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Physiological Changes of Longkong Fruit during Different Storage Conditions

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ABSTRACT

Background: Longkong fruit is one of the well-known tropical fruits in the South East Asia and widely produces in the southern region of Thailand. Its unique taste, aroma, nutritional and medicinal properties increase the export values to several continents. However, the shelf life of longkong fruit is not sustainable (3-5 days) at ambient temperature due to pericarp browning and quality loss. **Objective:** The present study, fruits stored in polyethylene (PE) package at 18°C (treatment) and at 70% relative humidity (RH) were used to sustain the shelf life and quality. The fruits stored without package at 18 and 25°C (70% RH) were served as the controls. **Results:** Fruits stored in PE package were effectively minimized the respiration rate than the controls. The higher activities of pericarp phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) were seen in the controls and thus, severely increased their pericarp browning than treatment. The increased in fruit weight loss was highly limited in the treatment. The severe loss in fruits titratable acidity and total soluble solids were observed in the controls. **Conclusion:** However, the treatment was extended the fruits shelf life for up to 24 days as compared to the controls in which the shelf life was limited to 15 (18°C) and 6 (25°C) days, and it mainly due to the mould growth and severe pericarp browning.

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INTRODUCTION

Longkong (*Aglaia dookoo* Griff.) is a tropical and non-climacteric fruit that associate with the Meliaceae family. It is originated and widely cultivated in the peninsula of Thailand and its cultivation also seen in the different parts of South East Asia [1]. The globular shape of longkong is developed on catkin inflorescences and born in bunches. The ripened longkong fruit has five segments of white translucent flesh that covered by yellow rind. Longkong has almost no seeds, and the flesh has the combination of sweet and sour taste with a pleasant aroma. It has unique nutritional properties such as antioxidant and medicinal properties [2, 3]. Longkong is an economical fruit of Thailand and has a high potential value to export into several countries. However, the predominant drawback in longkong is its shelf life, which limited between 3 and 5 days at ambient temperature and that because of pericarp browning, loss of freshness, textural loss, off-flavour formation and nutritional loss [4]. The shelf life of fresh produce is linked to its respiration rate. Normally, plant respiration process involves in using of atmospheric oxygen, carbohydrates, organic acids and the consequent production of metabolic energy, heat, carbon dioxide and moisture vapour. The respiration rate is generally affected by several environmental factors such as storage temperature, atmospheric conditions, pathogenic attack and various stress. Storage at below ambient temperature is an important factor that support to increase the shelf life of various fresh fruits by controlling their respiration and metabolic rates [5].

The Van't Hoff Rule refers that the rate of biological reaction increases upto 2 to 3 folds with every rise of 10°C in temperature. Longkong fruit stored at 18°C was considered as an optimum condition to extend the shelf life to the maximum of 21 days [1, 6]. Besides, modified atmosphere packaging (MAP) is another important and efficient process that successfully reduces fruit respiration and metabolic rate to extending the shelf life of plant produce. MAP prolongs the shelf life of fruits by passively decreasing oxygen (O₂) and increasing carbon dioxide (CO₂) levels inside the polymeric film [7]. Passive changes in a packaged atmosphere depend on the

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interaction between respiration rate of packaged fruits and permeability characteristics of the film. The inappropriate film permeability condition could lead the accumulation of CO₂ level within the package and spoil the fruit quality. Longkong stored in a low gas permeability package was decreased the fruit freshness, increased the accumulation of package headspace CO₂ level, and consequently, induced the pericarp browning and off-odour development [2, 8]. Venkatachalam and Meenune [9] observed; longkong stored under the higher O₂ transmission rate package (11,000-12,000 cm³/m².d) was maintained its freshness, control the pericarp browning, sustain the fruit quality and shelf life. Although, still there are lacking of adequate evidences on longkong fruit quality changes under the optimum storage condition. Therefore, the present investigation is focused on comparing the different storage conditions on longkong fruit quality and enzymatic activity changes.

MATERIALS AND METHODS

Plant material and experimental design:

Longkong fruits in bunches were obtained from a commercial orchard in the southern Thailand and taken to the laboratory within two hours at ambient temperature. Fruits were carefully cut out from the racemes. The individual fruits were selected on the basis of uniform size and colour. Blemished and diseased fruits were discarded. After that, the fruits were thoroughly washed in distilled water and then, dried by an electric fan for 20 min. Then, thirty longkong fruits for each replication were placed on polypropylene (PP) tray and proceed for storage preparation. First group was stored at ambient temperature (25°C). Second group was stored at 18°C without polyethylene (PE) package. Third group was stored at 18°C with PE package (12 x 8 inches and 50 µM thickness). All the groups during storage were maintained at 70% relative humidity (RH). Fruits storage life was terminated if any visible mould growth or severe pericarp browning appeared during storage. Every two days of the interval, fruits were measured of following quality analysis.

Measurement of package environment:

Fruit respiration rate was determined as the amount of CO₂ developed by fruit during storage as described by Caleb, *et al.* [10] with the slight modifications. A 30 fruit per replication was kept in a 3 L sealed glass chamber for 4 hr. After 4 hr incubation, 1 ml of a gas sample was taken with a syringe and injected in a gas chromatography using an 80/100-mesh Pora pack-Q column and a thermal conductivity detector. The results were expressed in ml CO₂ kg⁻¹h⁻¹.

Headspace CO₂ concentration in fruit package was measured by gas chromatography. A 1 ml of the gas sample from the package was injected into Poro Pak N Column with a helium carrier flow of 50 ml/min and detected by using a thermal conductivity detector (TCD). The headspace gas concentration was identified and quantified by comparison with the external standard gas. The obtained results were expressed in percentage.

Measurement of fruit weight loss:

Longkong fruit weighed by using an electronic weighing balance during initial and after storage and then, they were calculated and expressed in percentage of weight loss with respect to the initial weight.

Fruit pericarp parameters:

Pericarp colour analysis:

The surface colour on four sides of longkong fruit was measured by using a Hunter Lab colorimeter in term of CIE (L*, a* and b*) values. The colour values were expressed in Hue angle (H°) and it calculated as arctangent (b*/a*) (0° = red-purple, 90° = yellow, 180° = bluish-green, 270° = blue).

Pericarp phenolics:

Total phenolic content of fruit pericarp tissue was analysed by the method of Singleton and Rossi [11]. Pericarp tissues 2 g were homogenized with 20 ml of 80% ethanol (1:1 w/v) in a mortar and pestle method. The homogenized sample was centrifuged at 12,000 x g for 20 min. A 0.4 ml of supernatant was mixed with 0.4 ml of Folin-Ciocalteu reagent and 1 ml of 7% sodium carbonate solution. The volume was increased to 10 ml in distilled water and vortexed the mixture, then incubated for 1 hr at room temperature. Absorbance was measured at 750 nm using a spectrophotometer. Total phenol contents were expressed as gallic acid equivalents mg per g fresh fruit.

Pericarp PAL, PPO and POD activities:

For PAL activity, pericarp tissues (2 g) from 20 fruits were homogenized at 4°C in 20 ml of 0.1M sodium borate buffer (pH 8.0) solution, which contained 0.2 g of polyvinyl pyrrolidone (PVP), 5 mM mercaptoethanol and 2 mM ethylenediamine tetra acetic acid (EDTA). The homogenized sample was filtered through cheesecloth, and the filtrate was centrifuged at 19,000 x g for 20 min at 4°C. The supernatant was collected for analyzing PAL activity by according to the method of Jiang and Joyce [12].

For PPO activity, pericarp tissues (2 g) from 20 fruits were homogenized at 4°C in 40 ml of 0.2 M sodium phosphate buffer (pH 6.4). The homogenized sample was filtered through cheesecloth and the filtrate centrifuged at 12,000 x g for 30 min at 4°C. The supernatant of crude extract was collected for analyzing PPO activity by according to the method of Tian, *et al.* [13].

For POD activity, pericarp tissues (2 g) from 20 fruits were homogenized at 4°C in 20 ml of 0.05 M phosphate buffer (pH 7) solution and 0.2 g of insoluble polyvinylpyrrolidone (PVP). The homogenate was filtered through cheesecloth and the filtrate centrifuged at 19,000 x g for 20 min at 4°C. The supernatant of crude extract was collected for analyzing POD activity by according to the method of Zhang, *et al.* [14].

Fruit TSS and TA:

Peeled and deseeded longkong flesh homogenate was prepared by blending, then filtered using cheesecloth and then, subjected to analysis TSS and TA. The TSS was determined by using an Atago 1E (Japan) hand refractometer at 25°C. The results were expressed as °Brix. The titratable acidity (TA) was determined in accordance to Sangkasanya and Meenune [8]. The results were expressed as percentages of citric acid content.

Statistical analysis:

All the experiments were done in triplicate. The data's were studied by analysis of variance (ANOVA) using SPSS v 16.0 (SPSS Inc., Chicago, IL, USA) and significance ($P < 0.05$) among means was determined by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSIONS

Fruit respiration:

Optimum storage temperature and packaging are the most prominent factors in longkong fruit storage. Fruit stored at 25 and 18°C without PE package had a higher prevalence of decays such as severe pericarp browning, visible mould growth and because of that, their evaluation period was discontinued after 6th (25°C) and 10th day (18°C without package) of storage. Whereas, fruits stored at 18°C with PE package were controlled in decays and their shelf life were extended to 16 days. Usually, changing storage temperature and atmospheric conditions can control most of the biochemical processes. Respiration is the primary factor in deteriorating longkong fruit. Longkong is categorized under a high respiration rate commodity, and it is one of the reasons for decreased in shelf life [4, 5]. Fruit respiration rate was decreased slowly in all storage conditions during storage. The control fruit and fruit stored at 18°C without PE package held a higher respiration rate than 18°C with PE package (Figure 1). Typically, respiration rate depends on the storage environment, particularly gaseous composition, relative humidity, temperature and physical stress [15]. Increasing the storage temperature would raise the respiration rate of the plant commodity. However, a high level of respiration rate found in longkong fruit stored at 18°C without PE package than with PE package ($P < 0.05$). It could be due to prolonged exposure of air blown induced stress that might be caused by the relative humidity incubator during storage. Additionally, fruit stored at 18°C with PE package was monitored the headspace CO₂ level, and it was found that 54% of headspace CO₂ level was seen in the fruit package during at the end of storage (Figure 1). Li and Zhang, [16] reported that the increased accumulation of headspace CO₂ could decrease respiration rate in fruits and vegetables. This finding is in accordance with a low respiration rate of longkong fruit stored with PE package in this study.

Fruit weight loss and pericarp colour changes:

Longkong fruit weight loss represented the percentage of moisture loss. Generally, the level of changes of moisture content in longkong determines the level of deterioration. The increase of fruit weight loss was steadily observed throughout storage in all the storage conditions (Figure 2A). The control fruits had a high level of fruit weight loss as compared to others, and this could induce by the fruit transpiration process. Fruit stored at 18°C with PE package was kept a low-level of fruit weight loss as compared to 18°C without PE package ($P < 0.05$). Additionally, longkong stored at 18°C without packaging was found less percentage of weight loss, but the severe desiccation detected on the pericarp surface during storage. Conversely, fruits stored at 18°C with PE package had no severe desiccation observed, and it could be due to packaging, which acted as a barrier against external stress. Notably, the increased weight loss of longkong fruit during storage was not more than 8% in all the storage conditions. Longkong pericarp colour (H°) had changed throughout storage in all different storage conditions (Figure 2B). The significant decreased of fruit pericarp colour (H°) indicated that the increased of fruit pericarp browning during storage. This in accordance with previous reports was on longkong fruit postharvest quality changes [9, 17]. Until the fourth day, fruit pericarp H° values were dropped a high level in the control fruits and afterwards, fruits stored at 18°C without package were rapidly decreasing in pericarp H° values as compared to other storage conditions. The discoloration of longkong fruit pericarp might be induced by prolonged exposure and the accelerated effect of oxidoreductase enzymes by chilling stress [2, 18]. However,

longkong stored with PE package was steadily maintained a high H^o values as compared to other storage conditions.

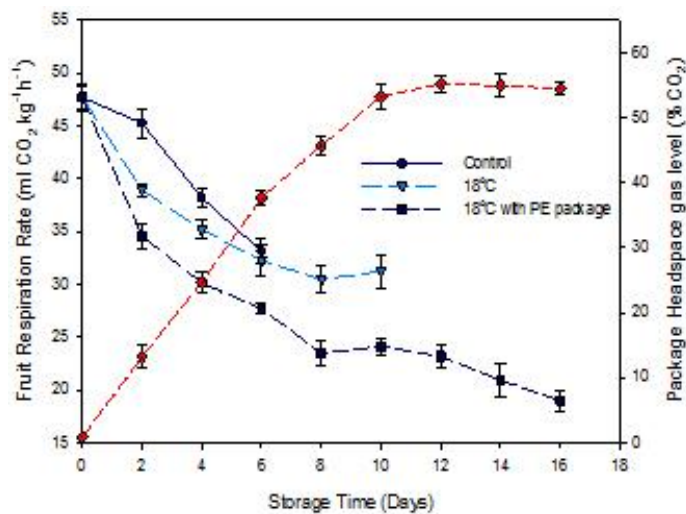


Fig. 1: Changes in fruit respiration rate and package headspace gas level during different storage conditions. The vertical bars represent standard errors.

Fruit TSS and TA:

TSS and TA are often used to identify the maturity level of longkong fruit. Changes in TSS and TA levels in longkong fruit are shown in Figure 2C-D. At the initial days of storage, a slightly increased level of TSS was seen in longkong fruit that stored in all different storage conditions. It could be due to the enzymatic degradation of starch into sugars by α -amylase, β -amylase and starch phosphorylase [19]. However, during the prolonged storage, fruit TSS level was gradually decreased. The control fruits and fruits stored at 18°C without PE package was found a decreased level of TSS than fruits stored at 18°C with PE package ($P < 0.05$). Besides, fruits stored at 18°C with and without PE package were retained a more level of TA as compared to control fruits ($P < 0.05$). However, TA level in all different storage conditions was steadily decreased throughout the storage. TA level in the control fruits was predominantly decreased as compared to other storage conditions. Fruit stored at 18°C with PE package was slow down the decrease of TA level throughout the storage period. Overall, the control fruits had an excessive reduction in TSS and TA as compared to other storage conditions. The alteration of TSS and TA levels in longkong fruit was due to the accelerated biological process by the influential factors, particularly external stress and temperature [20]. Fruit stored at 18°C without PE package could adversely speed up the metabolic process as compared to fruit stored in PE package at 18°C. The reduction in longkong fruit TSS and TA while storage was because of its respiration process, which utilized them as a substrate [19].

Pericarp total phenolics and browning related enzyme activities:

Total phenolic levels in longkong pericarp in all different storage conditions during storage are shown in Figure 3A. Pericarp total phenolic levels were steadily decreased during storage in all storage conditions ($P < 0.05$). At the initial storage period (2nd day), the control fruit pericarp phenolics were decreased more than the other storage conditions. Afterwards, fruit stored at 18°C without PE package was dropped the higher levels of pericarp phenolics than the control fruits and fruits stored at 18°C with PE package. Overall, fruit stored at 18°C with PE package was significantly preserved the pericarp phenolics and during prolonged storage it gradually decreased. Even though, the decreased level of pericarp total phenolics in fruit with PE package was still higher than the control and fruit stored at 18°C without PE package. It could be due to the barrier effect of package that minimized the degradation of phenolics, delay the damage of pericarp cell membrane and consequently, a low level of browning found on pericarp. Phenolic contents in longkong fruit are mainly affected by the biosynthesis of the phenylpropanoid pathway and also accelerated activities of the oxidoreductase enzymes. However, a high level of decrease in pericarp phenolics in 18°C without package could be induced by the activities of oxidative stress that generated by low-temperature stress conditions. Similar finding reported in the litchi fruit stored under low temperature [21]. Pericarp PAL activity in stored fruits in all different storage conditions are shown in Figure 3B. PAL activity in longkong pericarp was continuously increased and observed a fewer (< 0.9 unit) level during the course of storage in all storage conditions. Fruit stored at 18°C without PE package was raised a higher level of PAL activity than the other storage conditions ($P < 0.05$). Fruit stored at 18°C with PE package retained a least level of PAL activity during storage. PAL is the

predecessor for producing phenolics in longkong fruit and usually, it accelerated by various environmental stress conditions [3].

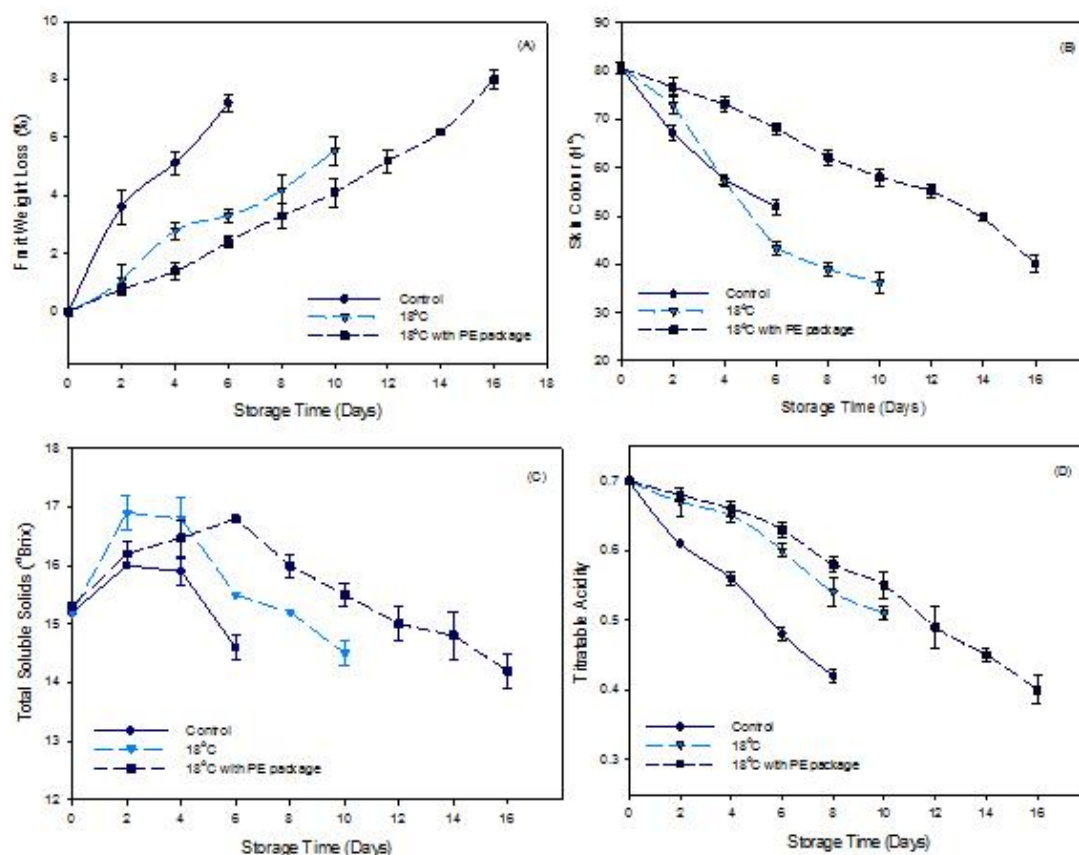


Fig. 2: Changes in fruit weight loss, skin colour, total soluble solids and titratable acidity during different storage conditions. The vertical bars represent standard errors.

Pericarp PPO activity in longkong fruit during storage at different storage conditions is shown in Figure 3C. PPO activity in longkong pericarp showed a similar trend in all different storage conditions, and they were steadily increased throughout the storage ($P < 0.05$). Fruit stored at 18°C without PE package had a higher PPO activity as compared to 25°C. Whereas, fruits stored at 18°C with PE package had a lower PPO activity as compared to other storage conditions during storage. Additionally, pericarp POD activity in all different storage conditions was increased till the end of storage (Figure 3D). Fruit stored at 18°C without PE package had a higher POD activity than the control and fruits stored at 18°C with PE package. At initially, a similar level of POD activity was absorbed in all different storage conditions. During the prolonged storage, POD activity was enormously increased in control and fruit stored at 18°C without PE package. Conversely, fruit stored at 18°C with PE package maintained a lower POD activity in all over the storage. PPO and POD are the key enzymes that induce the pericarp browning in longkong fruit [4]. Longkong fruit has an abundant level of oxidoreductase on the pericarp and especially in the trichomes [3]. Trichomes carry a high level of PPO and POD enzymes, and they are very sensitive to environmental changes such as gas and temperature [23].

Conclusion:

Longkong fruit stored under the different storage conditions were significantly influenced on its physiological qualities. Because of the visible mould growth and severe pericarp browning, fruits shelf life was shrunk to 6 (25°C), 10 (18°C without package) and 16 (18°C with package) days. The present investigation revealed that the fruits stored at 18°C without packaging had the severe pericarp browning and higher PAL, PPO and POD activities as compared to other storage conditions. Conversely, fruits with PE package held a better quality during all over the storage. However, further elaborated study required to elucidate clear mechanism based on chilling stress induced effects of longkong fruit storage under 18°C without package.

The contribution of this subject to knowledge:

Longkong fruit is one of the nutritionally rich fruits in the South East Asia. It has unique taste and varieties of health beneficial properties and particularly, antimicrobial and antioxidant activities. However, its shorter shelf life has made it unfamiliar to other regions in the world. The contribution of this research provides most useful knowledge and inexpensive and efficient technique to the field of postharvest handling of longkong and will increase the exportation values of longkong worldwide.

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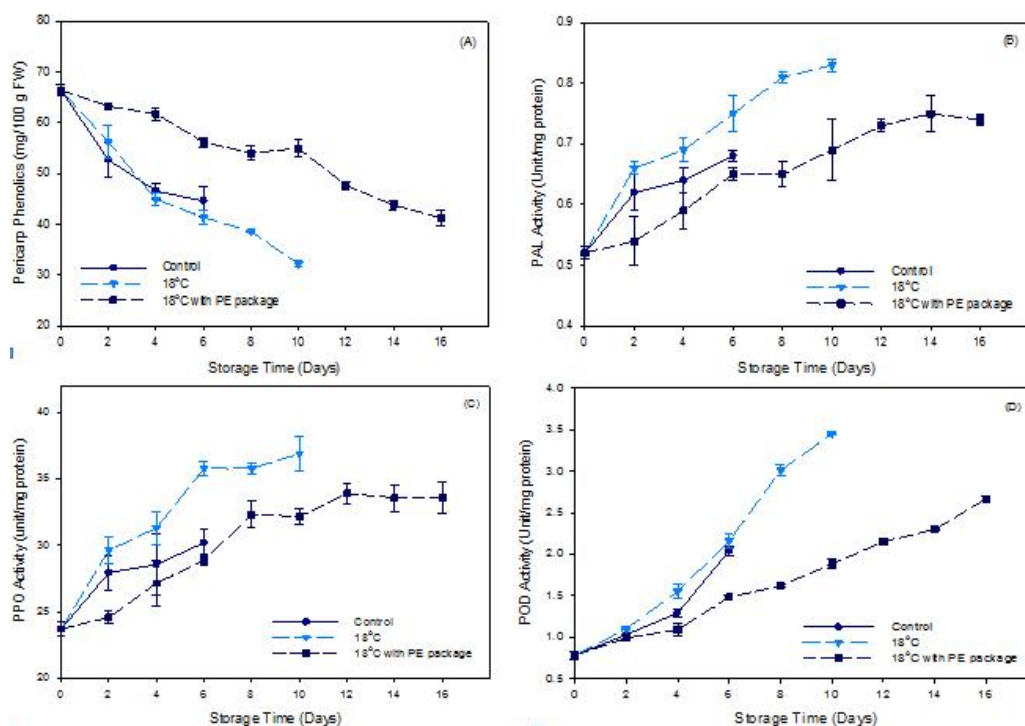


Fig. 3: Changes in fruit pericarp phenolics, PAL, PPO and POD during different storage conditions. The vertical bars represent standard errors.

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