride	163.7a	40.1b	4.1b
Calcium lactate	164.8a	38.3b	4.4b
Calcium propionate	168.9a	32.3c	5.4a

Values within columns followed by the same letter are not significantly different from each other at $p = 0.05$ (Duncan's multiple range test).

Table 2. Neutral sugar composition of CWM isolated from canned peaches treated with different calcium sources

	Non-cellulosic neutral sugar (μg mg^{-1} CWM)						
Treatment	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
No calcium	14.2a	6.2a	88.6a	34.0a	5.6a	35.4a	30.1a
Calcium chloride	14.6a	6.2a	93.9a	31.6a	5.8a	36.6a	29.7a
Calcium lactate	14.9a	6.1a	89.4a	28.4a	4.9a	35.3a	31.3a
Calcium propionate	13.7a	6.4a	87.6a	29.3a	5.4a	35.7a	31.1a

Values within columns followed by the same letter are not significantly different from each other at $p = 0.05$ (Duncan's multiple range test).

Calcium content

Total calcium content of calcium-treated peaches exhibited 5.8-fold higher content than non-treated peaches (Table 3). Calcium ions are supposed to be allocated in bridges among the galacturonic acid units of pectins that strengthen cell wall structure. Cell wall calcium was significantly higher in calcium-treated peaches (2.5-fold) (Fig 2). Calcium bound to insoluble pectin fraction differed statistically among all treatments. Calcium propionate exhibited the highest calcium amount bound to insoluble pectin (3.7-fold higher than control), followed by calcium lactate and calcium chloride.

The increase of total calcium did not correspond with the increase of cell wall calcium, and therefore the binding sites for calcium in the call wall had been saturated. Infiltration with various calcium concentrations in fresh fruits had previously led to an increase of cell wall calcium until saturation, and additional increase in the calcium concentration applied did not correspond to an increase of cell wall calcium in the fruits,[32,33] agreeing with our results. In addition, the calcium bound to water-soluble pectin fraction has been found not to participate in the increase of tissue retention.[32] Therefore, we argue that a lower concentration of calcium can be used ($<360\,mg\,l^{-1}$) in future experiments.

Calcium can be regarded as a softening–inhibiting ion that rapidly reduces pectin solubilization and loss of cellular turgor.[15] Calcium, as a constituent of the cell wall, plays an important role in interacting with pectic acid polymers to form cross-bridges, which influence cell wall strength.[12,14] In additional, it has been documented that calcium improved texture by maintaining greater levels of insoluble pectic substances and reducing pectin solubilization during prolonged heating in the canning process.[34] However, according to our data, a direct relationship cannot be established between calcium and uronic acid content in pectin fraction.

Table 3. Calcium content of canned peaches treated with different calcium sources

Treatment	Calcium ($\mu g\,g^{-1}$ FW)
No calcium	28.1b
Calcium chloride	161.0a
Calcium lactate	166.9a
Calcium propionate	160.9a

Values within rows followed by the same letter are not significantly different from each other at $p = 0.05$ (Duncan's multiple range test).

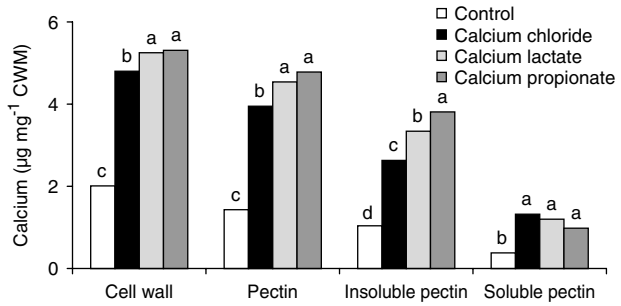

Figure 2. Calcium content of cell wall material and pectin fractions from canned fruits treated with different calcium sources.

Quality attributes — sensory evaluation

During heating of canned products, turgor and tissue integrity are quickly lost,[35] enhanced by dissolution of the cell wall and middle lamella.[36,37] Firmness showed that calcium-treated canned peach halves had 34.2–44.7% greater firmness retention than non-treated samples (Table 4). Overall calcium sources offer better tissue integrity than non-treated canned peaches, without statistically significant differences among them. Calcium ions seem to contribute to an increased membrane integrity and cell turgor pressure,[15] causing tissue hardening.[38,39] Firming effects accompanying calcium treatments have been attributed to increased calcium diffusion into the tissue.[40] Calcium sources applied in the fresh-cut industry resulted in significantly firmer samples than water dips.[7,9] Moreover, calcium ions have been proved to inhibit tissue weakening of processed fruit and vegetables.[4,8,41]

Sensory and quality attributes were evaluated in order to take advantage of the benefits that calcium additives offer regarding physicochemical parameters of the cell wall framework and tissue retention.

Objective measurements (total soluble solids, titratable acidity and their ratio) did not differ among treatments (Table 4). Although instrumental measurements are often preferred to sensory evaluations in research and commercial situations because they reduce variations in judgment among individuals,[42] use of sensory evaluation consists a critical part of fruit quality assessment in judging different attributes of fruit texture.

A significant difference was observed regarding crispiness among the calcium-treated and non-calcium-treated halved peaches, reflecting the generally recognized effect of calcium in tissue retention (Table 5). However, calcium propionate and calcium chloride proved to impart an undesirable flavour. Although beneficial for product texture, calcium chloride has been found to impart bitterness or flavour differences to other products as well.[5,7] No data is available regarding the effect of calcium propionate in sensory attributes of fruits, although it has been

Table 4. Tissue firmness, soluble solids content (SSC) and titratable acidity (TA) of canned peaches treated with different calcium sources

Treatment	Firmness (N)	SSC (%)	TA ($g\,l^{-1}$ malic acid)	SSC/TA
No calcium	0.38b	14.0a	5.21a	2.69a
Calcium chloride	0.51a	13.7a	5.24a	2.61a
Calcium lactate	0.54a	13.7a	5.04a	2.72a
Calcium propionate	0.55a	13.9a	4.96a	2.80a

Values within columns followed by the same letter are not significantly different from each other at $p = 0.05$ (Duncan's multiple range test).

Table 5. Mean sensory scores of canned peaches treated with different calcium sources

Treatment	Sensory attributes[a]			
	Appearance	Crispiness	Flavour	Aroma
No calcium	6.4c	5.6c	6.4a	6.1a
Calcium chloride	6.9b	6.3b	5.8b	5.8b
Calcium lactate	7.5a	6.6ab	6.3a	6.1a
Calcium propionate	6.1c	6.9a	5.7b	5.6c

Values within rows followed by the same letter are not significantly different from each other at $p = 0.05$ (Duncan's multiple range test).
[a] For each attribute a higher number represents higher intensity on a 0–10 scale.

reported to attract negative consumer perception as a chemical preservative.[43] These findings coincide with the data reported in the present paper. Calcium lactate exhibited the same score in flavour as non-treated halved peaches. An earlier report had shown that calcium lactate exhibited lower salty and bitter responses than equimolar concentrations of calcium chloride.[44] In addition, participants in quality evaluation of fresh-cut products could not distinguish differences between calcium lactate-treated and non-treated slices.[6,7] Aroma followed the same pattern as flavour. Calcium propionate and calcium chloride treatments differed statistically from calcium lactate and no calcium treatments. Moreover, appearance constitutes one of the major quality parameters, since visual properties are one of the most successful ways for assessing quality, and peach halves treated with calcium lactate exhibited the best appearance.

Overall, calcium lactate is suggested as a potential calcium source in the peach canning industry, since it provides both better textural features and sensory attributes.

ACKNOWLEDGEMENTS

The authors would like to thank the agricultural cooperation of Venus Growers for the canning process, Professor CG Biliaderis for critical reviewing of the manuscript and Professor S Raphaelides for Instron measurements. Results reported in the present paper are part of a PhD thesis by the senior author, with a grant from the State Scholarship Foundation of Greece.

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