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Optimization of bulk modified atmosphere packaging for long-term storage of 'Fuyu' persimmon fruit

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ABSTRACT

An optimal design procedure for bulk modified atmosphere packaging (MAP) that would enable the long-term storage of 'Fuyu' persimmon fruit for export market was proposed in this study. Initially, the lower O_2 limit of aerobic respiration was determined, based on the respiratory quotient breakpoint, and found to be 0.65 kPa. Next, the parameters of a Michaelis–Menten type respiration model were estimated, and they were used to predict the O_2 consumption rate at 1.5 kPa, which was set as the target O_2 partial pressure inside the package and is considered safe to avoid fermentation. Then, the values of the predicted respiration rate, the surface area of the packaging, and the sample weight were substituted into the mathematical model to calculate the gas composition change inside the package; as a result, the necessary O_2 permeance of the packaging film material was estimated as 2.59×10^{-7} L m^{-2} s^{-1} kPa^{-1} for the prospective bulk packaging system. Finally, the efficacy of the designed bulk MAP on the quality changes in the fruit was practically examined by comparing the MAP performance to that of fruit in an individual package (having 60-μm thick low-density polyethylene film bag) and to that of non-packed fruit (i.e., control). All the samples were stored under conditions simulating the long-term storage and subsequent transportation of fruit from Japan to Thailand. The results revealed that the O_2 concentration at a steady state in the designed bulk MAP could be established at 1.3–1.6 kPa, which successfully corresponded with the target value. Furthermore, losses in flesh firmness and color, and the external damage of fruits were reduced in the bulk MAP when compared to storage in the other two package treatments. Overall, the bulk MAP designed could be used for ca. 4 months storage of 'Fuyu' persimmon fruit and for its commercial application to maintain fruit quality and to export fruit to international markets.

1. Introduction

Persimmon (*Diospyros kaki* L.f.), in particular the cultivar 'Fuyu', is a popular fruit in many Asian countries and is highly regarded for its sweet taste and outstanding outward appearance. Crunchiness is also a key characteristic of the 'Fuyu' persimmon because of its non-astringency; otherwise, it would require a de-astringent treatment to become edible which always involves softening the fruit. Owing to its unique flavor, the consumer demand for 'Fuyu' has been increasing and is expected to become a profitable agricultural commodity. In Japan, the 'Fuyu' persimmon is recognized as a seasonal fruit because its harvest is limited from November to December; hence, the demand for its long-term storage is increasing to avoid an oversupply during the harvesting season and to continue its sale for as long as possible. In addition, there is a demand for export, which has increased recently due to the expansion of the sales of 'Fuyu' producers and distributers in markets abroad; for this reason, reliable long-term storage and long-distance transportation is required. Unfortunately, the 'Fuyu' persimmon has a short storage life, at no greater than 3 weeks at 20 °C due to the onset of ripening once harvested (Sargent et al., 1993). Generally, however, storage at low temperatures can prolong the shelf-life of a fruit by reducing both its respiratory activity and ethylene production; but fruit softening and skin darkening inevitably occur. Accordingly, improved postharvest handling techniques that extend the shelf-life of the 'Fuyu' persimmon are needed for the stabilization of its fruit supply in domestic markets and for realizing its long-term exporting potential to the overseas markets.

Modified atmosphere packaging (MAP), in combination with a low

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temperature, is a widely acceptable and suitable technology for the maintenance of the quality of fruits and vegetables during their long-term storage (Cia et al., 2006; Jeong et al., 2013; Fahmy and Nakano, 2013). The modified atmosphere condition slows down the respiration rate, ethylene production, and water loss, thus reducing the enzyme activities and metabolic rate of the packed fruit or vegetable (Kader and Watkins, 2000; Kim et al., 2010; Wang et al., 2011; Selcuk and Erkan, 2015). For the 'Fuyu' persimmon, the effectiveness of MAP for the reduction of weight loss and browning, and for the delay in the softening of its flesh, has been reported already, and a low-density polyethylene (LDPE) film has been often tested as the packaging material (Cia et al., 2006; Kim et al., 2010). Nonetheless, several disadvantages of MAP have been raised; for instance, Cia et al. (2003) suggested a risk of low-oxygen injury based on the acetaldehyde and ethanol accumulation detected when the 'Fuyu' persimmon was stored in an 80-μm LDPE bag at 1 °C for 40 d. Similarly, the ethanol production was found in the headspace of a 60-μm LDPE bag after 14 weeks of storage at 0 °C (Ben-Arie and Zutkhi, 1992). These metabolites originate from anaerobic respiration, and they are the causative agents of "off-odor", which inevitably lessens the market value of the fruit. Anaerobic respiration occurs when an extremely low O_2 condition is created in the package due to a careless packaging design. However, if the O_2 concentration in the package can be equilibrated just above the tolerance limits of the freshly packed produce through an appropriate packaging design, the risk of inducing anaerobic respiration may be avoided. Furthermore, in doing so, this also retains the greatest freshness because the physiological activities are suppressed at the maximum level in the range of aerobic metabolic processes. Hence, the determination of lower O_2 limit (LOL) for the target produce commodities is the first priority when the goal is to optimize the packaging condition. To date, however, most of the MAP studies on 'Fuyu' persimmon fruit were conducted on the basis of a trial-and-error method, and so they may not provide the best packaging condition. Ideally, to achieve the long-term storage and long-distance transportation of 'Fuyu' persimmon, the MAP should be precisely designed.

In the MAP system, as to how the atmosphere is modified inside the package depends on a complex combination of factors, such as the gas permeability of the packaging film material, the surface area of the package and the weight of the packed fresh produce, as well as its respiration rate. To date, the gas composition change in MAP has been modeled based on the mass balance of each O_2, CO_2, and N_2 gaseous between inside and outside of the packaging, and its reliable and versatile application confirmed by many researchers (Torrieri et al., 2009; Mangaraj et al., 2014; Fahmy and Nakano, 2014). However, a MAP for the 'Fuyu' persimmon based on a mathematical model that takes into consideration the fruit's respiratory characteristics is yet to be designed, neither has anyone yet evaluated the storability of this fruit when packed in a well-designed MAP.

In addition, to achieve a successful packaging design, the focus should not be only on quality preservation: the handling efficiency and a reduction in transport costs should also be considered. The bulk MAP is a promising method that fulfills all of the above requirements. This method has been tested at the laboratory scale to assist in the shelf-life extension of the persimmon fruit (Chaudhry et al., 2002; Jeong et al., 2013; Fahmy and Nakano, 2013). However, based on our literature review, there is little information on the long-term quality preservation of persimmon fruit when stored under a bulk MAP.

In this study, the respiration rate and LOL of the 'Fuyu' persimmon fruit were carefully examined. Following this, the optimum gas permeability of the packaging film material was estimated in a mathematical model that was priorly used to predict the gas composition changes occurring inside a MAP. Then, the effectiveness of the designed bulk MAP for the quality preservation of the 'Fuyu' persimmon was also evaluated, specifically when stored under conditions mimicking temperature changes that would occur in the long-term storage and subsequent transportation of 'Fuyu' from Japan to Thailand.

2. Materials and methods

2.1. Plant materials

The 'Fuyu' persimmon fruit (*Diospyros kaki* L.f.) was harvested at the commercial maturity stage from the Gifu Prefectural Agricultural Technology Center, Gifu, Japan. The fruits selected were uniform in shape and size, and they lacked any wound or decay symptoms. The average weight of the fruit sample was 0.33 ± 0.02 kg. After their selection, the fruits were packed in a corrugated fiberboard box (CFB) and carefully transported to the laboratory.

2.2. Determination of the respiratory quotient

Approximately 1.6 kg of 'Fuyu' persimmon fruits were placed into a 4.8 L hermetic chamber. The chamber was then set inside a 5 °C incubator (MIR-154-PJ Panasonic, Osaka, Japan). This chamber has both inlet and outlet ports that were linked individually to the plastic tube. The tube coupled to the outlet port of the chamber was connected to a semiconductor-type CO_2 sensor (GMP221 CO_2 probe Vaisala, Vantaa, Finland) equipped with an aspiration pump (GM70 Vaisala, Vantaa, Finland) and further along linked to a zirconia-type O_2 sensor (MC-8G Iijima Electronics Corporation, Aichi, Japan). The N_2 gas was flushed into the chamber via the inlet tube until the O_2 concentration inside the chamber dropped to 3 kPa. Then, the inlet tube of the chamber was removed from the N_2 gas cylinder and immediately reconnected to the outlet port of the O_2 sensor, to create a closed system. Changes in both the O_2 and CO_2 concentrations inside the chamber were then recorded at 10-min intervals by a data recorder (TR-V550 Keyence, Osaka, Japan). The measurements were stopped when the O_2 concentration inside the chamber fell to 0 kPa. The collected data on gas concentrations was used for estimating the respiratory quotient (RQ) value. The RQ value of the fruit was calculated from the slope of the regression line relating the increase of the CO_2 concentration with the decrease of the O_2 concentration inside the chamber, as shown in the following equation.

$$RQ = \Delta C_{CO_2}/\Delta C_{O_2} \quad (1)$$

where RQ is the respiratory quotient, and ΔC is the slope of the gas concentrations in the chamber (O_2 and CO_2) (% h^{-1}).

For determination of the slope of the gas concentrations in the chamber, sequential sets of 25 data points for each O_2 and CO_2 concentration were selected, and these were subjected to regression analysis to estimate ΔC_{O_2} and ΔC_{CO_2}, respectively. The calculation continued, by shifting down one step and computing the slope of the next dataset, as the method for obtaining a moving average. After the calculation of RQ values as mentioned above, the RQ values were then plotted against the moving average of 25 data points of the O_2 concentration to observe the changing pattern of RQ. This experiment was run in triplicate.

2.3. Determination of the respiration rate

The O_2 consumption rate of the 'Fuyu' persimmon at 1, 2, 5, 10, and 21 kPa O_2 were measured by the flow-through method (Nakamura et al., 2004). Briefly, approximately 1.6 kg of fruits were weighed and put into the 4.8-L hermetic chamber with inlet and outlet ports (as described in Section 2.2). The chamber was then placed inside the incubator (MIR-553 Sanyo, Osaka, Japan) and set at 5 °C. The gas mixture, which was generated by a gas blender (GB-3C Kofloc, Kyoto, Japan) consisting of mass-flow controllers connected to high purity O_2 and N_2 gas cylinders, was divided into two lines. The first line served as the inlet gas measurement, and the second line flowed into the chamber via the inlet port at the flow rate of 0.100 L min^{-1} that was maintained by the mass-flow controller (SEF-E40 Horiba Stec, Kyoto, Japan). The gas composition from the inlet port was exchanged by the sample's respiration, and flowed out via the outlet port. Both the inlet and outlet gases flowed to the channel selector: this consisted of solenoid valves that injected the sample gas into the gas chromatography (GC-14A Shimadzu, Kyoto, Japan) alternatively via a 0.2-mL volume-sampling loop. The O_2, N_2, and CO_2 in the sample gas was separated by a Molecular Sieve 5A and the Porapak Q column, and then analyzed by a thermal conductivity detector. Helium was used as the carrier gas. The O_2 consumption rate of the fruit was calculated using Eq. (2) (Fonseca et al., 2002).

$$R_{O_2} = |y_{O_2}^{in} - y_{O_2}^{out}|/100 \times F/W \times P/RT \times 10^6/60 \quad (2)$$

where R_{O_2} is the rate of O_2 consumption by the produce (μmol kg^{-1} s^{-1}), y_{O_2} is the volumetric concentration of O_2 in the chamber (*in* = inlet, *out* = outlet), F is the flow rate (L min^{-1}), W is the produce weight (kg), P is the atmospheric pressure (=101.3 kPa), R is the universal gas constant (=8.314 L kPa K^{-1} mol^{-1}), and T is the absolute temperature (K).

The measurement of the O_2 consumption rate was done in three replications for each O_2 concentration. The Michaelis–Menten type respiration model, described by Eq. (3), was applied in this study to predict the O_2 consumption rate at any O_2 concentration in the environment.

$$R_{O_2} = V_{max}[O_2]/(K_m + [O_2]) \quad (3)$$

where R_{O_2} is the rate of O_2 consumption by the produce (μmol kg^{-1} s^{-1}), V_{max} is the maximum respiration rate (μmol kg^{-1} s^{-1}), K_m is the apparent Michaelis-Menten constant, and $[O_2]$ is the oxygen concentration (kPa).

To obtain the V_{max} and K_m values, Eq. (3) was linearized into Eq. (4), and fitted with the measured O_2 consumption data by the least squares method.

$$[O_2]/R_{O_2} = [O_2]/V_{max} + K_m/V_{max} \quad (4)$$

2.4. Experimental verification of packaging performance

The bulk MAP, individual package, and non-packed conditions were used to verify the packaging performance on the quality maintenance of the 'Fuyu' persimmon fruit. For the bulk MAP condition, a micro-perforated polypropylene (PP) film bag (0.79 m × 0.80 m) was set as an inner packaging material inside a CFB (0.32 m × 0.39 m × 0.21 m). The O_2 permeance of the micro-perforated PP film was 2.59×10^{-7} L m^{-2} s^{-1} kPa^{-1} (obtained from Eq. (10) described in Section 3.1.2). A total 10-kg batch of fruits was packed in the micro-perforated PP film bag, with three layers separation by a molded fiber pulp tray to protect the fruit from impact and compression force. The bag was closed tightly using the rope. The closing margins were 0.20 m (Fig. 1A). For the individual package condition, a 60-μm thick LDPE film bag (0.20 m × 0.20 m) was used to separately pack each 'Fuyu' persimmon fruit (Fig. 1B). The non-packaged fruit served as the control for this experiment. All the samples were then stored in the incubator for 112 d under the conditions simulating a long-term storage environment (i.e., 0 °C for 60 d) and the subsequent temperature conditions in transporting the fruit from Japan to Thailand (7, 2, and 5 °C for 6, 16, and 30 d, respectively).

Changes in the O_2 and CO_2 concentrations inside the packaging were measured periodically by using the GC, as described previously in Section 2.3, with a manual injection technique. For the gas sampling by a syringe, two layers of polyvinyl chloride (PVC) tape were pasted on the surface of the packaging to avoid any gas leakage from the small hole caused by a needle. During the gas sampling, the top layer of tape was stripped off, and the needle of the syringe was then inserted into the package through the bottom layer of tape. After withdrawing the headspace gas, the small hole was immediately closed by the top layer of tape. A 0.2 mL gas sample was then injected into GC for the gas analysis. The results for these gas concentrations were expressed as the percentage of total gas volume (%).

The skin color at the equatorial zone of the fruit was measured by using a colorimeter (CR-13 Minolta, Osaka, Japan) calibrated with a standard white tile. The skin color was expressed as CIELAB color space (i.e., L^*, a^*, and b^*).

A compression test was conducted to evaluate flesh firmness. First, the fruit was cut transversely by a sharp knife, and the flesh was excised with an 18-mm diameter cork borer. Then, both edges were cut to obtain a cylindrical testing sample that was 20 mm in length. The testing sample was compressed on its topside by using a Rheometer (Compac-100 II Sun Scientific, Tokyo, Japan) that had a 30-mm diameter probe. The compression distance and test speed were respectively 1.5 mm and 0.05 mm s^{-1}. Young's modulus was obtained from the slope of the compressive stress and strain curve expressed by Eq. (5) (Khodabakhshian and Emadi, 2011).

$$E = \sigma/\varepsilon \quad (5)$$

where, E is Young's modulus (kPa), σ is a stress (kPa), and ε is a strain.

The color and flesh firmness measurements were done in five replications per packaging condition. The assumption of equality of variances was verified by the Bartlett's test. The effect of the three packaging condition groups on each response variable was tested by one-way ANOVA (followed automatically by Tukey's post hoc test at the 5% level of significance) in the R software platform v.3.1.0 (R Foundation for Statistical Computing).

3. Results and discussion

3.1. Designing bulk MAP for 'Fuyu' persimmon fruit

3.1.1. Respiration characteristics of 'Fuyu' persimmon fruit

Fig. 2 shows the relationship between the O_2 concentration and RQ of 'Fuyu' persimmon fruit at 5 °C. The change of RQ with the O_2 concentration was simply divided into two major phases: a constant state and an increasing state. The constant phase of RQ remained steady under the O_2 concentration when it ranged from 3 kPa to 1 kPa. An increasing phase of RQ was then observed when the O_2 concentration fell below 1 kPa and the abrupt increase appeared when the O_2 decreased to nearly 0. Normally, the RQ value of fresh produce usually ranges from 0.7 to 1.3 and is approximately constant under aerobic conditions (Kader et al., 1989; Ke and Kader, 1992; Wang and Long, 2014), whereas an abrupt increase in RQ is observed as fermentation occurs (Gran and Beaudry, 1993). To indicate the lowest O_2 concentration in the environment that does not induce fermentation in fresh produce, the LOL has been commonly used. The LOL can be estimated as the O_2 level at the RQ breakpoint. The RQ breakpoint is defined as the O_2 concentration at which the RQ increases; it can be specifically identified at 10% above the constant RQ value (Gran and Beaudry, 1993; Yearsley, 1996). In the present study, the RQ breakpoint of three replicates was 0.68, 0.58, and 0.68 kPa O_2 at 5 °C; therefore, the LOL of 'Fuyu' persimmon fruit could be estimated as 0.65 kPa O_2 (by taking the average).

Fig. 3 shows the relationship between the O_2 concentration and the O_2 consumption rates of 'Fuyu' persimmon fruit stored at 5 °C. The experimental data showed a gradual decline as the O_2 concentration decreased. The rate of O_2 consumption at 1 kPa O_2 was suppressed by 91% when compared with that at 21 kPa O_2. The predicted values were obtained by the Michaelis–Menten type respiration model (Eq. (3)) using the V_{max} and K_m values of 1.31×10^{-1} μmol kg^{-1} s^{-1} and 7.88 kPa, respectively. These predicted values could explain very well the experimental respiratory change under the different O_2 concentrations in the environment. Based on the high coefficient of determination ($R^2 = 0.90$) and the low root mean square error level (RMSE = 2.41×10^{-3} μmol kg^{-1} s^{-1}) found between the predicted values and the experimental values, the Michaelis–Menten type

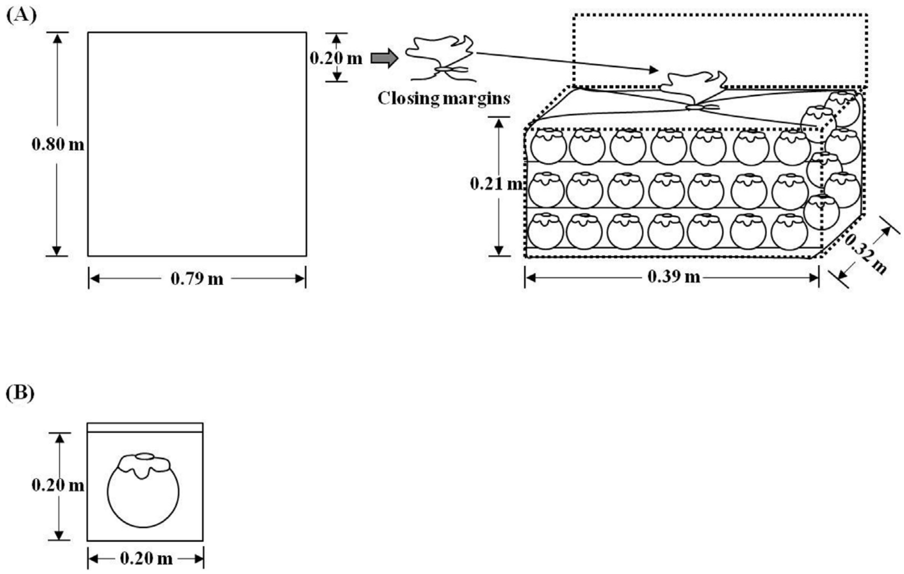

Fig. 1. Schematic diagrams of packaging dimension; bulk MAP design (A) and individual package design (B).

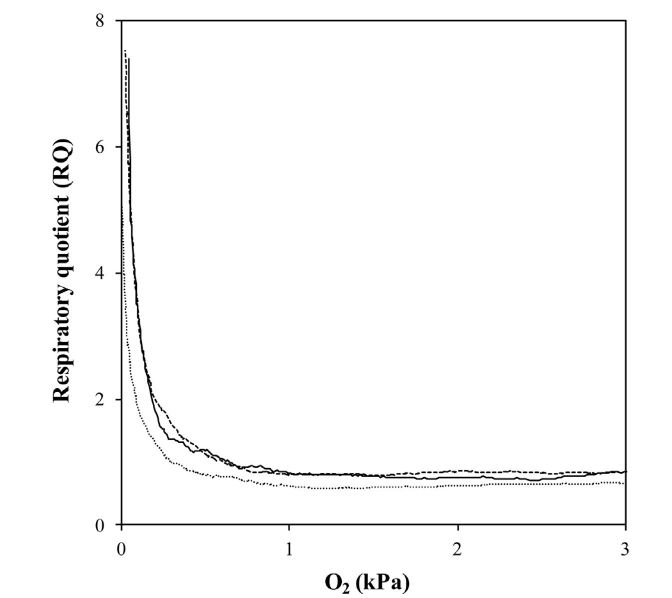

Fig. 2. Relationship between O_2 concentration and respiratory quotient (RQ) of 'Fuyu' persimmon fruit stored at 5 °C. Each line represents a different experiment (n = 3).

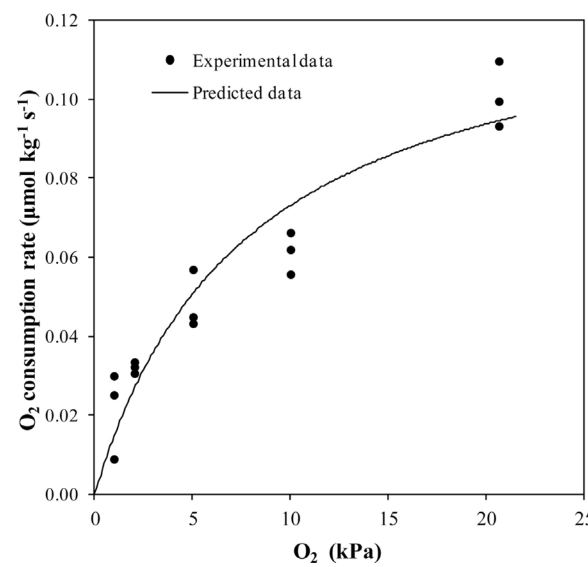

Fig. 3. The experimental and predicted O_2 consumption rates of the 'Fuyu' persimmon fruit at various O_2 concentrations when stored at 5 °C. Symbols represent the values of the experimental data. Solid lines indicate the predicted values obtained by Eq. (3).

respiration model can be used to reliably predict the O_2 consumption rate of the 'Fuyu' persimmon fruit under any O_2 concentration at 5 °C.

3.1.2. Mathematical approach for designing the bulk MAP

For the successful maintenance of fresh produce quality through MAP, the gas composition inside the given package must be controlled to meet the optimum level, whereby the produce's respiration is suppressed maximally without incurring anaerobic metabolism. The optimum gas condition in the package may be created by matching the gas permeability of the packaging film material to the respiration rate of fresh produce.

The gas composition change in the package has been modeled, as shown in Eq. (6) to Eq. (9). This model has been widely used to successfully predict the change in gas composition inside the package (Mahajan et al., 2007; Dash et al., 2012).

$$dV_{O_2}^{pkg}/dt = K_{O_2}\ A\left(p_{O_2}^{ext} - p_{O_2}^{pkg}\right) - R_{O_2}\ W \times RT/P \times 10^{-6} \tag{6}$$

$$dV_{CO_2}^{pkg}/dt = K_{CO_2}\ A\left(p_{CO_2}^{ext} - p_{CO_2}^{pkg}\right) - R_{CO_2}\ W \times RT/P \times 10^{-6} \tag{7}$$

$$dV_{N_2}^{pkg}/dt = K_{N_2}\ A\left(p_{N_2}^{ext} - p_{N_2}^{pkg}\right) \tag{8}$$

$$dV_{all}^{pkg}/dt = dV_{O_2}^{pkg}/dt + dV_{CO_2}^{pkg}/dt + dV_{N_2}^{pkg}/dt \tag{9}$$

where $V_g^{\rm pkg}$ is the volume of the gas g (g = O_2, CO_2, and N_2) inside the package (L); t is time (s); K_g is the film permeance for a gas g ($L\ m^{-2}\ s^{-1}\ kPa^{-1}$); A is the surface area of the packaging film (m^2); p_g^h is the partial pressure of a gas g in a location h (h = external (*ext*); package (*pkg*); $V_{\rm all}^{\rm pkg}$ is the total volume of the gas composition inside the package (L); and R_{CO_2} is the rate of CO_2 production by the fresh produce ($\mu mol\ kg^{-1}\ s^{-1}$); R is the universal constant gas (= 8.314 $L\ kPa\ K^{-1}\ mol^{-1}$); T is the absolute temperature of gas (K); and P is the atmospheric pressure (= 101.3 kPa).

Many researchers have suggested that the O_2 is directly related to the respiration rate of fresh produce (Andrich et al., 1991; Gong and Corey, 1994; Segall and Scanlon, 1996; Mahajan et al., 2016), whereas it is not significantly affected by CO_2 (Peppelenbos and Van't Leven, 1996). Therefore, in this study, only the O_2 concentration change in the package was considered. At the steady state, where the produce's respiration rate equals the gas permeation rate through the packaging film material, the left term of Eq. (6) is zero, and thus Eq. (6) can be rearranged for K_{O_2}, as shown in Eq. (10).

$$K_{O_2} = R_{O_2}\ W/\left(A\left(p_{O_2}^{ext} - p_{O_2}^{pkg}\right)\right) \times RT/P \times 10^{-6} \tag{10}$$

where $p_{O_2}^{ext}$ is the O_2 partial pressure outside the package (= 20.9 kPa), $p_{O_2}^{pkg}$ is the target equilibrium O_2 partial pressure inside the package (kPa), R_{O_2} is the product's respiration rate at equilibrium (μmol kg^{-1} s^{-1}), and T is the absolute temperature of gas (= 278 K).

For the 'Fuyu' persimmon fruits the target equilibrium O_2 partial pressure inside the package $p_{O_2}^{pkg}$ was obtained from the LOL measurement of 0.65 kPa O_2 (described in Section 3.1.1). However, according to a recent study of sweet cherry by Wang and Long (2014), its LOL was observed as the fermentation induction point. A similar result was reported by Lakakul et al. (1999) that the fermentation threshold, which was associated with the accumulation of ethanol production linked to the off-odor, occurred when the O_2 in the package fell below the LOL. Thus, to avoid any anaerobic metabolism, the target equilibrium O_2 concentration inside the package for the 'Fuyu' persimmon fruit was set at 1.5 kPa ($p_{O_2}^{pkg}$), which was slightly higher than the obtained LOL. This setup condition is supported by the work of Gran and Beaudry (1993) who found that the package with an O_2 concentration above the RQ breakpoint had no detectable ethanol in the package headspace. Based on the $p_{O_2}^{pkg}$ set here, the value of R_{O_2} in Eq. (10) was arrived at by calculating the O_2 consumption rate at 1.5 kPa O_2 using Eq. (3), which gave a result of 2.09×10^{-2} μmol kg^{-1} s^{-1}. Based on the testing package conditions, 10 kg and 9.48×10^{-1} m^2 were derived for W and A, respectively. The value of A was obtained from the calculation of the surface area of film bag considering the binding margin. By solving Eq. (10), the O_2 permeance (K_{O_2}) was calculated as 2.59×10^{-7} L m^{-2} s^{-1} kPa^{-1}.

3.2. Performance of the designed bulk MAP

3.2.1. Gas concentration change in the packaging

Fig. 4 shows the change in O_2 and CO_2 concentrations in the different types of packaging under the conditions simulating a low temperature storage and subsequent transportation from Japan to Thailand. The O_2 concentration of the individual package decreased slowly to 3 kPa in 2 months but then fluctuated in the range of 3–5 kPa in response to changes in the environmental temperature. By contrast, the designed bulk MAP decreased rapidly to 1.5 kPa within 1 month, but thereafter kept within a narrow range, fluctuating from 1.3 to 1.6 kPa (Fig. 4A). This greater stability of the O_2 concentration in the bulk MAP might come from the insensitivity of both the gas permeability of the packaging material and the temperature of the fruit in affecting the respiratory O_2 consumption response to the environmental temperature change. The gas permeability of LDPE, which was used in the individual packaging, is strongly related to the temperature, whereas that of the micro-perforated PP is not (Yaptenco et al., 2007). Moreover, the temperature of fruit in the bulk MAP might not be affected so much by the environmental temperature change, because the bulk MAP consisted of two layers: a CFB and a plastic film bag. Furthermore, they acted as a barrier to heat transfer, enhancing the thermal resistance of the bulk MAP when compared with the individual package having only one layer. In addition, a higher heat capacity of the bulk MAP would also reduce the potential changes in fruit temperature, because the bulk MAP contained more fruits than did the individual package.

The CO_2 concentration in the individual package increased to 4.3 kPa in 2 months, and rose to 7.1 kPa when the environmental temperature changed from 0 °C to 7 °C. Subsequently, it decreased and stayed at 4–5 kPa when the temperature changed from 2 °C and 5 °C. For the bulk MAP, the CO_2 concentration increased to 17 kPa in 2 months and rose rapidly to 24 kPa when the temperature changed to 7 °C. After that, however, it decreased to 20.5 kPa in response to a decreasing temperature, but then increased again gradually when the

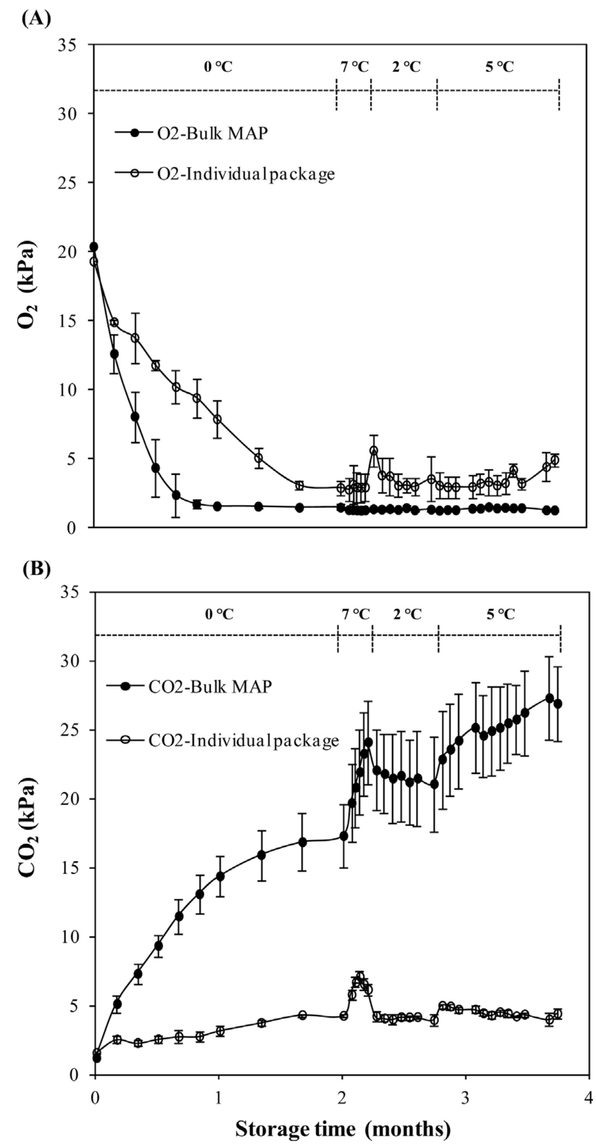

Fig. 4. Effect of different packaging conditions (individual package and bulk MAP) on the O_2 (A) and CO_2 (B) concentrations of 'Fuyu' persimmon stored at varying temperatures during 4 months. (n = 3, vertical bars represent standard deviation).

temperature changed from 2 °C to 5 °C (Fig. 4B). The higher CO_2 accumulation in the bulk MAP as compared to the individual package must have resulted from the lower CO_2 permeability of the micro-perforated PP film used in the bulk MAP; and conversely, from the higher selectivity of CO_2 in the gas permeation of LDPE in the individual packaging. Moreover, the CO_2 permeability of the micro-perforated PP film was less than that of O_2 (Yaptenco et al., 2007). This might explain why CO_2 in the bulk MAP tended to increase through the experimental period, whereas O_2 became almost steady after 1 month of storage.

It should be noted that the O_2 concentration in the bulk MAP was kept at an almost constant value, even though the ambient temperature fluctuated, and it also agreed well with our expected value set as a target equilibrium O_2 concentration in the mathematical model to predict the necessary O_2 permeability of the packaging material. Until now, unexpected temperature fluctuation, quite common during the storage and transportation of fruits and vegetables, has been pointed out as a major problem in the practical use of MAP because its reliability depends upon a rigorous temperature control (Jacxsens et al., 2000; Tano et al., 2007). However, based on the present results, it may be said that the bulk MAP offers a reasonable way to overcome this problem given the fact that the effect of the temperature fluctuation on

Table 1
Changes in L^*, a^*, and b^* of 'Fuyu' persimmon fruit packed in three different packaging conditions (non-packaged, individual package, and bulk MAP) on the first and last day of storage (4 months, 5 °C).

Treatments	Days in storage	Parameter		
		L^*	a^*	b^*
Initial	0	54.30 ± 1.05[b]	41.68 ± 1.73[b]	52.08 ± 8.30[b]
Non-packaged	112	35.96 ± 2.78[a]	23.92 ± 7.25[a]	22.98 ± 5.58[a]
Individual package	112	53.50 ± 3.00[b]	37.26 ± 1.49[b]	49.80 ± 5.07[b]
Bulk MAP	112	58.12 ± 2.91[b]	36.84 ± 3.04[b]	54.24 ± 6.27[b]

Note: Values represent the mean of five replicates with their standard error (SD). Values within columns followed by the same letter are not significantly different at $P = 0.05$ (ANOVA with a Turkey's post hoc test).

the O_2 concentration in the package was almost buffered due to its high insulating capacity. Additionally, the mathematical model approach employed here could be used to optimize the packaging parameters, such as the gas permeability of film according to the packaging size or volume, so as to achieve a suitable O_2 concentration in the package for quality maintenance.

3.2.2. Changes in fruit quality

The skin color (L^*, a^*, and b^* values) of the 'Fuyu' persimmon fruit stored in the different packaging conditions, before and after 112 d storage, is shown in Table 1. The L^* (lightness), a^* (redness), and b^* (yellowness) values in the non-packaged fruit were significantly decreased, representing the color change from light yellow-orange to dark red-orange. Conversely, the L^*, a^*, and b^* values of the fruit stored in both the bulk MAP and the individual package were similar and unchanged; that is to say, the skin retained its light yellow-orange color for 112 d. This prevention of color change may be primarily due to the low O_2 conditions created inside the two types of packages. Under a low O_2 condition the metabolic activity in the fruit is slowed down; this leads to a reduction in the respiration rate and peel browning, a slowing of the ripening process and chlorophyll degradation, and a delay in the senescence of the fruit (Kader, 1986; Sandhya, 2010). Although a significant difference in skin color was not observed between the fruit stored in the individual and the bulk packaging, in the former some black spots were found on the skin surface of the fruit after 3 months of storage. The same spots were also observed on the non-packed fruit after 2 months of storage. In stark contrast, the fruit packed in the bulk MAP kept their excellent appearance, and no signs of decay were observed throughout the storage period. The skin blackening is a physiological disorder that occurs easily in 'Fuyu' persimmon fruit; the pigment responsible is hypothesized to be a melanin compounds (Choi, 2013). These compounds were generated by the reaction of the polyphenol oxidase (PPO) enzyme with the phenolic compounds. This PPO activity is often suppressed in a low O_2 condition relative to under an atmospheric condition. Zhang and Quantick (1997) reported that PPO activity was slowed down when longan fruit was stored under 1 kPa O_2 + 5 kPa CO_2 and 3 kPa O_2 + 5 kPa CO_2 (when compared with its activity under 10 kPa O_2 + 5 kPa CO_2). As shown in Fig. 4A, the equilibrium O_2 partial pressure in the individual and bulk packages was 3–5 kPa and 1.5 kPa, respectively. Given the fact that the skin blackening was observed only in the fruit stored in the individual package, rigorous O_2 control in the package is required to accomplish the long-term storage of 'Fuyu' persimmon fruit.

Fig. 5 shows the changes in the Young's modulus value of the 'Fuyu' persimmon fruit stored in different packaging conditions for ca. 4 months. The Young's modulus is a property typically used to indicate the firmness of fresh produce (Avila et al., 2007), such that a decrease in its value is associated with the loss of flesh firmness. Evidently, the Young's modulus value of the non-packed fruit decreased rapidly and it reached 44 kPa after 2 months of storage. For the individually packaged

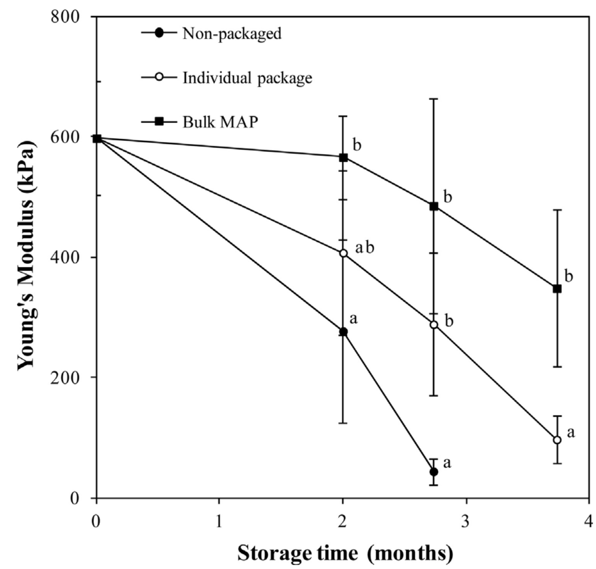

Fig. 5. Changes in Young's modulus value of the 'Fuyu' persimmon fruit packed in three different packaging conditions (non-packaged, individual package, and bulk MAP) stored for ca. 4 months. (n = 5, vertical bars represent standard deviation. Symbols with the same letter are not significantly different between them, Tukey test 0.05).

fruit, the Young's modulus also showed a decreasing trend, albeit slower than that of the non-packed fruit; however, the value eventually declined to 97 kPa after ca. 4 months storage, at which time the fruits were entirely softened. Young's modulus value of the fruit stored in the bulk MAP remained constant for 2 months, and then only gradually decreased to 348 kPa by the end of storage; however, no fruit softening was visually observed. Furthermore, its fruit firmness was apparently the same as before the fruit went into storage. Inside the MAP, the low O_2 and high CO_2 conditions were created, and this most likely reduced the rate of ethylene synthesis by decreasing the 1-aminocyclopropane-L-carboxylate (ACC) synthase activity, which catalyzes the oxidation of ACC to ethylene (Gorny and Kader, 1997). Based on our results, the bulk MAP was more effective than the individual package in preventing the flesh from softening. This may be caused by the higher CO_2 level which was created in the bulk MAP as compared with that of the individual packaging (as shown in Fig. 4B). According to Chavez-Franco and Kader's study (1993), an elevated CO_2 level up to 20 kPa had a stronger inhibitory effect on ethylene production, ACC synthase activity, and respiration rate in 'Bartlett' pear fruit when compared with those responses under 1 kPa CO_2.

4. Conclusion

In this study, a mathematical modeling approach was presented to estimate the appropriate O_2 permeability of packaging film material of the bulk MAP for the long-term storage of 'Fuyu' persimmon fruit. The O_2 concentration in the designed bulk MAP was successfully reduced to the target level, as determined based on the respiration characteristics; thereafter, it stayed almost constant despite the surrounding temperature fluctuations, ranging from 0 °C to 7 °C. By contrast, the O_2 concentration in the individual package varied more, closely following the surrounding temperature changes. Taken together, these results suggest that the bulk MAP is more beneficial and practical to use than the individual one due to its highly stabile O_2 level against unexpected temperature changes, which often occurs in the fruit distribution chain. Accordingly, the packaging design via the mathematical model approach would be more favorable for the bulk MAP than the individual one, since the consideration of a constant O_2 condition at the steady state is required in the design procedure of a MAP. In addition, our designed bulk MAP showed excellent performance at preserving fruit quality and extended the fruit shelf-life up to ca. 4 months. Therefore, the packaging design procedure proposed in the present study is an effective tool for designing bulk MAP. Moreover, the advantages of the bulk MAP shown in this study should promote and expand the practical use of bulk MAP for the high quality preservation of persimmon fruit during long-term storage and long-distance transportation to international markets.

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