Effect of different treatments with calcium salts on sensory quality of fresh-cut apple

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Summary

Tissue softening and oxidative browning at cut surface are serious problems with fresh-cut fruit products that can limit shelf life. Different techniques have been developed to extend the shelf life of minimally processed fruits, in particular refrigeration combined with antibrowning agents and calcium salts to reduce loss of firmness. The aim of the present study was to compare a commercial product, Natureseal (AgriCoat, Great Shefford, United Kingdom, control), with 2 different Ca salts (Calcium propionate 1%, w/v, and $CaCl_2$ 1%, w/v) combined with citric acid (1%, w/v) on 'Golden Delicious' apple diced, stored 5 days at 1 °C. The results obtained showed that Natureseal was highly effective in maintaining colour and firmness of fresh-cut apples, but also application of $CaCl_2$ + citric acid (CA) could be a good method to preserve the same product for 5 days. On the contrary, application of Ca propionate + CA resulted in acceptable values of firmness, but high browning, sometimes associated with off-flavours. The treatment with $CaCl_2$ + CA could be used in small fresh-cut industries as a cheap alternative to commercial products.

Keywords

fresh-cut apples; shelf life; Natureseal; Ca salts; firmness; browning; sensory analysis

Fresh-cut fruit is a ready-to-eat product increasingly demanded by consumers because of the health benefits and convenience associated with its consumption. The main aim of minimally processing the fruits is to maintain full nutritional value and ensure sufficient shelf life to allow commercialization. Unfortunately, fresh-cut fruits are highly perishable, undergo enzymatic browning (due to polyphenol oxidase activity) and softening. Different techniques were developed to extend the shelf life of fresh-cut products, in particular refrigeration associated with antibrowning agents [1].

Browning is an effect of cell disruption and release of polyphenol oxidases (PPO) that catalyse the oxidation of phenolic substances to quinones. These quinones are highly reactive and frequently continue to react with each other and with proteins, thus generating brown pigments. Its control have been extensively studied and reported in many fruits such as apples, pears and bananas [2–5]. The use of natural compounds and their derivatives was found to be effective in reducing browning in many fresh-cut fruits and vegetables. Ascorbic acid, citric acid and their derivates, or other compounds, were extensively studied in this application [6–11].

Another problem of fresh-cut fruits is the decrease in firmness during storage. The softening can be reduced by different treatments with Ca salts and derivatives [12–14]. In fact, Ca^{2+} provides rigidity to the cell wall and maintains the texture of the product [15, 16]. The decrease in cell-to-cell adhesion is considered as the main factor influencing firmness [17]. The middle lamella, the primary determinant of cell-to-cell adhesion, and primary cell walls are composed of rigid cellulose microfibrils held in concert by networks of matrix glycans (hemicellulose) and pectins. Large changes occur in both pectins and matrix glycans during fruit ripening, pectins becoming increasingly de-esterified. Pectin de-esterification leads

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to loss of integrity of cell walls, decrease in cell-to-cell adhesion, increase in intercellular spaces and a change of tissue structure. De-esterified negatively charged molecules can cross-link with divalent cations such as calcium, which adds rigidity to the cell wall and reduces its porosity [17, 18]. Calcium chloride was frequently and successfully used as a firming agent for strawberries [19], pears [20], apples [21, 22] and peaches [23]. The effect of calcium from different sources in maintaining the textural quality of produce is well known.

Many current studies agree on the need to combine the action of different substances in order to preserve the visual quality of fresh-cut fruit. The prevention of enzymatic browning and loss of firmness is frequently achieved using dipping treatments containing antibrowning agents such as antioxidants (ascorbic acid, calcium ascorbate, cysteine), acidulants (citric acid), enzyme inhibitors (4-hexylresorcinal), associated with different calcium treatments ($CaCl_2$, calcium ascorbate, calcium propionate; Tab. 1)

Recently, a new commercial product, Natureseal, was introduced to commercial use in Europe and elsewhere. The preparation contains calcium ascorbate, and was reported to be highly effective in maintaining the colour of fresh-cut apple slices as well as to increase wedge firmness during storage [24]. However, the commercial preparation may be sometimes taken as too expensive for the industries. The aim of the present study was to compare Natureseal (control) with 2 different Ca salts associated with citric acid on 'Golden Delicious' apple, diced and stored (1 °C) for 5 days.

MATERIALS AND METHODS

Apple samples and treatment

Golden Delicious apple cultivar was chosen for this study because of its extensive use. The apples were provided by Agrocompany, Chieri, Italy, at commercial ripeness stage (firmness 68.5 N; soluble solids 12.5 °Brix). Apples were selected, cleaned, peeled and diced (1.5 × 1.5 cm). The treatments studied were: Naturseal (AgriCoat, Great Shefford, United Kingdom, 3% w/v, control), citric acid (CA; Sigma-Aldrich, Munich, Germany, 1% w/v) + Ca propionate (Sigma-Aldrich, 1% w/v), CA (1% w/v) + CaCl2 (Sigma-Aldrich, 1% w/v). Apples samples were dipped for 2 min into the different aqueous solutions and drained. For each treatment, samples of about 150 g of apple were packaged in plastic bags in contact with air, and the samples were stored (1 °C) for 5 days.

Gas monitoring

In order to measure kinetics of respiration processes, concentrations of oxygen and carbon dioxide inside the packages were monitored by sampling the headspace using a Canal 121 instrument (Vizag – Gas Analysis, Croissy sur Seine, France). A sample of 0.5 ml was automatically withdrawn from the headspace atmosphere with a pin-needle connected to the injection system. Gases were analysed with an infrared sensor for CO_2 level and an electrochemical sensor for O_2 level. The instrument was calibrated towards air.

Analysis of soluble solids and titratable acidity

Soluble solids (*SS*) and titratable acidity (*TA*) were determined at the start of the experiment (day 0) and after storage (5 days) in triplicate using juice extracted from a 150 g apple sample (each treatment) blended at high speed in a tissue homogenizer. Soluble solids content was determined by a digital refractometer (Atago refractometer model PR-32; Atago Italia, Milan, Italy). Titratable acidity was measured by titrating diluted juice (1 : 10) using $0.1\,mol\cdot l^{-1}$ NaOH by an automatic titrator (Compact 44-00; Crison Instruments, Modena, Italy).

Firmness determination

Firmness was measured after 0, 1, 3, 5 days of storage on 30 pieces per treatment using a non-destructive penetrometer test by Durofel instrument (Copa Technologie, Cavaillon, France). The dynamometer was equipped with a bolt of diameter 3 mm (0.10 cm^2), on a scale of 1 (soft) to 60 (firm). Results were expressed as Durofel index (*DI*).

Colour measurement

Cut apple surface colour was measured daily with a Minolta colorimeter (Chroma Meter Model CR-400; Minolta, Tokyo, Japan). The colorimeter was calibrated using a standard white reflector plate. CIELAB values L^*, a^*, b^*, C^* and h^* were determined on 30 pieces per treatment. L^* indicates lightness, a^* indicates chromaticity on a green to red axis and b chromaticity on a blue to yellow axis. Chroma (C^*) and hue angle (h^*) were calculated using numerical values of a^* and b^*.

Browning potential

According to the method of Arias et al. [25], browning potential (*BP*) was determined by measuring soluble brown pigments (absorbance at 440 nm) generated by the oxidation of phenolic substrates by polyphenol oxidases. Apple samples (50 g each treatment) were homogenized with an Ultra-Turrax homogenizer (Ika-Werke, Staufen,

Tab. 1. Dipping treatments to maintain firmness and colour of fresh-cut apples.

Stabilizing treatment	Action	Reference
1% AA + 0.2% CA +/or 0.5 NaCl	Reduction of quinones + pH lowering	[43]
0.5% AA + 0.01% 4-HR	Reduction of quinones + enzyme inhibition	[44]
0.75% AA + 0.75% $CaCl_2$	Reduction of quinones + strengthening of the cell wall	[45]
0.5 mol·l^{-1} IAA + 0.001 mol·l^{-1} 4-HR + 0.005 mol·l^{-1} CaP + 0.0025 mol·l^{-1} Cys	Reduction of quinone + enzyme inhibition + strengthening cell wall	[12]
1% AA + 0.5% $CaCl_2$	Reduction of quinones + strengthening cell wall	[31]
2% AA + 1% CA + 1% NaHMP	Reduction of quinones + pH lowering	[46]
7% CaA	Reduction of quinones + strengthening cell wall	[10]
0.5% CaL	Strengthening cell wall	[47]
4% CaP	Strengthening cell wall	[33]
0.5% AA+ 1% $CaCl_2$ + 0.1% PA	Reduction of quinones + strengthening cell wall + pH lowering	[32]
1% NAcys + 1% Glut + 1% CaL	Reduction of quinones + strengthening cell wall	[48]
1% AA + 0.5% 4-HR + 1% Glut + 1% NAcys	Reduction of quinones + enzyme inhibition	[49]

AA – ascorbic acid, CA – citric acid, CaL – calcium lactate, CaA – calcium ascorbate, CaP – calcium propionate, 4-HR – 4-hexylresorcinol, IAA – isoascorbic acid, Cys – cysteine, NAcys – *N*-acetylcysteine, PA – propionic acid, Glut – glutathione, NaHMP – Na hexametaphosphate.

Germany). The homogenates were centrifuged for 10 min at 1789 ×*g* and then filtered through filter paper Whatman No. 4 (Whatman International, Maidstone, United Kingdom). The absorbance of the resulting clear juice was then measured at 440 nm using a spectrophotometer (DU 530, Beckman Coulter, Brea, California, USA). The determination was replicated three times at the start of the experiment and after 1, 3, 5 days of storage.

Sensory analysis

Sensory analysis of firmness and overall acceptability was conducted immediately after calcium salt solution treatment of apple pieces. Evaluations were made by 50 trained panelists using a five-point hedonic scale [26]. The panel was trained to recognize and score the quality attributes of the treated apple pieces. All assessments were compared to pieces freshly cut from whole air stored apples of the same cultivar and purchase date. The panelist were also asked if there were off-flavour or anomalous taste in the sample [27].

Statistical analysis

Statistical analysis was performed using Statistica 7.1 software package (Statsoft, Tulsa, Oklahoma, USA). Experimental data were processed with the variance analysis (ANOVA) according to Tukey's Honestly Significant Difference (HSD) test at $P = 0.05$ to compare means between treatments and control. Sources of variation were treatments and storage.

RESULTS AND DISCUSSION

Gas composition

During storage, an increase in CO_2 and a decrease in O_2 concentration occurred inside all packages (Fig. 1). The O_2 level decreased rapidly during the storage at all treatments. The highest concentration of O_2 on day 5 was found in the sample treated with Ca propionate + CA, the lowest level was found for Natureseal (about 0%). The CO_2 level increased rapidly at all treatments. On day 3, samples treated with Natureseal showed the lowest level of CO_2 (5.9% v/v) while at the end of the storage it became the highest (16.2%). Samples treated with Ca propionate + CA and $CaCl_2$ + CA showed the same trend reaching about 12% v/v on day 5.

The data suggest a high respiratory metabolism in apples treated with Natureseal. The apples consumed the oxygen of the headspace much more rapidly that the other two samples. These findings are in accordance with Gil et al. [28], who found that ascorbic acid dips increased the respiration rate of apple slices stored under air atmosphere (Natureseal contains derivatives of ascorbic acid). This effect might be pH-dependent as pointed out by Rocculi et al. [29] who reported an increase in the respiration rate of potatoes treated with ascor-

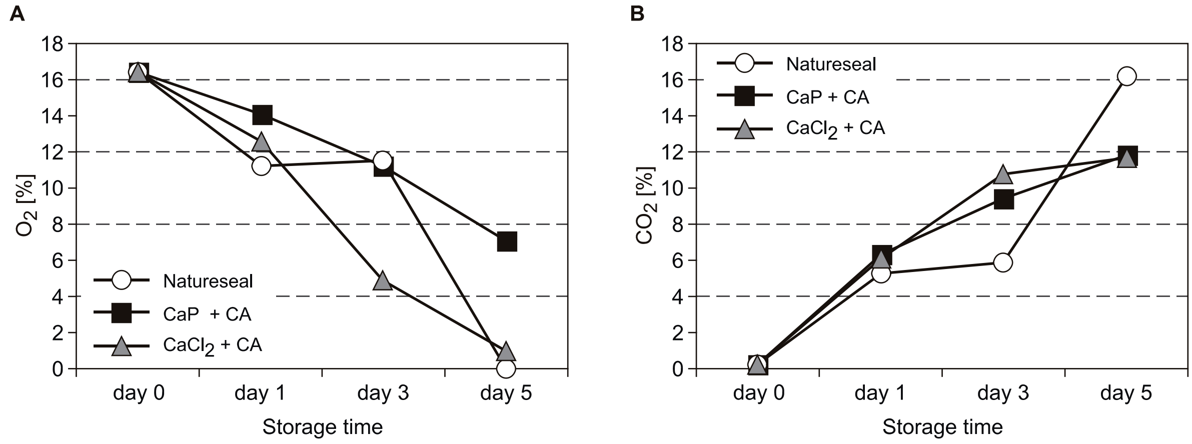

Fig. 1. Changes in O_2 and CO_2 levels measured in the plastic bags during five days of storage at 1 °C.

A – O_2, B – CO_2.
CaP – Calcium propionate, CA – citric acid.

Tab. 2. Effects of different treatments on titratable acidity and soluble solids content of minimally processed 'Golden Delicious' apples.

Treatment	Titratable acidity [meq·l⁻¹]		Soluble Solids [°Brix]	
	Day 0	Day 5	Day 0	Day 5
Natureseal	88.55 [a]	23.75 [c]	12.3 [a]	11.3 [c]
CaP + CA	87.36 [a]	32.90 [b]	11.8 [b]	12.1 [b]
$CaCl_2$ + CA	82.02 [a]	36.56 [a]	11.7 [b]	12.2 [a]

Different letters in the same column indicate significant difference in treatment effect ($p < 0.05$). Titratable acidity is expressed in milliequivalents of NaOH.

CaP – Calcium propionate, CA – citric acid.

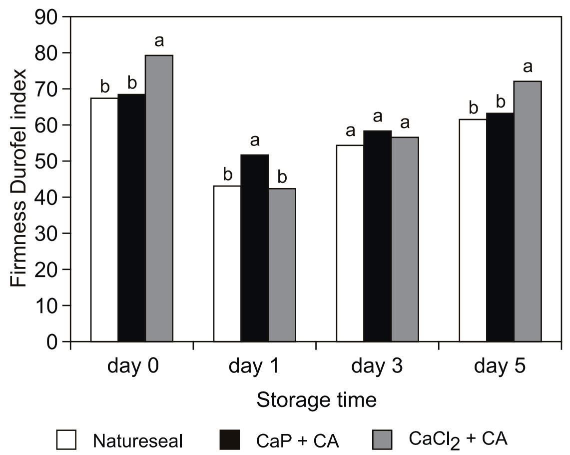

Fig. 2. Firmness Durofel index of minimally processed 'Golden Delicious' apples during storage (1° C).

Different letters show significant differences ($p < 0.05$) among treatments for each storage time.

CaP – Calcium propionate, CA – citric acid.

bic acid. The authors suggested that low pH was associated with an increasing demand for respiratory energy.

Titratable acidity and soluble solids content

Tab. 2 shows the effect of different treatments on titratable acidity (*TA*) and soluble solids content (*SS*). Results revealed that *TA* decreased during storage while *SS* increased or decreased slightly. On day 0, no significant differences were observed between samples concerning *TA*. At the end of storage, samples treated with Natureseal had lower acidity followed by Ca propionate + CA and finally by $CaCl_2$ + CA. Soluble solids content was found highest for Natureseal-treated sample on day 0, while on day 5, the same treatment showed the lowest value. The biggest changes in *TA* and *SS* probably reflected the high respiration rate of apples treated with Natureseal and the extreme values of CO_2 and O_2 reached at the end of storage seem to confirm this concept.

Firmness

The trend of the loss of firmness was the same for all samples. Firmness decreased on day 1 and then increased in the following days (Fig. 2). Samples treated with $CaCl_2$ + CA had the highest value of firmness on day 0 and day 5. No significant difference was found between samples on day 3. The other two treatments had a similar effect on firmness loss and the results were quite similar. As observed by Rössle et al. [24], Natureseal reduced firmness loss in consequence of cross-linking of both cell wall and middle-lamella pectin by calcium ions [30], but also Ca propionate + CA and $CaCl_2$ + CA were effective as firming agents.

In this study, best results were obtained on day 0 and 5 by $CaCl_2$ + CA. Calcium chloride has always been one of the most frequently used calcium salt when treating minimally processed fruits. SOLIVA-FORTUNY et al. [31] found that their use on fresh-cut apples maintained firmness over several weeks of storage. Also the results of VARELA et al. [32] showed that dipping of fresh-cut Fuji apple in 1% (w/v) $CaCl_2$ maintained the firmness of the sample for a week. In recent years, Ca propionate has been used as alternative source of calcium. Quiles et al. [33] found that fresh-cut apples treated with calcium propionate showed a reduced pectin methylesterase activity, probably as a consequence of the inhibiting effect of the propionate anions. In our study it was been possible to observe that all treatments maintained high values of firmness, which were close to the initial values.

Colour and browning potential

The L^* (lightness) and hue angle values decreased at all treatments during the storage and the differences found were significant. Sample treated with Natureseal showed always the highest L^* and hue values, so the results indicated that Natureseal inhibited browning most effectively (Fig. 3, Tab. 3).

Chroma describes the saturation of a colour and is presented in Tab. 4. Chroma values increased during storage in Ca propionate + CA and $CaCl_2$ + CA samples, while no significant differences were found in sample treated with Natureseal. Values were quite similar at every sampling date.

Browning was evaluated throughout a period of 5 days. Browning potential (*BP*) values were highest, for each treatment, on day 0 and lower at the end of storage period. Ca propionate showed greater absorbance than the other two treatments (Fig. 4) and the differences between treatments were always significant. Natureseal and $CaCl_2$ + CA samples were quite similar on day 1 and day 3. At the last sampling date, Natureseal showed the lowest *BP* values.

Appearance loss is the main factor that limits the shelf life of minimally processed fruits and vegetables [34–36]. Ca salts treatments are effective to reduce firmness loss [37, 38] and, in association with antibrowning agents, to improve the shelf life of fresh-cut products. In this study, regarding colour, Natureseal performed better than the other two Ca salts associated with citric acid though L^* and hue angle values decreased during storage. Our results are in accordance with published observations of RUPASINGHE et al. [39] and RÖSSLE et al. [24] on Natureseal, that found the

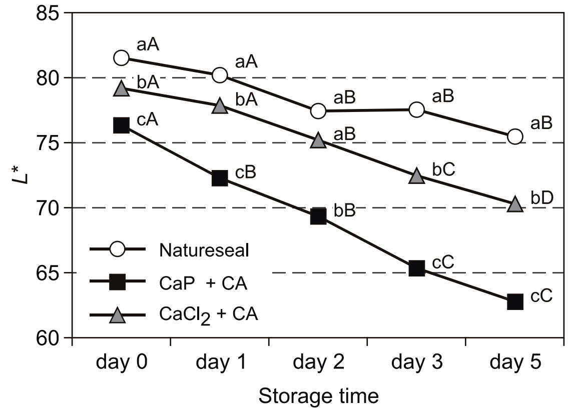

Fig. 3. L^* values of minimally processed 'Golden Delicious' apples during storage (1° C).

Minor and capital letters show significant differences (p < 0.05) among treatments for each storage time and during storage for each treatment, respectively.
CaP – Calcium propionate, CA – citric acid.

Tab. 3. Hue angle values of minimally processed 'Golden Delicious' apples during storage (1° C).

Storage time	Hue angle		
	Natureseal	CaP + CA	$CaCl_2$ + CA
day 0	104.62 ^{aA}	98.44 ^{aC}	102.55 ^{aB}
day 1	103.54 ^{aA}	94.59 ^{bcC}	101.36 ^{bB}
day 2	103.80 ^{aA}	94.18 ^{bcC}	100.50 ^{bcB}
day 3	104.25 ^{aA}	94.80 ^{bC}	99.69 ^{cB}
day 5	102.11 ^{bA}	93.37 ^{cC}	96.43 ^{dB}

Minor and capital letters show significant difference (p < 0.05) during storage for each treatment and among treatments for each storage time, respectively.
CaP – Calcium propionate, CA – citric acid.

Tab. 4. Chroma values of minimally processed 'Golden Delicious' apples during storage (1° C).

Storage time	Chroma		
	Natureseal	CaP + CA	$CaCl_2$ + CA
day 0	23.51 ^{aC}	29.73 ^{bA}	26.24 ^{cB}
day 1	24.32 ^{aC}	32.99 ^{aA}	27.08 ^{bcB}
day 2	24.17 ^{aC}	33.70 ^{aA}	28.37 ^{abB}
day 3	24.75 ^{aC}	33.21 ^{aA}	29.09 ^{aB}
day 5	24.78 ^{aC}	32.33 ^{aA}	30.07 ^{aB}

Minor and capital letters show significant difference (p < 0.05) during storage for each treatment and among treatments for each storage time, respectively
CaP – Calcium propionate, CA – citric acid.

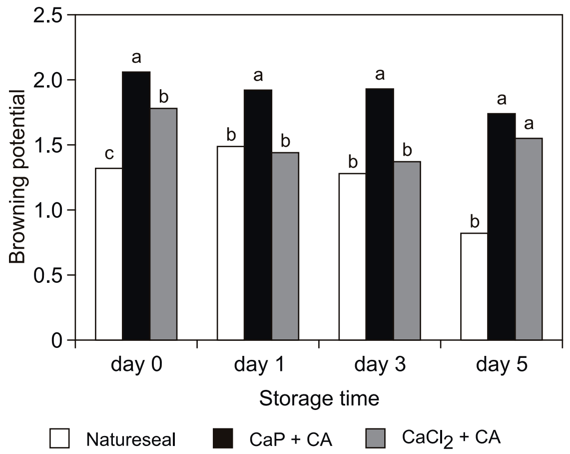

Fig. 4. Browning potential values of minimally processed 'Golden Delicious' apples during storage (1° C).

Different letters show significant differences (p < 0.05) among treatments for each storage time.
CaP – Calcium propionate, CA – citric acid.

same trend of the two parameters. $CaCl_2$ + CA was also reported to reduce browning [20, 40–42], probably due to polyphenol oxidase inhibition by the chloride ion. In our hands, Ca propionate + CA gave unacceptable results regarding *BP* determination as a method to measure the intensity of enzymatic browning. The low values of absorbance confirmed the best performance of Natureseal, and a similar efficacy of $CaCl_2$ + CA.

Sensory analysis

Differences between the treatments were found by panelist, in particular, apples treated with Ca propionate + CA obtained the lowest evaluation of firmness, while the other two treatments were found similar at this property. For overall acceptability, no difference was observed between the treatments. However, the scores assigned were quite low (Tab. 5). Moreover, off-flavours were found in Ca propionate + CA samples, while in Natureseal and $CaCl_2$ + CA samples, the panelists perceived anomalous tastes.

The sensorial analysis confirmed the results obtained by instrumental analysis. The results were in concordance with Luna-Guzman and Barrett [13], who observed that $CaCl_2$ + CA treated samples were significantly firmer than the other samples. However, regarding the overall acceptability, panelists assigned analogous scores to all treatments.

Tab. 5. Mean sensory scores of fresh-cut 'Golden Delicious' apples after treatment.

Sensory characteristic	Treatment	Mean values from sensory scale
Firmness	Natureseal	2.78 a
	CaP + CA	2.17 b
	$CaCl_2$ + CA	3.10 a
Overall acceptability	Natureseal	1.96 a
	CaP + CA	1.93 a
	$CaCl_2$ + CA	1.89 a

Different letters in the same column indicate significant difference (p <0.05). For each attribute, a higher value represents higher intensity on a 0–5 scale.
CaP – Calcium propionate, CA – citric acid.

CONCLUSION

In conclusion, this study showed that Natureseal was highly effective in maintaining the quality of fresh-cut apple, but also $CaCl_2$ + CA could be a good method to preserve the same product for 5 days, though sometimes anomalous tastes were perceived. The treatment with $CaCl_2$ + CA could be used in small fresh-cut industry as a cheap alternative (by 30% cheaper) to commercial products. Ca propionate + CA cannot be recommended for the purpose, though it was effective at maintaining firmness, *TA* and *SS*, but allowed high browning and was sometimes associated with off-flavours.

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