Effect of modified atmosphere packaging with varied gas combinations and treatment on the quality of minimally-processed litchi fruits

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Abstract

This study aimed to select the optimum oxygen (O₂) and carbon dioxide (CO₂) concentrations for storage of minimally-processed litchi fruit cv. ‘Jugkapat’ packed in a nylon laminated with linear low density polyethylene bag (Nylon/LLDPE). Litchi fruit (fully-red stage) and their arils were sanitized with peroxycetic acid solutions 100 mg L⁻¹, 3 min and 50 mg L⁻¹ for 1 min, respectively. Litchi arils were stored under nine combinations of O₂ (2.5, 5.0, and 7.5%) and CO₂ (5.0, 7.5 and 10.0%) with atmospheric nitrogen balance for 18 days at 2±1°C. The optimum concentration of O₂ and CO₂ for storage of minimally-processed litchi fruit was 5.0% and 5.0% due to the lowest levels of juice leakage, ethanol content, microbial growth and maintained firmness the best. The atmosphere of 5.0% O₂ and 5.0% CO₂ maintained the respiratory quotient value closely to 1.0 during storage. The microbial counts are in the microbiological limits for food safety.

Keywords: minimally-processed litchi fruit, modified atmosphere packaging, quality

1. Introduction

Litchi (Litchi chinensis Sonn.) is one of the economic fruit in Thailand. The fruit is naturally bright-red in color of the pericarp and the edible portion is surrounded with succulent aril and has a single dark brown seed. The arils are composed of aromatic compounds and contain higher sugar with several minerals and vitamins (Jiang et al., 2012). Browning of the peel occurs rapidly after harvest at room temperature and low relative humidity, thus shorten the shelf-life. The litchi fruit can be processed into minimally-processed or fresh-cut product. It is an alternative method to preserve the litchi arils as fresh-like fruit with high nutritional quality and create new marketing opportunities (Shah & Nart, 2006; 2008). However, the quality loss of minimally-processed litchi fruit was observed during storage at 4±1°C for 12 days in a polystyrene clamshell container (Phanumong et al., 2015). During processing, the wound and damaged tissues of minimally-processed fruit caused an increase in respiration rate and accelerated enzymatic cell membrane degradation (Toivonen & Brumell, 2008). Low oxygen atmospheres combined with adequate CO₂ concentrations in package help lowering the rate of respiration and ethylene production. It can also inhibit or delay enzymatic reactions, retard the microbial growth and preserve the product from quality loss (Rojas-Graü et al., 2009). Modified atmospheres storage (MAS) at low level of O₂ (1-5%) and high level of CO₂ (5-10%) has been approved to extend the shelf-life of fresh-cut fruit which results in a

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reduction of respiration rate, transpiration and ethylene production (Rojas-Gràu et al., 2009). MAS also has shown to effectively control enzymatic browning, a loss of firmness and decay of fresh-cut produces. However, the investigation of gas combinations for storage of minimally-processed litchi fruit has not been reported, whereas the vacuum packaging was extensively reported by Shah and Nath (2008) and Bolaños et al. (2010). Gas combinations and/or packaging material with different gas transmission properties to prolong the shelf-life of minimally-processed litchi fruit have gained increasing attention. Therefore, this study aimed to investigate the effect of oxygen and carbon dioxide levels on the quality of minimally-processed litchi fruit cv. ‘Jugkapat’ during storage at 2±1°C.

2. Materials and Methods

2.1 Litchi and minimal processing

Litchi (Litchi chinensis Sonn.) fruits cv. ‘Jugkapat’ at a commercially-harvesting stage (fully-red peel color) were bought from the local orchards in Fang district, Chiang Mai, Thailand. Fruits were packed in corrugated boxes with 10 kg per box and then transferred to the laboratory in Postharvest Technology Research Institute, Chiang Mai University, within 3 hrs and stored in a cold room at 4±1°C overnight before the experiment. Then, fruits were selected for uniform size (23-28 g), shape (oval shape), color (pinkish-red in color) and free from defects.

The experiment was designed as factorial in completely randomized design (CRD). Fruits were randomly divided into groups of 240 fruits per treatment. Whole litchi fruits were sanitized in 100 mg L\(^{-1}\) peroxycetic acid (PAA) solution, then de-seeded and peeled. The obtained arils were dipped in 50 mg L\(^{-1}\) PAA solution for 1 min (Phanumong et al., 2015) and drained before placing in a polystyrene plastic tray (twelve fruits per tray). Trays were packed in nylon laminated with linear low density polyethylene bag (Nylon/LLDPE; 72 µm thick, 21 cm width × 33 cm length, YOK, Chiang Mai, Thailand) and the bag was closed by heat sealer.

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2.2 Modified atmosphere storage

Modified atmosphere storage was used in nine treatments by combinations of O\(_2\) (2.5, 5.0, and 7.5%) and CO\(_2\) (5.0, 7.5 and 10.0%) with nitrogen balance atmosphere using a modified atmosphere packaging unit (Henkovac 150, Henkovac International’s-Hertogenbosch, Netherlands) and then stored at 2±1°C, 90-95% relative humidity (RH). Transmission rates of O\(_2\) and CO\(_2\) of nylon/LLDPE bags at 23°C were 73.6 and 161 ml m\(^{-2}\) day\(^{-1}\) respectively. All nine treatments of litchi arils were compared with the control which was stored in air in package (20.77% O\(_2\) and 0.03% CO\(_2\)). Litchi arils were analyzed at 2-day intervals with two trays per day per treatment for 18 days.

2.3 Analysis of headspace gases and respiratory quotient

Headspace gases, O\(_2\) and CO\(_2\) were monitored using headspace gas analyzer (Model 900151, Bridge analyzers Inc., California, USA) with non-dispersive infrared (NDIR) and electro chemical sensor for CO\(_2\) and O\(_2\), respectively, at 2-day interval. Gas sample was withdrawn from the package using a needle through an adhesive rubber septum, directly connected to the gas analyzer. Respiratory quotient (RQ) was also calculated as RCO\(_2\)/RO\(_2\), where RCO\(_2\) and RO\(_2\) are CO\(_2\) production and O\(_2\) uptake rates, respectively (Exama et al., 1993).

2.4 Evaluation of physical properties

Aril firmness was determined using a texture analyzer (TA.Xt plus; Stable Micro Systems Ltd., Surrey, UK) by a puncture test with a cylindrical probe of 2 mm diameter. The firmness was measured under 25°C at the test speed of 1 mm per sec. Twelve arils per treatment were cut along the length into two parts, so total 24 pieces were measured for the firmness per treatment. The juice leakage in the package was determined using 10 mL syringe. The juice was withdrawn manually and calculated as mL 100 g\(^{-1}\) fresh weight.

2.5 Ethanol content

The ethanol content of litchi arils was determined by gas chromatography according to a method developed by Davis and Chace (1969). Five grams of mashed litchi arils were placed in a 10 mL amber glass bottle with a cap. Then, the bottles were incubated in a hot water bath at 80°C for 45 min. Headspace gas was withdrawn using a 1 mL syringe through a rubber septum and then injected into a gas chromatography (TRACE GC, ThermoQuest Italia S.p.A., Milan, Italy) equipped with a flame ionization detector (FID) and capillary column (30 m x 0.53 mm i.d. x 1 µm OV-1 100% dimethylpolysiloxane). Conditions of the oven, injector, and detector were as follows: 150, 175 and 200°C, respectively. Ethanol content in litchi aril was compared with a standard absolute ethanol (0 to 3,600 mg L\(^{-1}\)).

2.6 Microbial evaluation

Aerobic bacteria and yeast-molds of minimally-processed litchi fruit were determined by spread plate method (BAM, 2001). Ten grams of sample were transferred to a sterilized bag containing 90 ml of 0.1% peptone water (Merck, Darmstadt, Germany) and macerated with a stomacher IVL Masticator 400 (IUL Instruments, Barcelona, Spain) for 30 sec. The homogenized sample was serially-diluted by a factor of ten in 0.1% peptone water. The undiluted mixture and serially-diluted mixture, 0.1 ml in duplicate, were spread on plate count agar PCA; Merck, Darmstadt, Germany, for aerobic bacteria, and on potato dextrose agar, PDA; Merck,
Darmstadt, Germany, for yeast-molds count. PDA was acidified with 10% tartaric acid (1.8 mL/100 mL) to pH 3.5 before used. PCA plates were incubated at 35°C for 48 hrs for aerobic bacteria count. PDA plates were incubated at 25°C for 5 days for yeast and molds count. Values were reported as log CFU g⁻¹.

2.7 Statistical analysis

The experiment was designed within 3x3 factorial and completely randomized design (CRD). Fruits were randomly distributed into nine treatments. Each treatment was carried out in duplicate of twelve arils with triplicate determinations. Data were analyzed using SPSS program (V.16; An IBM Company, Ontario, Canada) for analysis of variance at P < 0.05. Duncan’s multiple range test was used for comparison of mean values to determine the differences between treatments. Response surface methodology was carried out to describe the individual and interactive effects of the independent variables on the outcome of oxygen and carbon dioxide, using SigmaPlot software (V.13; Systat Software Inc., San Jose, California, USA).

3. Results and Discussion

3.1 Headspace gas composition

Headspace gas composition inside packaging of minimally-processed litchi at different gas ratios of O₂ and CO₂ are shown in Figure 1. The oxygen content in the packages decreased while the carbon dioxide content increased during the storage. The contents of O₂ and/or CO₂ were almost constant after reaching the plateau at Day 8. This result indicated that lower O₂ and higher CO₂ levels than ambient atmosphere compositions could reduce respiration rate of plant product (Sandhya, 2010). There was statistically significant difference (P<0.05) of O₂ content in the packages decreased and CO₂ content increased during storage. However, the reduction of headspace O₂ at each O₂ levels (2.5, 5.0 and 7.5%) with varying CO₂ concentration (5.0%, 7.5% and 10.0%) were not significant difference (P>0.05) during the storage periods. Similar results were observed in the changes of CO₂ content at the level of 7.5 and 10%. At 7.5% O₂ combined with 5% CO₂ help retarding the increase of CO₂ during storage when compared to 2.5% O₂ and 5.0% CO₂ (P<0.05). In the control, a rapid depletion of the O₂ was observed from 20.77 to 17.0%, whereas CO₂ production was significantly increased from 0.03 to 9.36% during 12 days of storage and then continuously decreased on day 14 throughout the storage periods (Figure 2). It indicated that respiration rate of litchi arils decreased, possibly due to the onset of the senescence process (Pogson & Morris, 2004).

3.2 Respiratory quotient

The consumption of O₂ and the production of CO₂ were reported in terms of respiratory quotient (RQ), as shown in Figure 3. RQ values of almost all treatments were changed in a similar pattern by increasing to the highest at the Day 6 and then remaining almost constant throughout the storage periods. There were only two treatments at 2.5% O₂ : 7.5% CO₂ and 2.5% O₂ : 10.0% CO₂, in which the RQ values were continuously increased until the end of the storage and showed the highest values (4.5 and 5.9), compared to other treatments. Results of those were differed from the treatment combination of 2.5% O₂ : 5% CO₂. Because of litchi is a non-climacteric fruit and has a low respiration rate after harvest and during storage in MAP. Thus, greater different ratio of O₂ and CO₂ could be occurred in both treatments. The combination of 5.0% O₂ : 5.0% CO₂ and 7.5% O₂ : 7.5% CO₂ could effectively maintain the RQ values close to 1.0 (1.1 and 1.2) throughout the storage periods.
In aerobic respiration, RQ values for fresh fruit and vegetables are usually range from 0.7 to 1.3. The RQ value is around 1 when carbohydrates are used as substrate for aerobic respiration, while it is <1 for lipids and >1 for organic acids (Saltveit, 2004). The oxygenated substrates such as malic acid, a main organic acid in litchi arils, cause the RQ value about 1.33 (Ramaswamy, 2014). At 2.5% O₂ in combination with all levels of CO₂ had the RQ values >2.0. The high RQ values indicated the onset of anaerobic respiration occurred in the tissues, thus resulting in the production of ethanol (Saltveit, 2004). However, the low RQ values may indicate incomplete oxidation of fat to CO₂ (Ramaswamy, 2014).

### 3.3 Ethanol content

The ethanol content of litchi arils during storage under different atmospheres is shown in Figure 4. The initial ethanol contents of litchi arils in all treatments were in the range of 204-264 µL kg⁻¹ and increased to 397-500 µL kg⁻¹ after storage for 18 days. This observation showed similar changes with the previous report that whole litchi fruit cv. ‘Heiye’ packed in MAP showed an increase in ethanol content from 250 µL kg⁻¹ to 750 µL kg⁻¹ after storage for 20 days at 3°C (Tian et al., 2005). The control showed the highest accumulation of ethanol during storage which was related with an increasing of CO₂ concentration. After storage for 8 days, the ethanol content of the control was higher than 500 µL kg⁻¹ and continuously increased about 2.5 folds until the end of storage. For MAP, the significant highest (P<0.05) accumulation of ethanol content were found in the combination of 2.5% O₂: 10.0% CO₂ followed by 2.5% O₂ : 7.5% CO₂ respectively. The ethanol content was increased 2.2 folds after storage for 18 days. Thus, the use of low concentration of O₂ and high concentration of CO₂ were associated with the onset of fermentation and accumulation of ethanol and acetaldehyde in fruit tissue (Beaudry et al., 1992). Other MAP treatments showed an increasing of ethanol content in the range of 1.6-1.9 folds at the end of the storage periods. Ethanol accumulation is related to the development of off-flavor and also links to tissue damage (Beaudry et al., 1992). In litchi fruit, ethanol content could be used as a predictor of over-ripeness because its accumulation was increased during maturation, which indicated that the premature fruit may respire both aerobically and anaerobically respirations (Sivakumar, 2011). The results revealed that MAP had the potential to delay an increase of ethanol content in litchi arils during storage periods.

### 3.4 Juice leakage

Juice leakage of litchi arils during storage in MAP is depicted in Figure 5. The use of 2.5% O₂ in combination with all concentrations of CO₂ showed the highest juice leakage and the optimum concentration of O₂ (5.0-7.5%) reduced juice leakage during storage. Thus, oxygen was the most effective factor involved in the juice leakage of litchi arils during storage. Mir and Beaudry (2004) noticed that various horticultural crops have different in the tolerance for O₂ and CO₂. Thus, the used of 2.5% O₂ might not appropriate for storage litchi arils in MAP which the injury can occur under the experiment condition. The juice leakage and firmness
3.4 Aerobic bacteria and yeast-mold counts

Aerobic bacteria count of minimally-processed litchi fruit is shown in Table 2. The control sample showed significantly higher aerobic bacteria count (2.4±2.6 log CFU/g) than other treatments because of an appropriate O₂ environment. Total aerobic bacteria counts in litchi arils in nine combinations of O₂ and CO₂ levels decreased continuously during storage from Day 2 to Day 10. This might be due to the reduction of O₂, CO₂, and the presence of microorganisms in the package and/or low temperature in a controlled atmosphere (CA) treatment. The aerobic bacteria count in CA treatments was below 2.0±2.6 log CFU/g during storage. Aerobic bacteria counts were comparable to the findings of Toivonen and Brummell (2008). The aerobic bacteria count in all treatments affected by the presence of O₂ and CO₂ during storage in 9 combinations of O₂ and CO₂ levels is shown in Table 2. It was observed that the presence of O₂ and CO₂ during storage affected the microbial growth and decreased aerobic bacteria counts by 2.0±2.6 log CFU/g. The aerobic bacteria count was significantly lower in the treatments where O₂ and CO₂ levels were reduced to 2.0±2.6 log CFU/g. The aerobic bacteria count in CA treatments was below 2.0±2.6 log CFU/g during storage.

3.5 Firmness

The firmness loss of minimally-processed litchi fruit during storage under the atmosphere of 9 combinations of O₂ and CO₂ levels is shown in Table 3. The firmness generally decreased in the corresponding treatment of the firmness decreased in the control decreased 40% after juice leakage. Firmness levels significantly affected by the presence of O₂ and CO₂ during storage in 9 combinations of O₂ and CO₂ levels is shown in Table 3. It was observed that the presence of O₂ and CO₂ during storage affected the firmness and decreased firmness by 2.0±2.6 log CFU/g. The firmness was observed in the treatments where O₂ and CO₂ levels were reduced to 2.0±2.6 log CFU/g. The firmness levels significantly affected by the presence of O₂ and CO₂ during storage in 9 combinations of O₂ and CO₂ levels is shown in Table 3. The firmness levels significantly affected by the presence of O₂ and CO₂ during storage in 9 combinations of O₂ and CO₂ levels is shown in Table 3. The firmness levels significantly affected by the presence of O₂ and CO₂ during storage in 9 combinations of O₂ and CO₂ levels is shown in Table 3.

Table 1. Changes in firmness (N) of litchi arils stored under the nine combinations of carbon dioxide and oxygen levels during storage in nylon/LLDPE bag at 2±1°C for 18 days.

<table>
<thead>
<tr>
<th>Gas concentration (%)</th>
<th>Firmness (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂</td>
<td>CO₂</td>
</tr>
<tr>
<td>------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>7.5</td>
<td>0.31±0.01</td>
</tr>
<tr>
<td>21.0</td>
<td>0.31±0.02</td>
</tr>
<tr>
<td>5.0</td>
<td>5</td>
</tr>
<tr>
<td>7.5</td>
<td>0.31±0.03</td>
</tr>
<tr>
<td>10</td>
<td>0.31±0.03</td>
</tr>
<tr>
<td>7.5</td>
<td>5</td>
</tr>
<tr>
<td>7.5</td>
<td>0.31±0.01</td>
</tr>
<tr>
<td>7.5</td>
<td>0.32±0.02</td>
</tr>
</tbody>
</table>

Values in each column with distinct lower cases letters represent the significantly different results (P<0.05). Values in each row with distinct upper cases letters represent the significantly different results (P<0.05).
### Table 2.

Changes in total bacteria count (log CFU g⁻¹) of litchi arils stored under the nine combinations of carbon dioxide and oxygen levels during storage in nylon/LLDPE bag at 2 ± 1°C for 18 days.

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>2.5% O₂</th>
<th>5.0% O₂</th>
<th>7.5% O₂</th>
<th>2.5% CO₂</th>
<th>5.0% CO₂</th>
<th>7.5% CO₂</th>
</tr>
</thead>
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<tr>
<td>0</td>
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<td>2.31 ± 0.03</td>
<td>2.37 ± 0.04</td>
<td>2.31 ± 0.03</td>
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<tr>
<td>3</td>
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<td>2.54 ± 0.08</td>
<td>2.56 ± 0.07</td>
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<td>2.47 ± 0.05</td>
<td>2.55 ± 0.07</td>
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Values in each column with distinct lower case letters represent the significantly different results (P<0.05). Values in each row with distinct upper case letters represent the significantly different results (P<0.05).
lowest juice leakage was found at 5.0% $O_2$ followed by 7.5% $O_2$ and 2.5% $O_2$, respectively. The $CO_2$ caused the lowest juice leakage at 5.0% $CO_2$, followed by 7.5% $CO_2$ and 10.0% $CO_2$, respectively (Figure 6C). Thus, application of 5.0% $O_2$ : 5.0% $CO_2$ was the best combination for reducing juice leakage during storage of minimally-processed litchi fruit.

The relationship of $O_2$ and $CO_2$ levels on the aerobic bacteria count is shown in Figure 6D. Aerobic bacteria count was reduced when the levels of $O_2$ decreased with the concomitantly increased levels of $CO_2$. However, $CO_2$ was only the main factor that affected ($P<0.05$) the growth of aerobic bacteria of minimally-processed litchi fruit. The highest 10.0% $CO_2$ concentration efficiently retarded the microbial growth during storage.

4. Conclusions

The storage of litchi arils in nylon/LLDPE bag under 5.0% $O_2$ and 5.0% $CO_2$ had a potential to be used for extending shelf-life and preserving the quality of minimally-processed litchi fruit. It reduced ethanol content, juice leakage and maintained arils firmness during storage periods at 2±1°C. At this gas mixture in combination with low temperature storage could retard an increase in aerobic bacteria and inhibit the growth of yeast and molds and remained under the microbiological limits for food safety during storage. The highest concentration of $CO_2$ (10%) and the lowest concentration of $O_2$ (2.5%) were not recommended for storage of litchi arils because it caused off-odor from fermentation and had the highest juice leakage. The use of firming agents in combination with MAP to reduce the juice leakage and to maintain the firmness property should be further investigated.

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References


Figure 6. Relationship between carbon dioxide and oxygen levels on ethanol content (A), firmness (B), juice leakage (C) and aerobic bacteria count (D) of litchi arils stored under the nine combinations of carbon dioxide and oxygen levels during storage in nylon/LLDPE bag at 2±1°C for 18 days.


