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Original Article

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 (O_2) and carbon dioxide (CO_2) concentrations for storage of minimally-processed litchi fruit *cv*. '*Jugkapat*' packed in a nylon laminated with linear low density polyethylene bag (Nylon/LLDPE). Litchi fruits (fully-red stage) and their arils were sanitized with peroxyacetic 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 O_2 (2.5, 5.0, and 7.5%) and CO_2 (5.0, 7.5 and 10.0%) with atmospheric nitrogen balance for 18 days at 2±1°C. The optimum concentration of O_2 and CO_2 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% O_2 and 5.0% CO_2 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 CO_2 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 O_2 (1-5%) and high level of CO_2 (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-Graü *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} peroxyacetic 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.

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 cylindered 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 $P \leq 0.05$. 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 O_2 and CO_2 are shown in Figure 1. The oxygen content in the packages decreased while the carbon dioxide content increased during the storage. The contents of O_2 and/or CO_2 were almost constant after reaching the plateau at Day 8. This result indicated that lower O_2 and higher CO_2 levels than ambient atmosphere compositions could reduce respiration rate of plant product (Sandhya, 2010). There was statistically significant difference ($P<0.05$) of O_2 content in the packages decreased and CO_2 content increased during storage. However, the reduction of headspace O_2 at each O_2 levels (2.5, 5.0 and 7.5%) with varying CO_2 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 CO_2 content at the level of 7.5 and 10%. At 7.5% O_2 combined with 5% CO_2 help retarding the increase of CO_2 during storage when compared to 2.5% O_2 and 5.0% CO_2 ($P<0.05$). In the control, a rapid depletion of the O_2 was observed from 20.77 to 17.0%, whereas CO_2 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 O_2 and the production of CO_2 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% O_2 : 7.5% CO_2 and 2.5% O_2 : 10.0% CO_2, 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% O_2 : 5% CO_2. 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 O_2 and CO_2 could be occurred in both treatments. The combination of 5.0% O_2 : 5.0% CO_2 and 7.5% O_2 : 7.5% CO_2 could effectively maintain the RQ values close to 1.0 (1.1 and 1.2) throughout the storage periods.

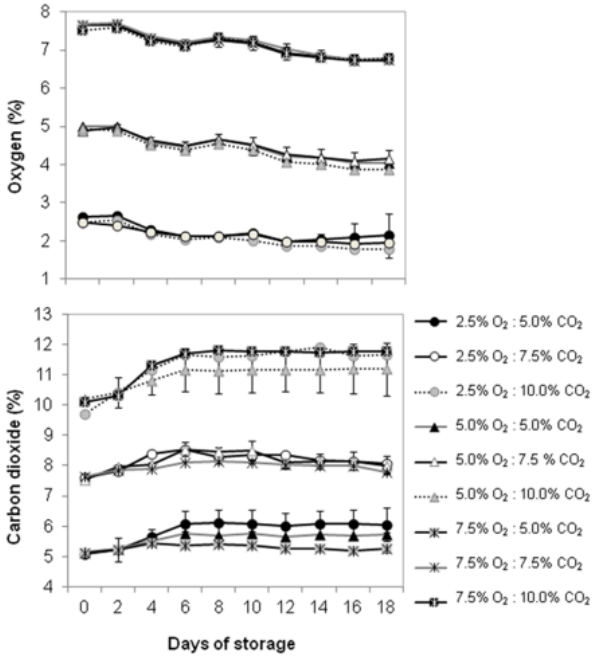

Figure 1. Changes in headspace gases inside package of litchi arils under the nine combinations of carbon dioxide and oxygen levels during storage in nylon/LLDPE bag at 2±1°C for 18 days.

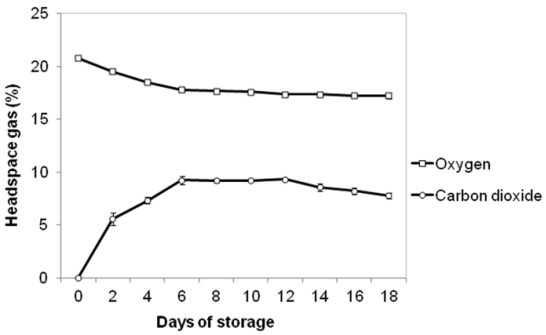

Figure 2. Changes in headspace gases inside package of litchi arils under ambient atmosphere (control) during storage in nylon/LLDPE bag at 2±1°C for 18 days.

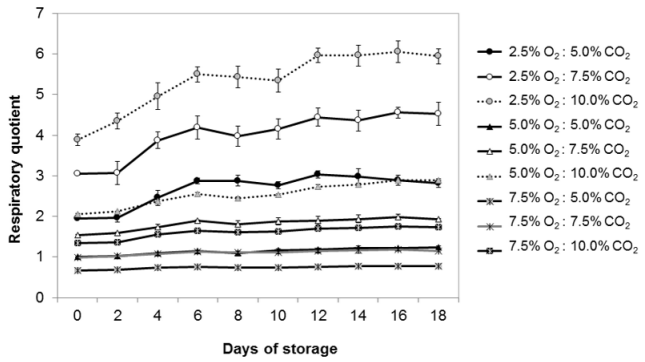

Figure 3. Respiratory quotient of litchi arils stored under the nine combinations of carbon dioxide and oxygen levels during storage in nylon/LLDPE bag at 2±1°C for 18 days.

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% O_2 in combination with all levels of CO_2 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 CO_2 (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 CO_2 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% O_2 : 10.0% CO_2 followed by 2.5% O_2 : 7.5% CO_2 respectively. The ethanol content was increased 2.2 folds after storage for 18 days. Thus, the use of low concentration of O_2 and high concentration of CO_2 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% O_2 in combination with all concentrations of CO_2 showed the highest juice leakage and the optimum concentration of O_2 (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 O_2 and CO_2. Thus, the used of 2.5% O_2 might not appropriate for storage litchi arils in MAP which the injury can occur under the experiment condition. The juice leakage and firmness

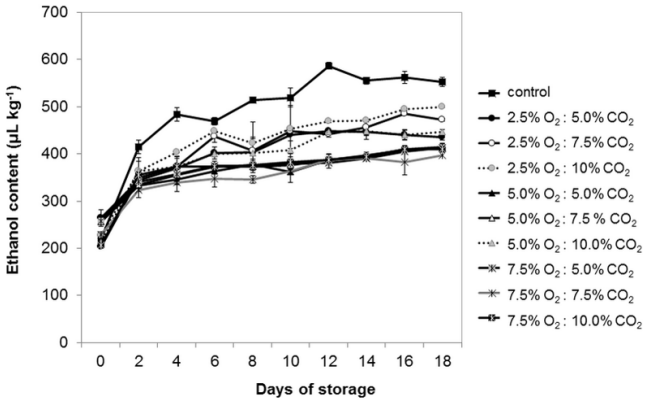

Figure 4. Changes in ethanol content (μL kg^{-1}) of litchi arils stored under the nine combinations of carbon dioxide and oxygen levels during storage in nylon/LLDPE bag at 2±1°C for 18 days.

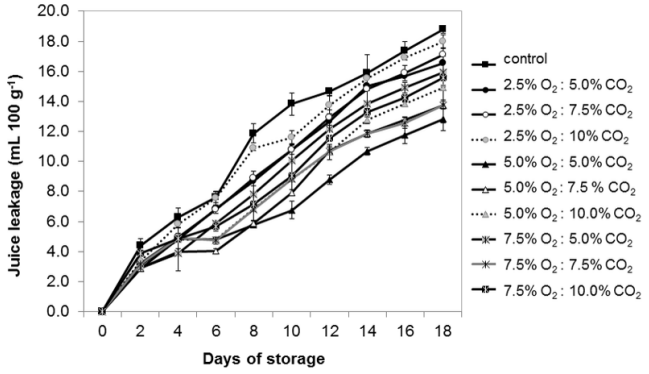

Figure 5. Changes in juice leakage (mL $100g^{-1}$) of litchi arils stored under the nine combinations of carbon dioxide and oxygen levels during storage in nylon/LLDPE bag at 2±1°C for 18 days.

were changed in the same manner, the lowest O_2 (2.5%) and the highest CO_2 (10.0%) resulted in the highest loss of both properties. For the control, the present of highest juice leakage was might be due to the higher respiration rate than in MAP system. Watada and Qi (1999) suggested that threshold levels causing the injury should be avoided because gas mixture of modified atmosphere packages cannot be closely controlled.

3.5 Firmness

The firmness loss of minimally-processed litchi fruit during storage under the atmosphere of 9 combinations of O_2 and CO_2 levels are shown in Table 1. Firmness generally decreased in corresponding to an increase in juice leakage. Firmness of the control decreased 40% after storage for 18 days. The O_2 levels significantly ($P<0.05$) affected arils firmness. Treatment with 2.5% O_2 in combination with all CO_2 concentrations (5.0, 7.5, and 10.0%) showed a decrease of firmness by 39, 33, and 32%, respectively. The best firmness was observed in the treatments of 5.0% O_2 : 5.0% CO_2 and 7.5% O_2 : 7.5% CO_2, where the firmness losses by only 10% in both treatments. However, in other treatments, the firmness decreased in the range of 13-18%.

Firmness loss in fresh-cut fruit has been attributed to the action of two types of pectolytic enzyme e.g., pectin methylesterase (PME) and polygalacturonase (PG). Their activities affected the solubilization of pectin substrates (Toivonen and Brummell, 2008). The action of PME is to remove methoxyl groups from methylated pectin, producing polygalacturonase substrate. This results in a loss of firmness (Prasanna *et al*., 2007). In apple and kiwifruit, the metabolic relationship between fruit softening and respiration rate were observed, in which low O_2 level reduced respiration rate and improved the firmness (Hertog *et al*., 2001, 2004). This is in contrast with the effect on O_2 level of fresh-cut pear, where no difference in fruit firmness was noticeable during storage under 0.25, 0.50 or 21.0 kPa O_2 for 10 days at 5°C (Gorny *et al*., 2002) or under 2.5 or 21.0 kPa O_2 for 21 days at 5°C (Oms-Oilu *et al*., 2007, 2008).

3.6 Aerobic bacteria and yeast-mold counts

Aerobic bacteria count of minimally-processed litchi fruit are shown in Table 2. The control sample showed significantly higher aerobic bacteria count (2.4-2.6 log CFU g^{-1}) than other treatments because of an appropriate O_2 environment. Total aerobic bacteria in litchi arils in nine combinations of O_2 and CO_2 levels decreased continuously during storage from Day 2 to Day 10. This might be due to the reduction of O_2 concentration inside the package and/or low temperature storage helps prolong a lag phase of microbial growth. Low temperature storage was an important factor to slow down a rate of microbial growth as well as retardation of microbial metabolism (Farber *et al*., 2003). The storage temperature below 4°C could inhibit the most of spoilage bacteria espe-

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.

Gas concentration (%)		Firmness (N)								
O_2	CO_2	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18
2.5	5	$0.33^{aA} \pm 0.06$	$0.31^{aB} \pm 0.03$	$0.29^{bcBC} \pm 0.04$	$0.29^{bBCD} \pm 0.01$	$0.27^{bCDE} \pm 0.06$	$0.26^{dE} \pm 0.02$	$0.27^{bcdDE} \pm 0.04$	$0.28^{bcCDE} \pm 0.02$	$0.23^{eF} \pm 0.02$	$0.20^{dG} \pm 0.01$
	7.5	$0.33^{aA} \pm 0.01$	$0.30^{abBC} \pm 0.02$	$0.30^{abB} \pm 0.02$	$0.29^{bB} \pm 0.02$	$0.27^{bCD} \pm 0.02$	$0.26^{dEF} \pm 0.02$	$0.25^{dEF} \pm 0.02$	$0.26^{dDE} \pm 0.01$	$0.24^{eF} \pm 0.02$	$0.22^{cG} \pm 0.01$
	10	$0.31^{abA} \pm 0.02$	$0.30^{abA} \pm 0.01$	$0.31^{abA} \pm 0.03$	$0.27^{cB} \pm 0.02$	$0.27^{bB} \pm 0.03$	$0.26^{dBC} \pm 0.03$	$0.26^{cdB} \pm 0.01$	$0.25^{cdBC} \pm 0.02$	$0.24^{eC} \pm 0.02$	$0.21^{cdD} \pm 0.02$
5.0	5	$0.30^{bAB} \pm 0.04$	$0.31^{aAB} \pm 0.02$	$0.31^{abA} \pm 0.02$	$0.31^{aA} \pm 0.02$	$0.29^{abABC} \pm 0.02$	$0.28^{cCD} \pm 0.03$	$0.28^{abcABC} \pm 0.03$	$0.29^{abBCD} \pm 0.03$	$0.27^{bD} \pm 0.02$	$0.27^{aD} \pm 0.02$
	7.5	$0.31^{abA} \pm 0.03$	$0.31^{aAB} \pm 0.04$	$0.31^{abAB} \pm 0.02$	$0.3^{abAB} \pm 0.03$	$0.31^{aAB} \pm 0.02$	$0.29^{bcBC} \pm 0.02$	$0.30^{aAB} \pm 0.02$	$0.30^{aAB} \pm 0.02$	$0.29^{aABC} \pm 0.02$	$0.27^{aC} \pm 0.01$
	10	$0.31^{abA} \pm 0.03$	$0.31^{aA} \pm 0.02$	$0.30^{abAB} \pm 0.02$	$0.3^{abAB} \pm 0.01$	$0.28^{bABC} \pm 0.4$	$0.29^{bcABC} \pm 0.04$	$0.29^{abABC} \pm 0.4$	$0.28^{bcABC} \pm 0.02$	$0.28^{aABC} \pm 0.2$	$0.27^{aC} \pm 0.01$
7.5	5	$0.30^{abA} \pm 0.02$	$0.29^{cdAB} \pm 0.1$	$0.28^{cdAB} \pm 0.02$	$0.3^{abA} \pm 0.01$	$0.29^{aAB} \pm 0.04$	$0.31^{abA} \pm 0.01$	$0.28^{abcAB} \pm 0.02$	$0.28^{bcBC} \pm 0.01$	$0.26^{cBC} \pm 0.01$	$0.25^{bD} \pm 0.02$
	7.5	$0.31^{abA} \pm 0.01$	$0.30^{abA} \pm 0.01$	$0.32^{aA} \pm 0.02$	$0.31^{aA} \pm 0.01$	$0.31^{aA} \pm 0.02$	$0.32^{aA} \pm 0.03$	$0.28^{abcB} \pm 0.02$	$0.29^{abB} \pm 0.02$	$0.29^{cB} \pm 0.01$	$0.28^{aB} \pm 0.02$
	10	$0.32^{abA} \pm 0.02$	$0.30^{abB} \pm 0.01$	$0.30^{abB} \pm 0.02$	$0.3^{abB} \pm 0.01$	$0.28^{bC} \pm 0.02$	$0.28^{cC} \pm 0.03$	$0.28^{abcC} \pm 0.02$	$0.26^{cdE} \pm 0.02$	$0.27^{bD} \pm 0.01$	$0.27^{aD} \pm 0.02$
control		$0.31^{abA} \pm 0.02$	$0.28^{dB} \pm 0.01$	$0.27^{dB} \pm 0.01$	$0.25^{dC} \pm 0.03$	$0.23^{cD} \pm 0.02$	$0.23^{eD} \pm 0.05$	$0.20^{eE} \pm 0.03$	$0.21^{cDE} \pm 0.01$	$0.20^{dE} \pm 0.01$	$0.17^{eF} \pm 0.01$

Values in each column with distinct lower cases letters represent the significantly different results ($P\leq0.05$). Values in each row with distinct upper cases letters represent the significantly different results ($P\leq0.05$).

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.

Gas concentration (%)		Firmness (N)									
O_2	CO_2	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18
2.5	5	$2.33^{abAB} \pm 0.06$	$2.35^{bcdA} \pm 0.03$	$2.31^{bcABC} \pm 0.03$	$2.21^{bD} \pm 0.03$	$2.08^{cE} \pm 0.03$	$1.86^{gF} \pm 0.03$	$2.09^{cdE} \pm 0.04$	$2.24^{bCD} \pm 0.04$	$2.28^{bcBCD} \pm 0.01$	$2.36^{bA} \pm 0.01$
	7.5	$2.29^{bcA} \pm 0.02$	$2.29^{deA} \pm 0.03$	$2.23^{dAB} \pm 0.02$	$2.14^{bcdC} \pm 0.02$	$2.00^{cdD} \pm 0.04$	$1.90^{fgE} \pm 0.05$	$2.05^{dD} \pm 0.05$	$2.19^{cdBC} \pm 0.04$	$2.20^{dBC} \pm 0.03$	$2.29^{bcdA} \pm 0.03$
	10	$2.30^{bcA} \pm 0.02$	$2.20^{fBC} \pm 0.04$	$2.15^{eC} \pm 0.03$	$2.09^{deD} \pm 0.02$	$1.97^{dE} \pm 0.02$	$2.01^{efG} \pm 0.06$	$2.10^{eF} \pm 0.05$	$2.16^{eBC} \pm 0.01$	$2.19^{dBC} \pm 0.01$	$2.21^{dB} \pm 0.01$
5.0	5	$2.23^{abBC} \pm 0.04$	$2.36^{bcA} \pm 0.02$	$2.25^{cdB} \pm 0.03$	$2.15^{bcdCDE} \pm 0.04$	$2.01^{cdF} \pm 0.02$	$2.10^{bE} \pm 0.06$	$2.13^{cDE} \pm 0.04$	$2.21^{bcBCD} \pm 0.01$	$2.23^{bcdBC} \pm 0.04$	$2.35^{bA} \pm 0.04$
	7.5	$2.34^{cA} \pm 0.03$	$2.31^{cdeA} \pm 0.03$	$2.16^{eBC} \pm 0.04$	$2.15^{bcdBC} \pm 0.07$	$2.09^{bcCD} \pm 0.05$	$2.05^{bcdD} \pm 0.04$	$2.12^{bcdBCD} \pm 0.04$	$2.19^{cdeBC} \pm 0.01$	$2.18^{dB} \pm 0.03$	$2.30^{bcA} \pm 0.02$
	10	$2.23^{cAB} \pm 0.04$	$2.25^{efA} \pm 0.02$	$2.13^{eC} \pm 0.03$	$2.13^{bcdC} \pm 0.03$	$1.97^{dE} \pm 0.05$	$1.97^{deE} \pm 0.02$	$2.05^{deD} \pm 0.02$	$2.12^{cdeC} \pm 0.02$	$2.16^{dBC} \pm 0.04$	$2.19^{dABC} \pm 0.04$
7.5	5	$2.33^{abABC} \pm 0.03$	$2.39^{abA} \pm 0.01$	$2.32^{bCD} \pm 0.04$	$2.20^{bcDE} \pm 0.07$	$2.17^{bDE} \pm 0.05$	$2.14^{bF} \pm 0.02$	$2.19^{bDE} \pm 0.01$	$2.23^{bcD} \pm 0.02$	$2.29^{bC} \pm 0.03$	$2.39^{bAB} \pm 0.01$
	7.5	$2.30^{bcAB} \pm 0.07$	$2.31^{cdeA} \pm 0.03$	$2.18^{e} \pm 0.04$	$2.10^{cdeDEF} \pm 0.03$	$2.06^{cdF} \pm 0.07$	$2.09^{bcEF} \pm 0.07$	$2.11^{bcdDEF} \pm 0.03$	$2.22^{cdeCDE} \pm 0.01$	$2.22^{cdBC} \pm 0.04$	$2.31^{bcA} \pm 0.03$
	10	$2.27^{bcA} \pm 0.03$	$2.25^{efAB} \pm 0.02$	$2.14^{eCDE} \pm 0.02$	$2.04^{deEF} \pm 0.06$	$2.06^{cdEF} \pm 0.07$	$1.99^{cdeF} \pm 0.08$	$2.08^{dDEF} \pm 0.03$	$2.12^{cdeCDE} \pm 0.02$	$2.17^{dBCD} \pm 0.03$	$2.19^{dABC} \pm 0.06$
Control		$2.41^{aBC} \pm 0.06$	$2.44^{aB} \pm 0.05$	$2.43^{aB} \pm 0.01$	$2.35^{eCD} \pm 0.01$	$2.29^{dDE} \pm 0.02$	$2.25^{aE} \pm 0.02$	$2.26^{a} \pm 0.01$	$2.31^{aDE} \pm 0.01$	$2.39^{aBC} \pm 0.02$	$2.57^{aA} \pm 0.04$

Values in each column with distinct lower cases letters represent the significantly different results ($P \leq 0.05$). Values in each row with distinct upper cases letters represent the significantly different results ($P \leq 0.05$).

cially in acidic food (pH<4.6) such as litchi arils. However, the microbial number increased after 10 days storage which was a result of a present of juice leakage leads to an increasing of moisture content and juice was turned to a nutrient supplement for microbial growth. In this study, treatments with 10.0% CO_2 in combination with all levels of O_2 showed the lowest number of aerobic bacteria by 0.39-2.36 log CFU g^{-1} at the end of the storage periods (Table 2). The results were also in the agreement with Farber *et al*. (2003) who found that CO_2 level was only one factor for retardation the growth of aerobic bacteria in MAP. However, the numbers of aerobic bacteria in all treatments did not exceed the standard of Department of Medical Sciences of Thailand (2010) throughout the storage periods where total aerobic bacteria and yeast-molds count were less than 10^{-6} CFU g^{-1} and 10^{-4} CFU g^{-1}, respectively. Fungi were not detected under MAP conditions during storage (data not shown) because of limited O_2 concentration. However, they could be able to grow under the high O_2 content in the control (0.71 to 1.03 log CFU g^{-1}). The results were in an agreement with Oms-Oilu *et al*. (2008) who reported that low O_2 atmosphere was beneficial for controlling the proliferation of yeast and molds on fresh-cut pear. On the other hand, the rate of microbial growth on fresh-cut pear was not affected by O_2 level (0.5, 2.0 and 18.2 kPa) (Gomes *et al.*, 2012). However, these results were not in the same way of litchi arils treated with sucrose solution in combination with anti-browning agents by osmo-vacuum drying method. Arils showed a higher number of total bacteria (5-9 log CFU g^{-1}), yeast-molds (2-7 log CFU g^{-1}), lactic acid bacteria (2.5-7.5 log CFU g^{-1}) and psychrophilic bacteria (2-7 log CFU g^{-1}) under storage temperature of 4±2°C (Shah and Nath, 2008). Differences in the results might depend on the hygiene of process operations after harvesting and during minimal processes along with chemical treatment efficacy.

3.7 Relationship of O_2 and CO_2 on ethanol content, firmness, juice leakage, and aerobic bacteria

The surface plot in Figure 6A-D show the relationships between O_2 and CO_2 levels on the ethanol content, arils firmness, juice leakage, and total bacteria count of minimally-processed litchi fruit during storage for 18 days. The concentration of O_2 at 2.5% caused the increase of ethanol content ($P>0.05$) but the concentration of O_2 at 5.0 and 7.5% showed no significant differences ($P>0.05$) between treatments. The concentration of CO_2 at 10.0% has the highest ethanol content but the concentrations of 5.0 and 7.5% had no pronounced impact on ethanol content. Thus, the concentration of O_2 and CO_2 in the range of 5.0% and 7.5% were the optimal condition to obtain the lowest ethanol content for storage of minimally-processed litchi fruit and maintained the greatest firmness value. However, all levels of CO_2 did not affect the firmness in each O_2 level (Figure 6B).

At 2.5% O_2 in combination with all concentrations of CO_2 resulted in the highest juice leakage and significant differences ($P>0.05$) when compared to other treatments. The

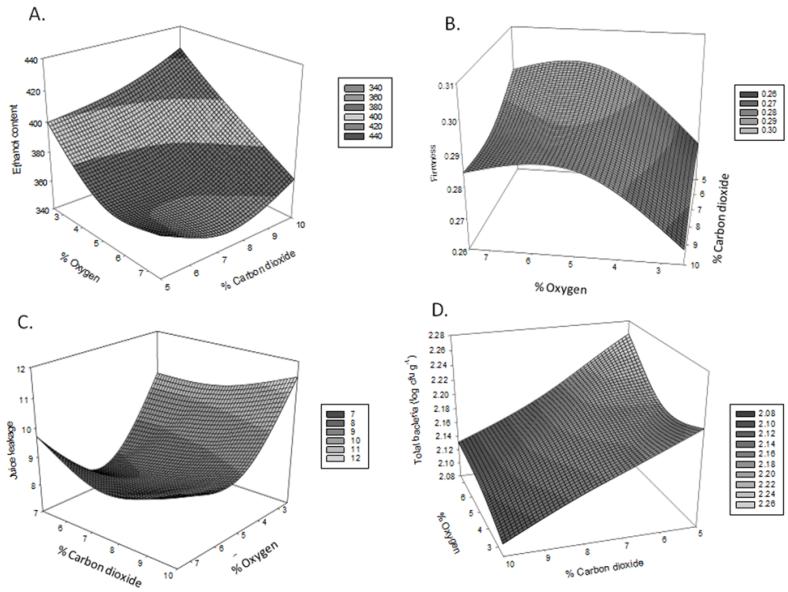

Figure 6. Relationship between carbon dioxide and oxygen levels on ethanol content (A), firmness (B), juice leakage (C) and aerobic bacteria count (D) of litchi arils stored under the nine combinations of carbon dioxide and oxygen levels during storage in nylon/LLDPE bag at 2±1°C for 18 days.

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 ($P \leq 0.05$) 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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