Chiang Mai J. Sci. 2016; 43(3) : 556-569
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Combined Effect of Calcium Chloride and Modified Atmosphere Packaging on Texture and Quality of Minimally-Processed Litchi Fruit

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Received: 22 June 2015
Accepted: 30 October 2015

ABSTRACT

The combined effect of calcium chloride treatment (1%, 25 °C) and modified atmosphere packaging (MAP) (5% O_2 + 5% CO_2 with N_2 balance) were studied in litchi arils *cv.* Jugkapat, compared to the control which was packed in normal atmosphere (20.43% O_2 + 0.03% CO_2). The physical, biochemical, physiological, microbiological and morphological properties were investigated during the storage for 18 days at 2±1 °C. The combined effect of calcium chloride and MAP reduced the loss of firmness and juice leakage, and retarded the increasing of ethanol content, total bacteria and yeast-molds counts. PME and PG activities in modified atmosphere (MAP) storage were lower than those in the control throughout the storage period. According to microstructure analysis, the combined effect of calcium chloride and MAP reinforced the cell wall and retarded the loss of cell turgor during storage period.

Keywords: minimally-processed litchi, calcium chloride, modified atmosphere packaging, pectin methylesterase, polygalacturonase

1. INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is an exotic subtropical fruit originated from Southeast Asia. The fruit is naturally bright-red in color surrounding the succulent edible aril and a single seed in the center. The fruit possesses a large amount of aromatic compounds and high sugar contents with several minerals and vitamins. It can be eaten fresh or as a process product [1]. The processing of litchi fruit into minimally-processed or fresh-cut product is an alternative method to preserve the arils as fresh-like fruit with high nutritional quality which could create marketing opportunities [2]. However, litchi arils become softened after three days of storage and lead to juice leakage during storage [1]. Litchi fruit *cv.* 'Jugkapat' is the most appropriate cultivar for minimally-processed product because of larger fruit size,

ease of deseeding, absence of brown color on the internal surface and high vitamin C content. However, a loss of firmness of delicate arils is observed during the storage period [1].

Because of the response to wounding and damage during processing, minimal processing of fruits causes increase in respiration of tissue, accelerating cell membrane degradation which results in the loss of product quality [3]. The application of atmosphere low in oxygen (O_2; 1-5%) and high in carbon dioxide (CO_2; 5-10%) has been shown to extend the shelf-life of fresh-cut fruit by reducing respiration, ethylene biosynthesis and product transpiration. Moreover, there have been reports on inhibition or delay of enzymatic reactions, retardation of the microbial growth and preservation of the product from quality loss [4].

Use of various calcium salts has been proven to be effective in improving or retaining the firmness of minimally-processed fruit and vegetables. Calcium ions can form cross-links or bridges between free carboxyl groups of the pectin chain, and make complexes to cell wall and middle lamella polygalacturonic acid residues, resulting in improving structural integrity [5]. Calcium chloride ($CaCl_2$) is one of the most frequently-used calcium salts for treating minimally-processed fruit. The concentration used is usually in the range of 0.5-1% for 1-5 min. Other calcium salts which provided antimicrobial effect and are recognized to have GRAS status (Generally Regarded as Safe) are calcium lactate and calcium propionate [6].

Thus, the middle lamella is pectin-rich which helps bind the cell wall of two adjoining cells together. The enzymes related with the fruit texture are pectin methylesterase (PME, EC 3.1.1.11) and polygalacturonase (PG, EC 3.2.1.15). PME is a cell wall-bound enzyme, which deesterify pectin, converting it into low methoxy pectin or pectic acids. PME is specific for galacturonide esters and its action is to remove methoxyl groups from methylated pectin. After PME action, partially-demethylated pectins are later depolymerised by PG. PG is a hydrolytic enzyme which acts on pectic acid (polygalacturonic acid, PGA). It hydrolyses the α-1,4-glycosidic bonds between the galacturonic acid residues in galacturonans, causing a loss of firmness [5].

There is a limit reported in the used of calcium salt in minimally-processed litchi fruit. The application of 0-2% calcium lactate (CL) in combination with 4-hexyl resorcinol (4-HR) and 1% potassium sorbate stored in package vacuum; CL reduced drip losses significantly and sensory color scores were improved with addition of 4-HR. Furthermore, in-package vacuum was not influence affected on drip loss and sensory color score [7]. The packing of litchi arils in a polystyrene clamshell container [1] and polyethylene bag under vacuum [2] have been reported. However, the investigation into other gas combinations has not been reported.

Thus, the objective of this research work was to study textural and quality changes of minimally-processed litchi fruit preserved by calcium chloride treatment and modified atmosphere packaging (5% O_2 + 5% CO_2) during storage at 2±1 °C. Furthermore, microstructure of arils and cell wall enzyme activities were also investigated to understand the mechanism clearly.

2. MATERIALS AND METHODS

2.1 Materials

Litchi (*Litchi chinensis* Sonn.) fruit *cv.* 'Jugkapat' at the fully-red peel color (commercially-harvesting stage) was harvested from local orchards during July-August,

2014, Fang district, Chiang Mai province, Thailand. Fruits were packed in cardboard boxes and transferred to laboratory room within 3 h under the temperature of 25 °C. After an overnight storage at 4±1 °C, fruits were selected for uniform size, shape, color and free of defects for the experiment.

For minimal processes, all equipment were sanitized with 200 mg L^{-1} sodium hypochlorite (CLOROX®, California, USA). Fruits were randomly distributed into groups of 240 fruits per treatment. Whole fruits were dipped in 100 mg L^{-1} peroxyacetic acid (PAA) solution (BLRLOX-5®, Thai Peroxide Co., Ltd., Saraburi, Thailand) for 3 min [8] to reduce initial microbial populations on the surface of pericarp. The seed was removed with sterilised sharp stainless steel knife by coring around the stem-end of fruit, followed by manual peeling. Arils were dipped in 1% calcium chloride for 3 min, followed by sanitizing in 50 mg L^{-1} PAA for 1 min and finally drained to remove excessive water.

2.2 Packaging and Experimental Setup

Twelve arils were placed in polystyrene tray and then packed in nylon laminated with linear low density polyethylene bag (Nylon/LLDPE) under 5% O_2 + 5% CO_2 with nitrogen balance atmosphere, using a modified atmosphere packaging unit (Henkovac International, Netherlands) and stored at 2±1 °C. Twenty bags per treatment was used and the weight of the bags including twelve arils was around 310-330 g. The Nylon/LLDPE bags had the dimensions of 72 µm thickness, 33 cm length × 21 cm width. O_2 and CO_2 transmission rates of Nylon/LLDPE bags were 73.6 and 161 $cc.m^{-2}/day^{-1}$, respectively, at 23 °C. Treatment was compared with those packed in air (20.43% O_2 + 0.03% CO_2) as the control.

2.3 Oxygen and Carbon Dioxide Concentration Inside Package

Concentration of O_2 and CO_2 were monitored using headspace gas analyzer (Model 900151, Bridge analysers Inc., California, USA) with non-dispersive infrared (NDIR) and electro chemical sensor for CO_2 and O_2, respectively, every 2 days. Gas samples were automatically withdrawn through an adhesive rubber septum with a sampling needle directly connected to the injection system. Data were reported as percentage.

2.4 Ethanol Content

The ethanol content in the fruit was determined by gas chromatographic analysis of head space according to the method developed by Davis and Chace [9] with slight modification. Five grams of blended arils were placed in a 10 mL amber glass bottle with rubber cap and incubated in a water bath at 80 °C for 45 min. Headspace gas was withdrawn using a 1 ml syringe and injected into a TRACEGC gas chromatograph (ThermoQuest Italia SpA., Milan, Italy) equipped with a flame ionisation detector. The temperature of the oven, injector and detector were 150, 175 and 200 °C, respectively. The column used was a 30 m × 0.53 mm i.d. × 1 µm OV-1 (100% dimethylpolysiloxane) capillary column. Retention time and standard curve of absolute ethanol in water solutions concentration (0 to 3,600 mg L^{-1}) were used for peak identification and quantification.

2.5 Juice Leakage and Textural Properties

Juice leakage was separated from the package by withdrawing, using 10 mL syringe and was defined as mL 100 g^{-1} fresh weight. The firmness of arils was evaluated by a puncture test using texture analyzer (TA.Xt plus; Stable Micro Systems Ltd., Surrey,

UK) at 25 °C, equipped with 50 kg load cell. A 2 mm diameter stainless steel cylindrical probe was set to penetrate 5.0 mm into the arils and the test speed before and after the measurement of the penetration test was 1 mm per second. Aril was cut along the length into two parts. Each treatment was measured for 48 samples. The unit of force was expressed as Newton (N).

2.6 Scanning Electron Microscopy

Scanning electron microscopy was used to observe the effect of calcium chloride combined with modified atmosphere packaging on morphological changes of minimally-processed litchi fruit during storage at 2±1 °C. Cross-section of litchi aril was cut into the dimension of width × length × thickness at 0.5 × 0.5 × 0.2 cm^3. Nine sections of tissue from each treatment were fixed with a 2.5% glutaraldehyde solution for 1 h, post-fixed with 10 g L^{-1} osmium tetroxide solution for 2 h and dehydrated, using a graded ethanol series (30, 50, 70, 80, 90 and 100%) for 15 min at each step. Samples were subjected to critical point drying (CPD7501, Polaron, UK) [10]. The specimens were examined with a scanning electron microscope (SU-70; Hitachi, Tokyo, Japan), using low vacuum mode (LV) at an accelerating voltage of 3 kV, with the magnification of ×200.

2.7 Pectin Methylesterase Activity

Pectin methylesterase (PME) was extracted and assayed according to the method of Hagerman and Austin [11]. All solutions, pectin, bromothymol blue, and crude enzyme were adjusted to pH 7.5 in advance. For the enzymatic activity assay, the reaction mixture was composed of 2.00 ml of 0.5% pectin (w/v), 150 µL of 0.1% bromothymolblue (w/v) and 750 µL of distilled water. The reaction was started by adding 100 µL of crude enzyme, and the rate of decrease in absorbance at 620 nm (A_{620}) of the mixture was recorded versus water for 1 min using a spectrophotometer (Jasco V-530, Hachioji, Tokyo, Japan). Protein content was analyzed using Bradford method [12]. Specific activity was calculated by the activity of an enzyme per milligram of total protein per min (µmol min^{-1} mg^{-1}).

2.8 Polygalacturonase Activity

Polygalactoronase (PG) was extracted according to the method of Gross [13]. PG activity was determined by measuring the reducing groups released from polygalacturonic acid (Sigma-Aldrich, Darmstadt, Germany). Reducing groups were quantified using 3, 5-dinitrosalicylic acid according to the slightly modified method of Luchsinger [14]. The reaction mixtures, containing 1 ml of 0.5% polygalacturonic acid, 1 ml of 0.1 M sodium acetate buffer (pH 4.5), 100 µl of crude enzyme, and 900 µl of deionized water, were incubated at 30±2 °C for 30 min. After incubation, 1 ml of 3, 5-dinitrosalicylic acid solution was added and then boiled at 95±3 °C for 10 min. After cooling the reaction mixture to room temperature using ice water, the change in the absorbance at 575 nm was determined. Galacturonic acid (0-1.0 mg mL^{-1}) was used as the standard reference. Protein content was analyzed according to the method of Bradford [12]. Specific activity was calculated by the activity of an enzyme per milligram of total protein per min (µmol min^{-1} mg $protein^{-1}$).

2.9 The Degree of Esterification of Pectin

The degree of esterification (DE) was calculated using the moles ratio of the amount of methanol and the amount of galacturonic acid [15] as follows;

$$DM = [((N_{methanol})/(AIR)/((N_{galacturonic\ acid})/(AIR)] \times 100$$

where $N_{methanol}$ is the number of moles of methanol, $N_{galacturonic\ acid}$ is the number of moles of galacturonic acid and AIR is the amount (g) of alcohol insoluble residue.

2.9.1 Alcohol-insoluble residue preparation

Alcohol-insoluble residue (AIR) of the cell wall was extracted according to the method of Mcfeeters and Armstrong [16]. The litchi aril was blended with 5 volumes of 95% ethanol using a blender (Philips, Eindhoven, Netherlands). The blended aril was filtered using Whatman No. 1 filter paper under vacuum. The insoluble cell wall extract was re-suspended in 70% ethanol (2.5-fold v/w, compared to the fresh tissue), blended and filtered again. The cell wall was re-suspended in 2.5-fold (v/w) acetone relative to flesh tissue, blended again, and filtered. Finally, the residues were dried in an oven at 25 °C and stored at -30 °C.

2.9.2 Galacturonic acid analysis in cell wall extracts

Insoluble material of AIR was first solubilized in the concentrated H_2SO_4 according to the method of Ahmed and Labavitch [17]. The obtained solution was used for the assay of uronic acid according to the method of Ibarz et al. [18]. Samples were diluted by adding 0.1 ml pectin solution into 0.9 ml distilled water. Then, 5 ml of 0.0125 M sodium tetraborate ($Na_2B_4O_7$) was added and the mixture was incubated for 6 min at 80 °C in a water bath, followed by cooling immediately in an ice water. After cooling to room temperature, 100 µL of 0.15% *m*-hydroxydiphenyl (in 0.5% NaOH) was added in the mixture solution. Then, the mixture solution was measured at 520 nm, using a spectrophotometer (Jasco V-530, Hachioji, Tokyo, Japan) and compared with blank (using 100 µL of 0.5% NaOH replace of *m*-hydroxydiphenyl).

2.9.3 Methanol analysis in cell wall extracts

The AIR was de-esterified by addition of 2.0 M NaOH as described by Ng and Waldron [19]. The amount of methanol formed was determined according to the method of Klavons and Bennett [20]. One mL of alcohol oxidase (Sigma-Aldrich, Darmstadt, Germany) (1 unit/ml water) was added to 1 mL of the obtained solution. The mixture was gently mixed and incubated at 25 °C for 15 min. Then, 2 mL of pentanedione solution (0.02 M 2, 4 pentanedione in 2.0 M ammonium oxalate and 0.05 M acetic acid) was added. Afterward, the mixture was incubated at 60 °C in a water bath for 15 min and cooled to room temperature. The absorbance at 412 nm was read using a spectrophotometer (Jasco V-530, Hachioji, Tokyo, Japan). For the blank sample, 2 mL of distilled water (instead of 2 mL pentanedione solution) was added.

2.10 Microbiological Analysis

The change in the microbial population of minimally-processed litchi was evaluated using aerobic plate and yeast-mold counts. A 10 g from four arils per replication were cut using a sterilised stainless steel scissors. Ninety mL of 0.1% peptone water (Merck, Darmstadt, Germany) was added to the weighed sample to achieve 10^{-1} dilution. Then, the mixture was homogenised in a stomacher (IVL Masticator 400; IUL Instruments, Barcelona, Spain) for 30 s. The homogenised sample was serially-diluted by a factor of ten in 0.1% peptone water to make appropriate dilution. Aerobic plate and yeast-mold counts were evaluated by

spread plate method, using plate count agar (PCA; Merck, Darmstadt, Germany) and potato dextrose agar (PDA; Merck, Darmstadt, Germany), and were incubated at 35 °C for 48 h and 25 °C for five days, respectively [21]. Values were reported as log CFU g^{-1}.

2.11 Statistical Analysis

Each treatment was done in duplicate of 12 fruits per bag. At 2-days interval, 2 bags were used for analysis in triplicate (4 fruit per determination). Data were analyzed using SPSS program (V.16; An IBM Company, Ontario, Canada) for analysis of variance at $P \leq 0.05$. Duncan's multiple range test was used for comparison of mean values to determine the differences between treatments.

3. RESULTS AND DISCUSSION

3.1 Headspace Gas

Changes in headspace gas composition (O_2 and CO_2 concentrations) of minimally-processed litchi fruit were measured over 18 days. The O_2 content in the packages decreased during storage while the CO_2 content increased as expected (Figure 1 a-b). For 5% O_2 + 5% CO_2 atmosphere, changes in O_2 and CO_2 concentrations were more dramatic from day-1 to day-6 than from day-6 to day-18. The biochemical reaction occurred faster during day-1 to day-6 due to the atmosphere inside package was changed to reach equilibrium. After the 6 days of the storage, the percent O_2 decreased to 4.46 and CO_2 increased to 5.76 and almost unchange throughout the end of the storage. In whole 'Shahi' litchi fruit which packed in MA packages made from laminates of bi-axially oriented polypropylene (BOPP) and polyvinyl chloride (PVC), levels of O_2 and CO_2 at equilibrium were in the range of 5.92-8.60% and 7.14-9.35%, respectively [22]. For the control, the O_2 concentration was continuously decreased until the end of the storage and the CO_2 rapidly increased up to 10.32% on day-4. However, after 12 days of the storage, the CO_2 dropped to 8.12% at the end of the storage which was indicated that respiration rate of litchi arils came down, possibly due to the onset of the senescence process.

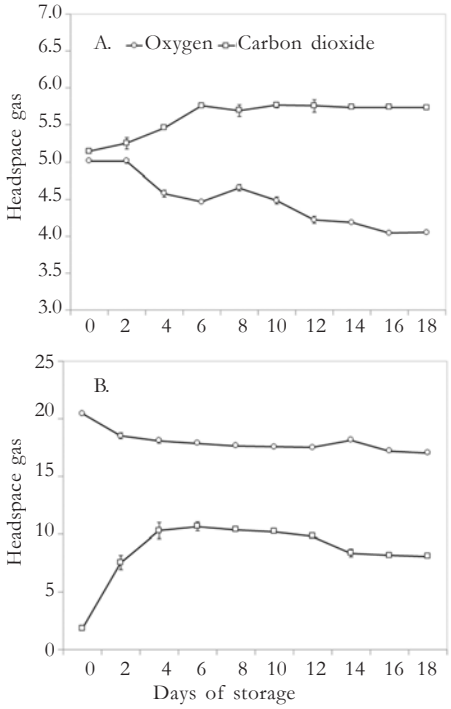

Figure 1. Changes in oxygen and carbon dioxide headspace gas inside package of minimally-processed litchi fruit during storage in modified atmosphere packaging (A) for 18 days at 2±1 °C compared with control which was packed in normal atmosphere (control; B).

The composition of the atmosphere inside the packaging results from the interaction of several factors including the permeability characteristics of the packaging material, the respiratory behavior of the plant material and the environment [4]. Litchi is a non-climacteric fruit which the behavior

of respiration rate slows gradually after harvest. However, wounded stress from deseeding process is the main factor make the increasing of respiration rate. Litchi arils showed the respiration rate by 58.26 mg kg^{-1} h^{-1} compared to whole fruit and deseeded fruit (with peel) by 35.82 and 51.39 mg kg^{-1} h^{-1}, respectively, whereas ethylene production was very low (0.23-0.25 µL kg^{-1} h^{-1}) (data not shown). The analysis of gas composition of whole litchi fruit within three types of package, film coded BOPP-1, BOPP-2 and BOPP-3, which have the percentage material perforate was 0.00565%, 0.00313%, and 0.00939%, respectively, showed the gas composition revealed higher O_2 (17.0%) and lower CO_2 (6.0%) concentrations in BOPP-3 whereas in BOPP-1, the concentrations of O_2 and CO_2 were 11.47% and 12.07%, respectively [23].

3.2 Ethanol Content

The 5% O_2 + 5% CO_2 atmosphere significantly ($P \leq 0.05$) reduced ethanol content as compared to samples storage under the normal atmosphere. Ethanol content of the control increased from the initial 203 to 561 µL kg^{-1} after 18 days of storage. However, 5% O_2 + 5% CO_2 atmosphere was more beneficial to delay ethanol production which showed an increasing from 195 to 438 µL kg^{-1} during storage periods (Figure 2). The result showed similarity with 'Heiye' litchi fruit which was stored in polyethylene film bag in 5% O_2 + 5% CO_2 atmosphere. The initial ethanol content was 250 µg kg^{-1} and increased to 600 µL kg^{-1} after 42 days storage in controlled atmosphere cabinet at 3°C [24]. They reported that ethanol content of 500 µl kg^{-1} was a detection limit for the unpleasant odor in litchi arils. Refer to that limit, only the control showed the higher ethanol content than that of the treatment after storage for 8 days. However, ethanol content of litchi arils during storage in 5% O_2 + 5% CO_2 atmosphere was considered to be under the limit throughout the storage periods.

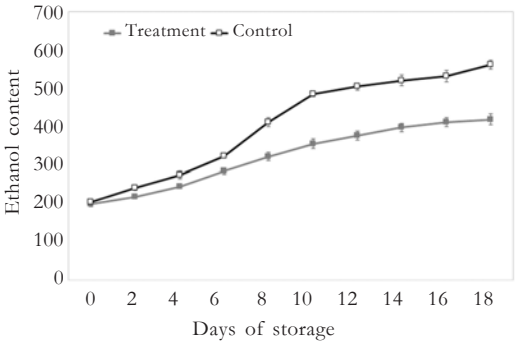

Figure 2. Changes in ethanol content (µL kg^{-1}) of minimally-processed litchi fruit during storage at 2±1 °C for 18 days. Treatment: the sample treated with 1% calcium chloride and packed in modified atmosphere (5% O_2 + 5% CO_2). Control: the sample treated with 0% calcium chloride and packed in normal atmosphere (20.43% O_2 + 0.03% CO_2).

Analysis of ethanol content in both treatments indicated that relatively high levels of ethanol was presented in the arils. Generally, the accumulation of acetaldehyde and ethanol content in litchi fruit increased during the maturity stage. The present of those indicated that the premature fruit may respire both aerobically and anaerobically. There has a reported that the green litchi fruit showed the O_2 uptake by the aril is much lower than in the partially ripe red fruit. In over-mature fruit, the increase in anaerobic respiration could be found. Thus, the ethanol release from the fruit may be consider as a predictor of over-ripeness [25]. In this study, high ethanol content of the control stored in high O_2 atmosphere (20.48%) was correlated with the decline in total soluble solid and increase in titratable acidity [1]. High O_2 in the control treatment also promoted a bacteria and yeast-molds growth (Figure 8 A, B). Some

fermentative microbe is able to convert pyruvate which the end product of glycolysis into acetaldehyde before finally catalyst to ethanol [26].

3.3 Juice Leakage

Water loss of fresh produce is an important parameter because it leads to the economic losses [3]. Juice leakage of treated litchi aril was found to be 2.44 mL 100 g^{-1} after 2 days of storage and significantly ($P \leq 0.05$) increased to 6.09 mL 100 g^{-1} after 18 days of storage. The control showed a dramatic increase in juice leakage to 7.21 mL 100 g^{-1} after 18 days of storage (Figure 3). The increasing of juice leakage showed a correlation with a declining of aril firmness. Juice leakage or osmotic solutes from vacuoles of the wounded tissue is the main factor causing a loss of aril firmness. Juice can move from inner cell to outer cell, and the mixing of substrates with enzymes can enhance the hydrolytic reactions. Moreover, fresh-cut processing of fruit increases wound-induced ethylene (C_2H_4), water activity and surface area per unit volume, which may also increase water loss [3]. The mechanism of beneficial effect of low O_2 on the reduction of juice leakage was explained by the rate of respiration. Respiration in plant is the oxidative breakdown of starch, sugar and organic acids to simpler molecule including CO_2 and H_2O, with a concurrent production of energy [4]. Thus, low level of O_2 in MAP helps reduce the water loss which was a product from the metabolism of respiration. The recommended O_2 and CO_2 levels for litchi fruit to maintenance relative high humidity and reduce water loss were 5% for O_2 and 5-8% for CO_2 [27]. Litchi arils dipped in 0-2% calcium lactate with 0-0.02% 4-hexyl resorcinol stored in vacuum packaging presented juice leakage in the range of 19-33% after 20 days of storage at 4 °C [7].

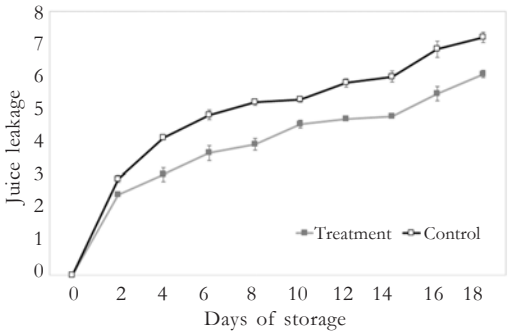

Figure 3. Changes in juice leakage (mL 100 g^{-1}) of minimally-processed litchi fruit during storage at 2±1 °C for 18 days. Treatment: the sample treated with 1% calcium chloride and packed in modified atmosphere (5% O_2 + 5% CO_2). Control: the sample treated with 0% calcium chloride and packed in normal atmosphere (20.43% O_2 + 0.03% CO_2).

3.4 Firmness of Litchi Arils

The firmness of both treated and control litchi arils decreased throughout the storage periods for 18 days at 2±1 °C. After 2 days of storage, firmness value of treatment and the control decreased by 3.80 and 19.47% and declined continuously to 20.58 and 52.87% at the end of storage, respectively (Figure 4). The control aril showed significantly ($P \leq 0.05$) lower firmness than the treated sample during storage periods. The loss of firmness was related to cell-wall structure. It was confirmed by SEM micrographs as shown in Figure. 5. The effect of MAP on retarding the firmness loss was attributed to the reduction of cell wall degrading enzymes, such as PME and PG as discussed in subtitle 3.6 and 3.7. Both enzymes are involved in a softening process in fresh-cut fruit and vegetables [3]. Calcium ion helps maintain the structure of cell wall by increasing the cross linkages with both cell wall and middle lamella pectin. It is interacted with carboxylic groups of pectic acid polymers between pectin molecules to form an insoluble salts [28].

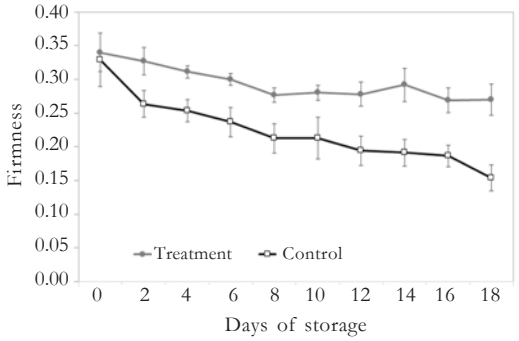

Figure 4. Changes in firmness (N) of minimally-processed litchi fruit during storage at 2±1 °C for 18 days. Treatment: the sample treated with 1% calcium chloride and packed in modified atmosphere (5% O_2 + 5% CO_2). Control: the sample treated with 0% calcium chloride and packed in normal atmosphere (20.43% O_2 + 0.03% CO_2).

3.5 Microstructure Study

SEM micrograph showed aril tissue composing of parenchyma tissue with very thin-walled and polyhedral-shaped cell of various diameters. Cells are formed by the conjunction of 4-5 cells (Figure 5 a-b). At the initial day of storage, the structure of both treatment and control tissues was well-organized with firm texture (Figure 5 a). The morphological changes could be observed after storage for 4 days. The structure of the control showed a loss of cell integrity (Figure 5 d) while the treatment maintain a cell turgor and remained well-organized (Figure 5 c). After 8 days of storage, cells shrinkage was observed in the control resulting in a loss of cell turgor (Figure 5 f). The extensive cell shrinkage mostly occurred in the control after storage for 14 days (Figure 5 h) and finally collapsed at the end of storage (Figure 5 j). In case of the treatment, the SEM images showed that structures maintained cell-to-cell contact and turgor during storage. However, cell showed a loss in the rounded shape after 14 days of storage, probably due to water loss during storage (Figure 5 g). This phenomenon occurred continuously until 18 days of storage (Figure 5 i).

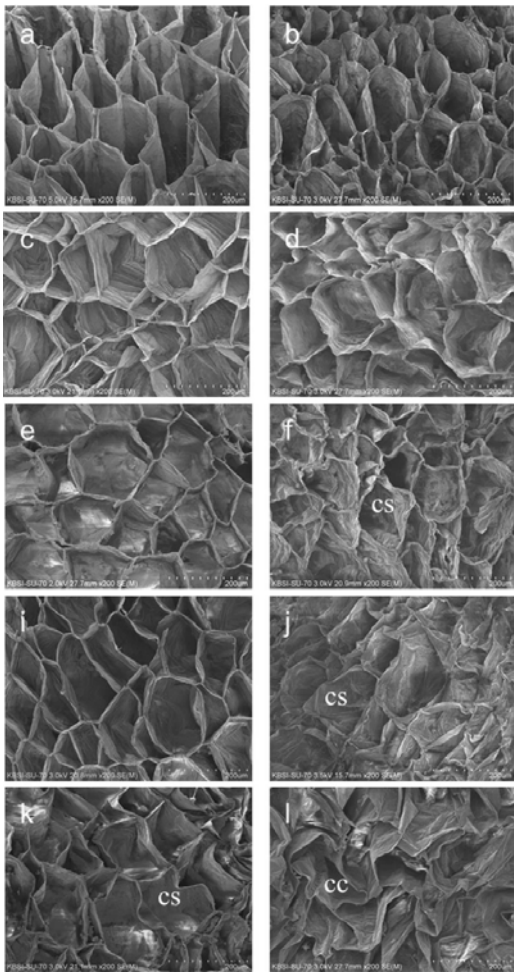

Figure 5. SEM micrographs (magnification ×200) of litchi arils which were dipped in 1% calcium chloride + 50 mg L^{-1} PAA and stored under 5% O_2 + 5% CO_2 (left side) compared with storing in normal atmosphere (right side) at initial day (a, b) and after storage for 4 (c, d), 8 (e, f), 12 (g, h) and 18 days (i, j) at 2±1 °C. cs: cell wall shrinkage, cc: cell wall collapse.

3.6 Pectin Methylesterase Activity

PME activities of the control and treatment during storage are shown in Figure 6. The initial activity of both treatments were 0.49 and 0.44 μmole min^{-1} mg protein, respectively. PME activity of the control increased signicantly ($P \leq 0.05$) during storage, although showing fuctuations within the storage period (Figure 6). At the end of the storage, the activity of the control increased to 0.83 μmole min^{-1} mg $protein^{-1}$ and was significantly ($P \leq 0.05$) higher than the treatment. The high PME activity of the control indicated that the pectin in cell wall was deesterified into low methoxy pectin which become ready to be a substrate for PG action. The litchi arils kept in modified atmosphere packaging showed a lower PME activity than the control throughout the storage periods. During storage, the activity of PME increased to the highest value (0.68 μmole min^{-1} mg $protein^{-1}$) on day-8 and reduced to the range 0.54-0.62 μmole min^{-1} mg $protein^{-1}$ throughout the storage periods. This irregular behavior might be due to a wounding response and/or to change in the solubility of the enzyme during storage by the formation of calcium pectates [5]. Santana *et al.* [29] also reported the reduction of PME activity in peach fruit packed in low density polyethylene (LDPE) bags (50 and 60 μm of thickness) with active modified atmosphere (10 kPa CO_2 + 1.5 kPa O_2, balance N_2). Since PME and PG are played an important role in fruit ripening and softening processes which involved in the increasing of respiration and ethylene production. Wounding from the fresh-cut processing activated the ACC synthase and ethylene production [3]. Thus, low O_2 could help retard the metabolism of both enzymes compared to normal atmosphere.

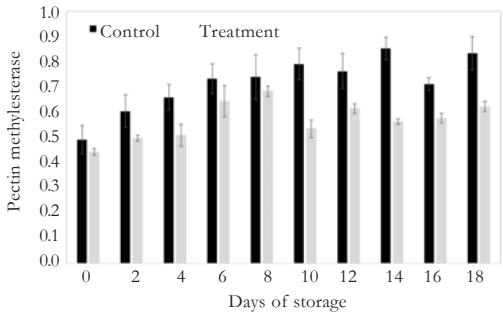

Figure 6. Changes in pectin methylesterase (μmole min^{-1} mg $protein^{-1}$) of minimally-processed litchi fruit during storage at 2±1 °C for 18 days. Treatment: the sample treated with 1% calcium chloride and packed in modified atmosphere (5% O_2 + 5% CO_2). Control: the sample treated with 0% calcium chloride and packed in normal atmosphere (20.43% O_2 + 0.03% CO_2).

3.7 Polygalacturonase Activity

The initial PG activity of the control and treatment were 15.68 and 14.85 μmole min^{-1} mg $protein^{-1}$, respectively. The activity of the treatment was lower than that of the control throughout the storage periods. The PG activity changed in the same trend correlating with the PME activity (Figure. 7). The PG acts as hydrolytic enzyme and the product obtained from the PME activity results in the loss of fruit firmness [5]. During the storage periods, the use of modified atmosphere packaging helps retard the increasing of PG in comparison to the control by 11-31%. It is well known that PG is an ethylene-regulated enzyme and that its activity is increased by damage especially from cutting or deseeding processes of fresh-cut fruit and vegetables [3]. Low O_2 atmosphere generally reduced respiration rate and ethylene production, then PG also suppressed.

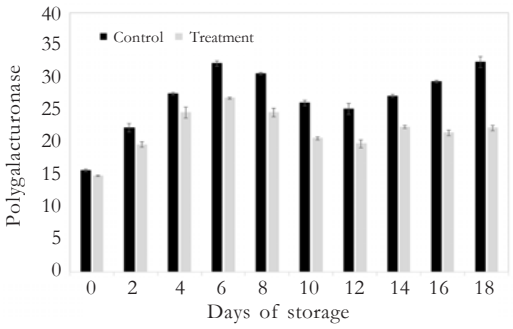

Figure 7. Changes in polygalacturonase (µmole min^{-1} mg $protein^{-1}$) of minimally-processed litchi fruit during storage at 2±1 °C for 18 days. Treatment: the sample treated with 1% calcium chloride and packed in modified atmosphere (5% O_2 + 5% CO_2). Control: the sample treated with 0% calcium chloride and packed in normal atmosphere (20.43% O_2 + 0.03% CO_2).

3.8 Degree of Methyl Esterification of Pectin

Degree of methyl esterification of pectin in litchi arils stored in modified atmosphere packaging is shown in Table 1. An initial degree of methyl esterification of pectin in the cell wall extract was quite low (39%) in both treatments. This type of pectin was classified as the low methoxyl pectin. The low methoxyl pectin has high available carboxyl group to combine with calcium ion. Calcium ions can combine to non-methylated galacturonic acid residues and form calcium bridges between adjacent pectin polymeric chains, resulting in reinforcing the cell walls [28]. Thus, the application of calcium chloride could be used to retain firmness of the minimally-processed litchi fruit *cv.* 'Jugkapat'.

The percent degree of methylation of pectin in both treated and control arils were continuously decreased during storage for 18 days. The changes in degree of methyl esterification was the result of the increase of PME activity during storage. Dipped litchi arils in calcium chloride solution which were stored in 5% O_2 + 5% CO_2 atmosphere showed the reducing degree of methylation from 39 to 32% after storage for 18 days while the drastic reduction was found in the control by 39 to 22%. The stress of wounded aril tissue activated the increase of PME and PG activity. The high activity of both enzymes resulted in the decrease of methyl esterification degree and a loss of firmness. The MAP treatment showed the little changes of the degree of methylation compared to the control which was correlated with the changes of both enzyme activities. However, Alonso *et al.* [30] reported that the correlation of the degree of methyl esterification decreased from 58.8 to 55.7% with the increase of PME activity and firmness of thermal and calcium pretreatment of sweet cherries.

Table 1. Changes in degree of methyl esterification of minimally-processed litchi fruit during storage in 5% O_2 + 5% CO_2 atmosphere for 18 days at 2±1 °C compared with control which was packed in normal atmosphere (20.43% O_2 + 0.03% CO_2).

Treatment	Storage days									
	0	2	4	6	8	10	12	14	16	18
Control	39^{a}±3.07	34^{b}±1.15	29^{c}±3.07	25^{cd}±3.68	26^{cd}±2.49	25^{cd}±4.77	25^{cd}±3.00	24^{def}±1.34	23^{def}±2.32	22^{f}±4.40
Treatment	39^{a}±3.06	37^{ab}±0.92	38^{abc}±2.41	37^{bcd}±3.22	37^{bcd}±1.84	36^{def}±0.73	35^{def}±0.58	35^{ef}±0.78	34^{f}±0.68	32^{g}±1.64

Data represent mean ± standard deviation.

Mean followed by values in each row (day) with the distinct letters represent the significantly difference ($P \leq 0.05$).

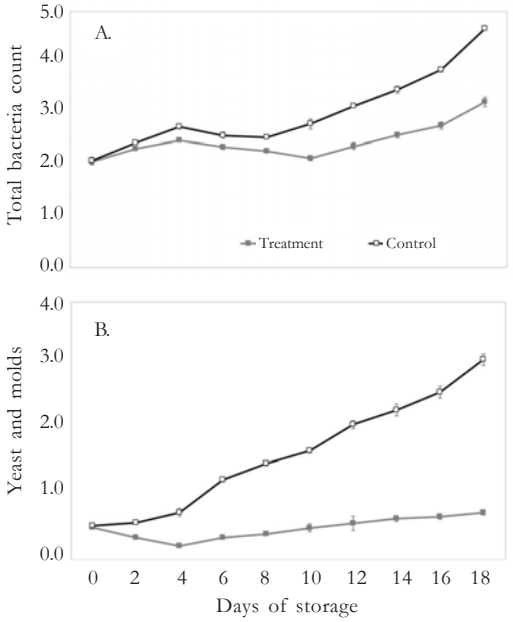

Figure 8. Changes in total bacteria count and yeast-molds (log cfu g^{-1}) of minimally-processed litchi fruit during storage at 2±1 °C for 18 days. Treatment: the sample treated with 1% calcium chloride and packed in modified atmosphere (5% O_2 + 5% CO_2). Control: the sample treated with 0% calcium chloride and packed in normal atmosphere (20.43% O_2 + 0.03% CO_2).

3.9 Microbial Populations

Aerobic bacteria and yeast-mold counts of both MAP and control were relatively low about 2.0 log cfu g^{-1} and 0.5 log cfu g^{-1}, respectively at the beginning of storage (day 0). During storage, aerobic bacteria and yeast-mold counts of the control increased progressively to 4.67 and 3.11 log cfu g^{-1}, respectively, after 18 days of storage (Figure. 8) while in the treatment were 3.23 and 0.73 log cfu g^{-1}, respectively (Figure. 8). Aerobic bacteria in the control was significantly ($P \leq 0.05$) higher than MAP treatments because they can grow in the high O_2 condition which was mostly spoilage bacteria [26]. Furthermore, yeast and molds grew slowly in MAP condition. However, the microbial counts was not exceed than 7 log cfu g^{-1} for total bacteria count and 4 log cfu g^{-1} for yeast and molds as established by Thai regulation [31]. The use of modified atmosphere packaging had positive effect to retard the growth of aerobic spoilage organisms in some fresh-cut fruits such as fresh-cut honeydew melon [32], fresh-cut apple [33] and fresh-cut pear [6]. The combination of chemical treatments of calcium chloride (1% w/v) and citric acid (2% w/v) and packed in an atmosphere of 5% O_2 + 10% CO_2 + 85% N_2 in fresh-cut papaya retarded the growth of mesophilic bacteria and yeast-molds up to 25 days at 5°C [34].

4. CONCLUSION

The combination of calcium ion treatment and MAP is more useful for maintaining the qualities of litchi arils which reduced the loss of juice leakage and firmness, retarded the increasing of ethanol content and the microbial populations during storage. The combined effect also presented a low level of both PME and PG and reinforced the cell wall and retarded the loss of cell turgor during 18 days of storage at 2±1°C.

ACKNOWLEDGEMENTS

Financial support from the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0348/2552) to Putkrong Phanumong and Nithiya Rattanapanone is heartfully acknowledged.

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