Vacuum infusion of pectin methylesterase and calcium maintains firmness of the fresh-cut strawberry

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Abstract

Fruit quality is related to maintenance of texture. Fruit firmness is a critical factor for successful harvest and handling. Solutions of pectin methylesterase (PME) and/or calcium were vacuum infused into fresh-cut strawberries to determine the potential to better maintain cellular integrity. The calyx and peduncle of strawberries (*Fragaria* x *ananassa*) fruit were removed and strawberries were cut in half (longitudinally). After cut strawberries were immersed in cooled chlorinated (5±0.5 °C, 200 mg L^{-1}) during 10 min. After the sanitization the strawberries were submitted to rinse and to drainage during 5 min perforated trays at the processing environment temperature, 5±1°C under 79±2 % RH. Infusion treatments were: non-vacuum treated (C, control), water (WI), PME solution (10 U mL^{-1}), PME + 1% (w/v) calcium lactate (PME + Ca) and 1% (w/v) calcium lactate (Ca). After infusion, each drained replicate (100 g fresh cut strawberries) was packaged on PP trays with a layer of napkin in the bottom and wrapped in OPA PP (ORVED) film and stored at 5 ± 1 °C and 78-82% RH. After 4 and 8 days 4 samples each treatment were analyzed for quality (decay, freshness, bruising, firmness) and for composition (alcohol insoluble solids), electrolytic leakage, galacturonic acid (GalA) content and degree of methylation (DM). Infusion increased mass retention of the fruit by 4%, indicating water uptake occurred. Infusion of PME increased electrolytic leakage after 4 days of storage. PME + Ca decreased DM of pectin and increased firmness. The infusion in all treatments did not alter significantly the GalA content (200 $mg.g^{-1}$ AIS). Calcium lactate resulted in maintenance to DM indicated possible calcium lactate may inhibit the PME endogenous or promote higher "egg-box" conformation. The use of the vacuum infusion of PME + Ca shows potential as a method to preserve quality and firmness of strawberries.

Keywords: *Fragaria* x *ananassa,* postharvest, vacuum infusion, calcium lactate, PME, fruit quality.

Resumo

Infusão a vácuo de pectina metilesterase e cálcio na manutenção da firmeza de morangos minimamente processados.

A qualidade de frutos está relacionada com a manutenção da textura. A firmeza dos frutos é um fator crítico para a colheita e manuseio eficiente. Soluções de pectina metilesterase (PME) e/ou cálcio foram aplicadas por meio de infusão à vácuo em morangos minimamente processados para manter a integridade e qualidade celular. O cálice e o pedúnculo de morangos (*Fragaria* x *ananassa*) foram removidos e os frutos cortados longitudinalmente. Após cortados foram imersos em água clorada (5 ± 0,5 °C, 200 mg L^{-1})

por 10 min. Após higienização, morangos foram submetidos ao enxágue e drenagem por 5 minutos em bandejas perfuradas mantidos a 5 ± 1 °C e 79 ± 2% de UR. Os tratamentos de infusão foram: tratamento sem vácuo (C, controle), água (WI), solução de PME (10 U mL^{-1}), PME 1% (p/v) + lactato de cálcio (PME + Ca) e 1% (p/v) lactato de cálcio (Ca). Após a infusão, a solução foi drenada e cada repetição (100 g) foi embalada em bandejas PP (100 x 140 x 40 mm) com uma camada de guardanapo no fundo, selada com filme OPA PP (ORVED) e armazenada a 5 ± 1 °C e 78-82% de UR. Após 4 e 8 dias, 4 amostras de cada tratamento foram analisadas quanto à qualidade (deterioração, frescor, danos e firmeza) e composição (sólidos insolúveis em álcool (AIS), extravazamento eletrolítico, teor de ácido galacturônico (GalA) e grau de metilação (DM). A infusão de PME aumentou o extravazamento eletrolítico após 4 dias de armazenamento. PME + Ca reduziu a DM e aumentou a firmeza. A infusão não alterou o teor de GalA (200 mg.g-1 AIS). O lactato de cálcio resultou em manutenção do DM indicando uma possível inibição do lactato de cálcio na PME endógena ou promoção de uma maior conformação de "caixa de ovo". O uso da infusão a vácuo com PME + Ca é um método potencial para preservar a qualidade e a firmeza dos morangos.

Palavras-chave: *Fragaria* x *ananassa,* pós-colheita, infusão a vácuo, lactato de cálcio, PME, qualidade de frutos.

Introduction

Strawberry (*Fragaria* x *ananassa)* is appreciated for its characteristic color, flavor, and aroma, however, are among the most perishable fruits and susceptible to mechanical injury, water loss, decay, and physiological deterioration (Mitcham, 2016).

Fresh-cut fruit are generally more perishable than whole fruits because they have been subjected to physiological stresses caused by wounding and minimally processing methods (Ma et al., 2017). The fresh cut fruit quality result of reducing on deterioration rate and maintenance of texture, color, flavor and aroma, to keep them attractive to the consumer as long as possible (Rico et al., 2007). The texture of fruit is one of the most important factors to determine the acceptance by consumers which may limit the acceptance aspect when food is very different from what the consumer expects (Shewfelt, 1999).

The decrease on firmness (texture of the fruit pulp) in intact or fresh cut fruits has been attributed to changes and degradation of cell wall components such as cellulose, hemicellulose and pectin (Ma, et al, 2017). Decrease on firmness during ripening is associated with the degradation of pectin in the cell wall, caused by the activity of hydrolases, such as pectin methylesterase (PME, EC 3.1.1.11) and polygalacturonase (PG) (Kohli et al., 2015).

The degradation of the polysaccharide can be performed by primary demethylation with pectinesterase, releasing methanol and the formation of pectates by depolymerization (decrease on the size of the polymer chain) with hydrolysis (acid or enzymatic) of bonds α (1 → 4) PG, or β-elimination reactions under the action of pectin lyase and pectate lyases of microbial origin (Singthong et al, 2004). The demethylation of pectin results in a higher number of carboxylic acid groups, which can facilitate the action of polygalacturonase, which degrades pectin (Michelli, 2001). Pectin demethylation can improve the texture of fruits and vegetables since the resulting free carboxyl groups can be crosslinked with

divalent ions such as Ca^{+2}, forming a fortifying network. Moreover, a decrease on the degree of methoxylation (DM) reduces the sensitivity of pectin to thermal depolymerization by β-elimination (Kohli et al., 2015).

Recently studies have been done using the PME infusion or PMR plus Calcium infusion in fresh-cut papayas (Yang et al., 2017), apples (Guillemin et al., 2008) and strawberries fruits (Fraeye et al., 2009). Fraeye et al. (2010) evaluated the evolution of the texture of strawberries infused with PMEs and calcium, and verified increase on firmness and preservation on microstructure under high pressure. Guillemin et al. (2008) verified on apple that incorporated PME, or by dipping or by vacuum impregnation, resulted in better penetration and more even distribution of the enzyme in the fruit and the use de PME plus calcium resulted in a firming effect.

Calcium forms covalent bonds with homogalacturonan loaded, thus strengthening the cell wall. The calcium and the pectin complex ("egg box" model) acts as a cement providing firmness to the plant tissue and contributing on the maintaining cell membrane integrity, promotes delaying maturation and senescence and is a signaling in ethylene biosynthesis and signal transduction (Aghdam et al., 2012).

Calcium chloride is commonly used as a firming agent with many products in industries, such as apples (Sams et al., 1993) and strawberries (Garcia et al., 1996). Studies have shown that the presence of residual calcium found at the food surface confers bitter taste (Luna-Guzman & Barrett, 2000). The use of calcium lactate can be an alternative to solve this problem. It was tested in processed strawberries (Morris et al., 1985) and mangoes (Kirtil et al., 2014). In addition, Luna-Guzman & Barrett (2000) evaluated the use of calcium lactate and calcium chloride on maintaining the firmness of minimally processed cantaloupe, they verified the same hardening effect, but firmness was higher on samples treated with calcium lactate. In fresh cut "Gaia melon", a considerable loss of flavor was verified, except the treatments with Ca chlorine, lactate, and ascorbate, found acceptable from the consumer point of view (Silveira et al., 2011).

The aim of this work was to evaluate the utilization of PME and Ca^{+2} as an alternative treatment for maintaining the firmness and quality of the fresh cut strawberries in order to provide a product with proper texture for the market.

Material and Methods

Fresh-cut processed

Strawberry fruits were obtained from a local market. The calyx and peduncle were removed by hand and the peduncle using a sharp blade, 3 mm from the base. Strawberries were cut in half (longitudinally) using sharp blades. After cut strawberries were immersed in cooled chlorinated water at 5±0.5°C at the concentration of 200 mg L^{-1} during 10 min. After the sanitization the strawberries were submitted to rinse at in cooled chlorinated water at 5±0.5°C at the concentration of 5 mg L^{-1} during 10 min, then strawberries were submitted to drainage during 5 min perforated trays at the processing environment temperature, 5±1°C under 79±2 % RH.

Vacuum infusion of strawberries

The infusion was done by dipping fresh cut strawberries in a solution (100 g strawberries per 150 ml at 5° C). The beaker containing the fruits was placed in a desiccator connected to a vacuum pump; when switching on the pump, pressure decreased quickly.

Starting from the moment a pressure of 12Kpa was reached, the strawberries were left in the vacuum for 10 minutes. After this time the pressure slowly dropped to 0 kPa and was built up slowly (ca 1min) to atmospheric pressure. The infusion solution was discarded. Each group of strawberries was drained and weighed just after infusion.

The fresh cut strawberries were infused divided into 5 treatments: fresh cut strawberries without vacuum infusion (C) this treatment was used as control, only water vacuum infusion (WI), Pectin methylesterase (PME) infusion solution, Pectin methylesterase plus 1% (w/v) Calcium lactate (PME+ $(C_3H_5O_3)_2Ca$) infusion solution and only 1% (w/v) calcium lactate ($(C_3H_5O_3)_2Ca$) infusion solution. A commercial preparation of *Aspergillus oryzae* PME (Novoshape, NovozymesBagsvaerd, Denmark) was diluted till a final enzymatic activity of 10 U mL^{-1}.

After the infusion, the fresh cut strawberries were dried for at 5 min on perforated trays at the processing environment temperature, 5±1°C under 79±2 % RH. After dried 100 g fresh cut strawberries were placed on PP trays (100 x 140 x 40 mm) with a layer of napkin in the bottom and wrapped in OPA PP (ORVED) film. The OPA PP film was perforated so that the O_2 and CO_2 concentration stay in 21 and 0.03 %, respectively and the kept in a cold room at 5±0.5ºC, under 79±2 % RH from 8 days. Each 4 days samples were removed to isolation of alcohol insoluble solids (AIS), electrolytic leakage, quality analysis (decay, freshness and bruising), firmness analysis, determination of galacturonic acid (GalA) content and degree of methylation (DM).

Isolation of alcohol insoluble solids (AIS)

The alcohol insoluble solids, with cell wall material were isolated from raw samples (Huber, 1984). 20 g of strawberries were homogenized in 80 mL of 100% ethanol using a Sorvall Ominimixer for 3 min. The homogenate was refluxed for 30 min in boiling water bath. When necessary 100% ethanol was added to keep ethanol at the initial level. Then it was storage overnight at -20 ^oC. The suspension was filtered through glass microfiber filter (GF/C 55.5mm) in an aspiration flask. The material was washed with 200 mL 80% ethanol under slow aspiration. Then washed with 100 mL 100% acetone. Acetone was removed by aspiration and the powder-air dried in an oven (39 ^oC) for one day.

Electrolytic Leakage

Mesocarp of fresh cut strawberries was excised in cubes (5mm x 2mm), 2g this tissue was rinsed with water distilled, dried on paper and placed into glass tubes with 35mL of 0.4M isotonic mannitol solution and kept at 23^o C for 4h (Villalta & Sargent, 2004). The conductivity of the bathing solution was measured after 4 h of incubation at 25^oC by an YSI-31, conductivity bridge equipment with a conductivity cell (Model 3403, Yellow Springs, OH, USA). The total electrolyte content was determined after (24h at -20 ^oC), thawing and heating the cubes and in a boiling water bath for 30 min. Electrolyte efflux was expressed as a percentage of total tissue electrolytes.

Quality analysis (freshness, decay, and bruising)

The freshness was determinate in according to the following scale: 9 = excellent, full fresh appearance, high sheen; 7=good: still looks fresh, still shiny; 5=fair: not fresh appearance, low sheen, limit of marketability; 3=poor: dull, limit of usability; 1= extremely poor, shriveled appearance. The decay was determinate on the number of pieces of fruits

with no incidence of postharvest decay, especially visible mycelia growth. The bruising was determinate on the number of pieces of fruits with no postharvest mechanical damage.

Firmness analysis

The pulp firmness was measured on opposing sides of each fresh cut fruits (n=4 fresh cut fruits/clamshell; 16 fruits/treatment/analysis). Using an Instron universal Testing Instrument model 1132 (Instron Corp, Canton, MA) with a 5 kg load, crosshead speed of 10 cm min-1 and a 4mm diameter convex probe. The maximum force necessary to penetrate 3mm into the pulp was determinate and results were expressed in Newton (N).

Determination of galacturonic acid (GalA) and degree of methoxylation (DM)

The GalAc content of AIS was determined as a measure for pectin content. DM of the pectin in AIR was calculated as the ratio of the molar amount of methanol esters to the molar amount of GalA residues. To determine the GalA content, pectin was hydrolyzed using sulfuric acid and next to the concentration of GalA was quantified using the spectrophotometric method as described by Ahmed & Labavitch (1977). To estimate the amount of methanol esters, a pectin solution was saponified to pectate and methanol according to the procedure described by Ng and Waldon (1997). The amount of methanol release was measured using the spectrophotometric method of Wood and Siddiqui (1971).

Statistical analysis

The experiment was performed according to a completely randomized design with 4 repetitions each treatment and the experiment was made two time and the data were analyzed by ANOVA and the averages were compared by least significant difference test for multiple comparisons test, where differences between two greater than the sum of two standard deviations treatments were considered significant at the 5% probability (Shamaila et al., 1992).

Results and discussion

Influence of infusion on weight, cell integrity and quality

Upon infusion treatment the strawberries weight increased by 4% (fig. 1). This weight gain of strawberries was due to water uptake by the strawberries and retained by the strawberries. This results also verified by Fraeye et al. (2010) to strawberries after enzyme infusion. After 4 days of storage the weight was similar to initial weight because the fresh-cut strawberries were stored in a cold room at 5 ^{o}C with 79% RH in perforated trays. This condition did not affect the strawberries appearance.

Electrolyte leakage (fig. 2) from fresh-cut fruits remained constant (about 50%) in all treatments during storage. By contrast, fruit treated with PME exhibited an increase in electrolyte leakage on 4 days of storage. The role of PME has been intensively examined in relation to modification in the cell wall structure (Pelloux et al, 2008). The demethylesterification due to the PME activities can release protons that promote the action of endopolygaracturonases and contribute to cell wall loosening (Micheli, 2001). This effect can also modify the structure of the cell membrane and facilitate the loose of the electrolytes.

No decay was found on fresh-cut fruit at 4 days after vacuum infusion, however, all treatments showed about 8,5% decay at 8 days at storage. Strawberries were lower sensitive to decay. This lower decay might be related to the efficiency of the sanitization prior the infusion and to the storage temperature (5 ^{o}C).

Bruising was verified on fruits after 8 days storage. These results are probably a consequence of good handling and the type of used trays which protected the fresh cut strawberries from bruising during storage.

Strawberries appeared fresh during cold storage, but they lost the fresh appearance after 8 days of storage. The freshness was rated as excellent (around 9=full fresh appearance, high sheen) after cut in all treatments but decreased to around 8 (good) when without treatment or only water or PME infusion. When treated with only calcium the fresh-cut freshness was 9 after 4 days. A storage period of 8 days to fresh cut was too long to maintain the good appearance of the product. Normally the shelf life to fresh cut strawberries is shorter (Costa et al., 2011).

Firmness

The initial firmness of the fresh cut strawberries varied slightly during the storage time (fig. 2). For treatments with PME plus calcium lactate infusions the firmness increased significantly. The use of the PME or calcium lactate increased the firmness when compared with the controls, but less than the use of the PME plus calcium lactate (fig. 3). Fraeye et al. (2010) had similar results, they verified that strawberries firmness increased significantly upon PME+Ca^{+2} infusion. This behavior was attributed to the first PME action on the demethylation of pectin results in a higher number of carboxylic acid groups, which facilitate the bridging by Ca^{+2} of antiparallel homogalaturonic chains with negatively charged carboxyl groups to pectin form structure called “egg-boxes” increase the firmness to product (Aghdam et al., 2012, Guillemin et al, 2008).

Galacturonic acid (GalA) content and degree of the methoxylation (DM)

The infusion did not alter significantly the GalA content in AIS , the average of the GalA was 200 mg.g^{-1} AIS (fig. 3). Similar results were obtained by Fraeye et al. (2010), with the GalA content was about 175 mg GalA g^{-1} AIR and the infusion did not alter de GalA content of AIR of the strawberries.

The DM of pectin in AIS from fresh cut strawberries varied from 35 to 73% (fig. 3). Fraeye et al., (2009) verified the DM of pectin in AIS from fresh strawberries was 74% and Duvetter et al. (2005) found that strawberries non-infused the DM was 67%. Nonetheless the use of PME plus calcium lactate the DM was 35%, indicating that PME had acted on the pectin and facilitated links with calcium and pectin (“egg-boxes” model) increased the firmness of the product (fig. 3).

The use of calcium lactate resulted in maintenance to DM in fresh-cut strawberries (fig. 3) indicated possible calcium lactate may inhibit the PME endogenous or promote higher “egg-box” conformation which hinders the activity of the PME increasing the methylation degree (Aghadam et al., 2012).

Conclusions

Infusion resulted in increased the mass of strawberries indicated water uptake by the strawberries. The use of PME exhibited an increased in electrolyte leakage in 4 days of storage.

The infusion of PME+Ca^{+2} in fresh-cut strawberries resulted in a decrease in DM of pectin and increase in firmness probably due to the action of the PME on the demethylation facilitating the link of calcium.

The use of the vacuum infusion and the PME plus calcium on fresh cut strawberries showed as an alternative to preserve the firmness of the product. However further experiments should be done to investigate with more details the effect of the vacuum infusion with PME and calcium on fresh cut strawberries.

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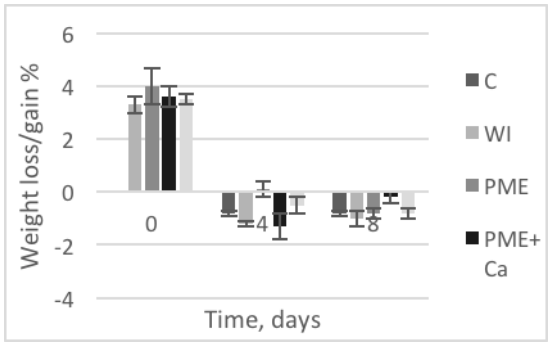

Figure 1 - Weight gain/loss (%) after vacuum infusion (day 0) and during storage (4 and 8 days) of strawberries at 5º C. C-control, WI-Water infusion; PME- only PME infusion, PME + Ca -PME plus Calcium lactate infusion and Ca-only calcium lactate infusion.

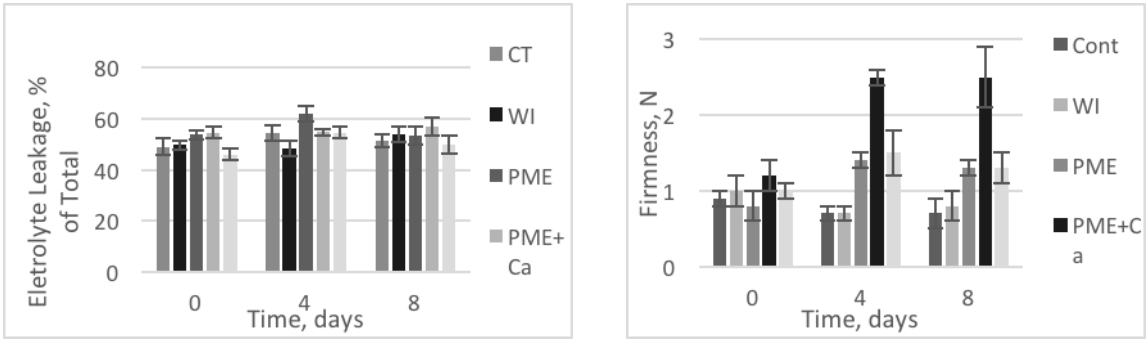

Figure 2 - Electrolyte leakage (%) and Firmness after vacuum infusion (day 0) and during storage (4 and 8 days) of strawberries at 5º C. C-control, WI-Water infusion; PME- only PME infusion, PME + Ca-PME plus Calcium lactate infusion and Ca-only calcium lactate infusion.

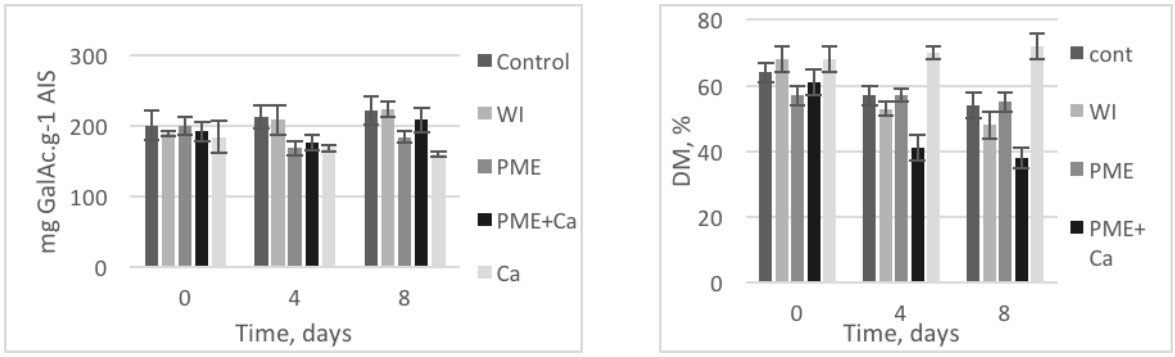

Figure 3 - Galacturonic acid (GalAc) content (mg GalAc. g^{-1} AIS) and Degree of the methylation - DM (%) of fresh cut strawberries after vacuum infusion (day 0) and during storage (4 and 8 days) of strawberries at 5º C. C-control, WI-Water infusion; PME- only PME infusion, PME + Ca-PME plus Calcium lactate infusion and Ca-only calcium lactate infusion.