

Effect of Packaging on Postharvest Quality Changes of Longkong

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Abstract

Longkong has a short shelf life (3 to 5 days) at ambient temperature due to its pericarp browning and biochemical quality loss. The present study investigated the quality changes of longkong fruit during storage under passive modified atmospheric packaging (MAP) at ambient temperature (25 °C and at 85 % relative humidity). Fruit stored without MAP served as a control. The accumulation of headspace gas (% CO₂) in the fruit package was observed less than 65 % during all over the storage. The increase of fruit weight loss was effectively controlled in passive MAP storage than the control. Passive MAP storage maintained the lowest browning index values as compared to the control during storage. Throughout the storage, pericarp phenylalanine ammonia lyase, polyphenol oxidase and peroxidase had higher activities in the control fruits. The significant changes in fruit pH, titratable acidity (TA), total soluble solids (TSS) and TSS/TA content were observed in both the control and passive MAP during the storage. Fruits stored under passive MAP had more scavenging ability than reducing power during storage. However, due to lower surface mould growth, the passive MAP stored longkong had a quality and shelf life of up to 12 days at ambient temperature.

Keywords: Longkong fruit, packaging, quality, temperature, storage

Introduction

Longkong (*Aglaia dookkoo* Griff.) is one of the well-known commercial fruits in Thailand, and it is mostly cultivated in the southern part of Thailand. It is also produced in Borneo, India, Sri Lanka, Philippines, Australia and Puerto Rico. It is a non-climacteric and tropical fruit and belongs to the Meliaceae family [1]. Longkong is globular in shape and size of between 1.2 to 2.4 inches in diameter. It develops in clusters and contains 15 to 25 fruits in per raceme. The immature longkong peel has green colour, and it turns to yellow when it ripens and frequently with brown blemishes. The mature longkong fruit contains 5 separate segments of white translucent pulp covered on green seeds [2]. Longkong include a variety of nutrients such as carbohydrates, proteins, vitamins, antioxidants, low fat and high minerals [1,3]. The sweet and sour taste of longkong pulp and along with its pleasant aromatic smell makes the fruit more valuable to export [4].

Longkong fruit is mostly handled at ambient temperature during shipping. Nonetheless, the shelf life of longkong is limited to between 3 to 5 days at ambient temperature. This is due to its respiration and mechanical injury that accelerates deterioration by increasing the pericarp surface browning, weight loss and off-flavour [5]. Pericarp browning decreases the commercial value of fruits and is considered as the most important postharvest problem. Enzymatic browning is predominantly responsible for the browning of several postharvest fruits and vegetables. The postharvest browning of most fruits and vegetables is

majorly attributed to oxidation of phenolics by polyphenol oxidase (PPO), peroxidase (POD) and phenylalanine ammonia lyase (PAL).

Modified atmosphere packaging (MAP) is used to prolonging the shelf life of fresh and/or minimally processed fruits and vegetables. Normally, the MAP technique helps to reduce browning, controls postharvest diseases and maintains a high-humidity environment in several fruits and vegetables that are sealed inside a plastic film [6]. It also prevents cross-contamination during handling, transportation and storage [7]. MAP consists of the enclosure of respiring produce in polymeric films in which the gaseous environment is passively altered to slow respiration [6,8]. There is little published data available concerning longkong quality changes under passive MAP. However, there is no available research on changes of longkong fruit antioxidant and browning related enzyme activities under passive MAP storage. Therefore, the present objective is to characterize the changes associated with longkong fruit quality under passive MAP at ambient temperature.

Materials and methods

Reagents and chemicals

The following reagents and chemicals were used for the experiments. All of the solvents were purchased from Fluka, Folin-Ciocalteu's reagent (Fluka, 2N), gallic acid (Fluka, 98 %), 1-diphenyl-2-picrylhydrazyl (Sigma, 90 %), 2,4,6-tripyridyl-s-triazine (Sigma, 98 %), L-phenylalanine (Sigma, 98 %), 4-methyl catechol (Fluka, 95 %), guaiacol (Merck, 98 %), bovine serum albumin (Sigma, ~66,000 Da) citric acid (Fluka, 99 %), L-ascorbic acid (Sigma, 99 %), Sodium carbonate (Fluka, 99 %), hydrochloric acid (Fisher), ferric chloride hexahydrate (Sigma, 97 %), ethylenediamine tetra acetic acid (Fluka), polyvinyl pyrrolidone (Sigma, PVP40), hydrogen peroxide (Merck, 30 %), sodium hydroxide (Fluka, 98 %), sodium acetate (Fluka, 99 %) and sodium phosphate (Fluka, 96 %).

Plant material

Longkong fruits at commercially mature stage [3] were purchased from a contact garden at Natawee, Songkhla province, in southern Thailand. The fruits were cut off from raceme and selected uniform in size (2 inches in diameter). The defective fruits were discarded. Fruits were thoroughly washed in distilled water and then dried at ambient temperature by using an electric fan for 15 min. The dried fruits were processed for packaging and storage.

Passive modified atmospheric packaging and storage conditions

The 20 individual fruits were placed into a polypropylene (PP) tray for each replication and passively MAP with the polyethylene (PE) bags. The bags were 8×15 inches in size and 25 µm thickness. PE bags were obtained from Thantawan industry public company limited, Thailand. Fruit in the PE bags were sealed by an impulse sealer (Hand impulse sealer, model PCS 300C) and then, stored at ambient temperature (25 °C and at an 85 % relative humidity) in an incubator for the quality analysis. Fruits without packaging served as a control. The storage was terminated, when any fruits displayed more than 4 browning index score and visible mould growth on the fruit pericarp. Every 3 days the fruits were measured according to the following determinations.

Physical quality

Fruit pericarp browning index was visually observed and accessed by measuring the extent of the total browning area on each fruit pericarp using 40 fruits per interval during shelf life evaluations based on the method of Kumar *et al.* [9]. Longkong fruit weight loss was determined before and after storage using an electrical weighing balance. Weight loss was calculated and expressed in percentage with respect to the initial weight.

Fruit respiration gas

Determination of headspace CO₂ and O₂ gas concentration in the fruit bag was measured by gas chromatography (Perkin Elmer (Auto system XL) USA) in accord with Sangkasanya and Meenune [10].

A gas sample (1 mL) from the headspace inside the bag was injected directly into a Poro pak N column (Sigma Aldrich, Singapore) with a helium carrier flow of 50 mL/min and a thermal conductivity detector. The internal package atmosphere was identified and quantified by comparison with an external standard gas.

Chemical quality

The deseeded longkong pulps were homogenized by blending at 4 °C and then filtered using a cheesecloth and then used for chemical analysis. The pH was measured by using a Sartorius PB-20 (Germany) digital pH meter. Total soluble solids (TSS) were determined by using an Atago 1E (Japan) hand refractometer at 25 °C. The results were shown as in °Brix. Titratable acidity (TA) was determined by according to the method of Sangkasanya and Meenune [10]. The results were expressed as a percentage of the citric acid content.

Antioxidant analysis

Longkong pulp (25 g) was homogenized at 4 °C in accordance with Lim and Lim [11] with a slight modification. The homogenized sample was transferred into a 100 mL volumetric flask and volume made up with 50 % ethanol. The mixture was shaken with a vibrator for 10 min and then centrifuged at 10,000 g at 4 °C for 10 min to obtain a clear supernatant solution. The supernatant was immediately used for antioxidant analysis such as total phenol content (TPC), and with 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability and ferric reducing ability power (FRAP).

Total phenol content (TPC)

TPC was determined by using the Folin-Ciocalteu's reagent [11]. A 0.3 mL of sample was placed in a test tube followed by 1.5 mL of Folin-Ciocalteu's reagent (1:10 dilution with distilled water) and then 1.2 mL of sodium carbonate (7.5 % w/v) added. The tubes were vortexed and covered with parafilm and then kept at room temperature for 30 min. Absorption at 765 nm was measured. Total phenol contents were expressed in gallic acid equivalents (mg/100 g fresh fruit (FW)).

1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability

DPPH assay was measured according to the method of Binsan *et al.* [12]. A 1.5 mL of the sample was added to a test tube, followed by 1.5 mL of 0.15 mM DPPH in 95 % ethanol. The reaction mixture was thoroughly mixed and kept in a dark place for 30 min at room temperature and then a sample was measured at 517 nm. Distilled water was used instead of the sample for the blank. The activity was expressed as ascorbic acid equivalents (mg/100 g FW).

Ferric reducing ability power

The ferric reducing ability power was determined in accordance with the method of Benzie and Strain [13]. The stock solution included a 300 mM acetate buffer (pH 3.6) and 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM hydrochloric acid and along with 20 mM ferric chloride hexahydrate (FeCl₃.6H₂O) solution. A FRAP solution was freshly prepared by adding 25 mL of acetate buffer, 2.5 mL of TPTZ solution and 2.5 mL of FeCl₃.6H₂O solution. The mixed solution was incubated at 37 °C for 30 min. A sample of 150 µL was mixed with 2,850 µL of FRAP solution and kept for 30 min in the dark. The absorbance was measured at 593 nm. The activity was expressed as ascorbic acid equivalents (mg/100 g FW).

Enzyme activity analysis

Extraction and assay of phenylalanine ammonia lyase (PAL)

Pericarp tissues (2 g) from 20 fruits were homogenized in 20 mL of 0.1M sodium borate buffer (pH 8.0) solution contained 0.2 g of polyvinylpyrrolidone (PVP), 5 mM β-mercaptoethanol and 2 mM ethylenediamine tetra acetic acid (EDTA) at 4 °C. The homogenate was centrifuged at 19,000 g and at 4 °C for 20 min and then, the supernatant was collected and used for measuring PAL activity in accordance

with Jiang and Joyce [14]. PAL activity was determined by incubating a 0.1 mL mixture of enzyme extract and 2.9 mL of a 0.1 M sodium borate buffer (pH 8.0) solution containing 3 mM L-phenylalanine for 1 h at 37 °C. An increase in the PAL activity was measured at 290 nm. The specific activity was expressed as unit/mg protein.

Extraction and assay of polyphenol oxidase (PPO)

Pericarp tissues (2 g) from 20 individual fruits were homogenized in 40 mL of 0.2 M sodium phosphate buffer (pH 6.4) at 4 °C. After that, the homogenate was filtered through one layer of cheesecloth, and the filtrate centrifuged at 12,000 g for 30 min. The supernatant used for measuring PPO activity, in accordance with the method of Tian and Xu [15]. The reaction mixture contained 3 mL of 0.5 M 4-methylcatechol in a 0.2 M sodium phosphate buffer (pH 6.4) and 100 µL of the crude enzyme sample. The absorbance was measured at 398 nm at 25 °C for 1 min. The specific activity was expressed as unit/mg protein.

Extraction and assay of peroxidase (POD)

Pericarp tissues (2 g) from 20 individual fruits were homogenized in 20 mL of a 0.05 M phosphate buffer (pH 7) solution and 0.2 g of PVP at 4 °C. The homogenate was filtered through cheesecloth; the filtrate centrifuged for 20 min at 19,000 g at 4 °C. The supernatant was collected and used for measuring the POD activity. POD activity, using guaiacol as a substrate, was assayed by the method of Zhang and Quantick [16]. A 3 mL reaction mixture contained 25 µL of supernatant, 2.78 mL of 0.05 M phosphate buffer (pH 7.0), 0.1 mL of 20 mM hydrogen peroxide (H₂O₂) and 0.1 mL of 20 mM guaiacol. The absorbance was measured at 470 nm at 25 °C for 2 min. The specific activity was expressed as unit/mg protein.

Protein determination

The crude enzyme extracts were used to determine the protein content in accordance with Bradford's [17] method with bovine serum albumin (BSA) used as a standard.

Statistical analysis

The experiment was done in triplicates. Data were analyzed by completely randomized design (CRD). Significant differences between means were estimated by Duncan's new multiple range tests (DMRT), with a level of significance of 0.05. Statistical analyses were performed by using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

Results and discussion

Fruit physical quality and respiration gas

Pericarp browning index of longkong fruit was rapidly decreased during storage (**Table 1**). The browning index of the control fruits was 2.35 and 4.65 after 3 and 6 days of storage. The shelf life evaluation of the control fruits was discontinued after 6 days of storage due to them reaching the maximum level of browning on the pericarp surface. Whereas, the fruit stored under passive MAP storage showed only a gradual increase in pericarp browning, being 1.23 and 3.4 after 6 and 12 days of storage, respectively. The shelf life period of fruits stored under passive MAP was shortened to 12 days due to visible mould growth appearing on the pericarp surface. The increased pericarp browning of the control fruits and fruits under passive MAP could be a breakdown of membrane and thus allow the enzyme and substrate to react with each other to cause the browning [18]. Trichomes on the longkong pericarp surface might be a precursor for activating oxidoreductase enzymes under stress conditions [3]. The oxidoreductase enzymes (PPO and POD) are predominant inducers of browning on the longkong pericarp surface [4]. Weight loss of longkong fruit was significantly increased ($P < 0.05$) throughout the storage period and in the end, the reduction was 5.22 % (6 days) and 3.80 % (12 days) in the control and under passive MAP, respectively (**Table 1**). Passive MAP storage significantly reduced weight loss relative to the control. The increase in fruits weight loss could be accelerated by a respiration induced transpiration

process. The increase in fruit weight loss has been reported as a cause of freshness reduction of fruit and thus, leads to pericarp browning [19]. Fruit respiration gases such as CO₂ and O₂ are shown in **Figure 1**. The percentage of CO₂ steadily increased ($P < 0.05$) throughout the storage and conversely, the O₂ level decreased throughout the storage ($P < 0.05$). However, at the end of storage, the accumulation of CO₂ under passive MAP package was observed to be less than 65 %, and it still had an acceptable eating quality.

Table 1 Physical and chemical quality changes of longkong during storage under MAP at ambient temperature.

Storage period (days)	Treatment	Browning index (0 - 5)	Weight loss (%)	pH	TA (% citric acid)	TSS (° Brix)	TSS/TA ratio
0	Control	0.00±0.00 ^f	0.00±0.00 ^f	4.43±0.11 ^a	0.55±0.02 ^a	15.00±0.00 ^c	27.27±0.02 ^e
	Package	0.00±0.00 ^f	0.00±0.00 ^f	4.43±0.11 ^a	0.55±0.02 ^a	15.00±0.00 ^c	27.27±0.02 ^e
3	Control	2.35±0.04 ^c	3.56±1.20 ^b	4.35±0.05 ^{ab}	0.40±0.08 ^c	17.5±0.80 ^a	43.75±0.12 ^b
	Package	0.49±0.01 ^e	0.51±0.08 ^e	4.31±0.03 ^{ab}	0.45±0.02 ^b	16.90±0.10 ^b	37.55±0.10 ^c
6	Control	4.65±0.10 ^a	5.22±1.20 ^a	4.36±0.02 ^{ab}	0.35±0.01 ^d	17.5±0.70 ^a	50.00±0.80 ^a
	Package	1.23±0.12 ^d	1.39±0.13 ^d	4.29±0.05 ^b	0.44±0.04 ^b	17.33±0.12 ^d	39.38±1.50 ^c
9	Control	NA	NA	NA	NA	NA	NA
	Package	2.2±0.56 ^c	2.73±0.07 ^c	4.24±0.04 ^b	0.51±0.04 ^{ab}	15.20±0.20 ^c	29.80±0.25 ^d
12	Control	NA	NA	NA	NA	NA	NA
	Package	3.4±0.80 ^b	3.80±0.23 ^c	4.14±0.14 ^c	0.53±0.02 ^{ab}	14.27±0.12 ^d	26.92±0.18 ^{ef}

Note: The superscript alphabets in the columns are represented the significant differences ($P < 0.05$).
NA: Not available

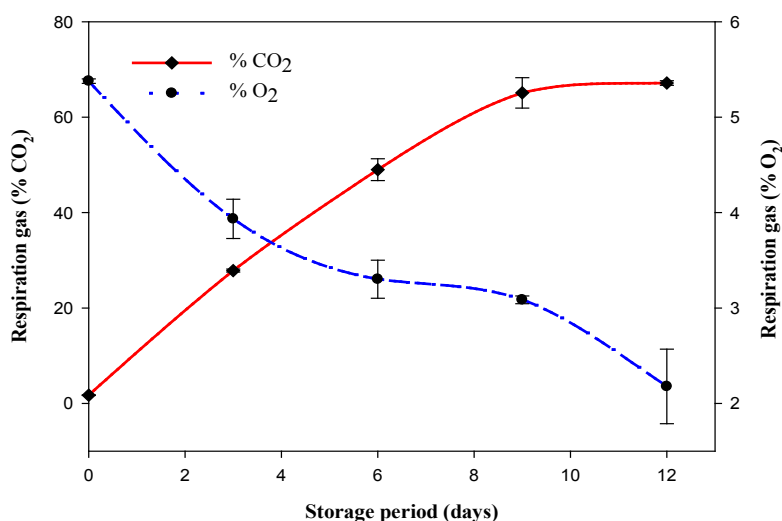


Figure 1 Changes in the inner package respiration gas (%CO₂ and %O₂) of longkong during storage under MAP at ambient temperature. The vertical bar specifies the standard deviation.

Fruit chemical quality

Fruit chemical quality such as pH, titratable acidity, total soluble solids and TSS/TA ratio are given in **Table 1**. Longkong fruit pH values gradually decreased throughout the storage ($P < 0.05$). The control fruits retained a higher pH than fruits under passive MAP storage. Fruits under passive MAP storage showed a decreasing pH as the storage period increased. Titratable acidity (TA) decreased up to 6 days of storage in both the control fruits and fruit stored under passive MAP and then, it gradually increased until the end of storage in fruits stored under passive MAP ($P < 0.05$). An increased accumulation of CO_2 could accelerate the impaired function of the citric acid cycle and thus gradually extend the shelf life of longkong fruit by decreasing the metabolic rate. Similarly, total soluble solids (TSS) in longkong increased up to 6 days of storage in both the control and passive MAP storage and then, it steadily decreased throughout the storage ($P < 0.05$). A TSS/TA ratio more than 20 could be an appropriate level for longkong fruit sweetness [3]. The increase in the TSS/TA ratio was observed in the control fruits during storage and at the end of storage it reached 50. Whereas, fruits under passive MAP storage had a gradual increase of TSS/TA ratio until 6 days (39.38) of storage, it then decreased slowly until the end of storage (26.92). The control fruits retained more sweetness than fruits stored in passive MAP, and it could be due to the accelerated activity of invertase in the control fruits. The decrease in TA and TSS levels could be taken as a substrate for the respiration process in longkong fruit during storage.

Table 2 Longkong fruit antioxidant abilities and pericarp browning related enzyme activities during storage under MAP at ambient temperature.

Storage period (days)	Treatment	TPC (mg/100 g FW)	DPPH scavenging ability (mg/100 g FW)	FRAP (mg/100 g FW)	PAL enzyme (units/mg protein)	PPO enzyme (units/mg protein)	POD enzyme (units/mg protein)
0	Control	42.58±1.18 ^d	6.27±0.49 ^b	4.21±0.28 ^c	0.60±0.00 ^b	21.23±0.62 ^c	0.74±0.07 ^d
	Package	42.58±1.18 ^d	6.27±0.49 ^b	4.21±0.28 ^c	0.60±0.00 ^b	21.23±0.62 ^c	0.74±0.07 ^d
3	Control	66.23±0.80 ^b	8.12±0.80 ^{ab}	6.23±0.50 ^a	0.66±0.10 ^b	37.85±0.23 ^b	1.12±0.30 ^c
	Package	67.57±0.29 ^b	8.62±0.24 ^a	5.23±0.21 ^b	0.56±0.01 ^c	31.41±2.11 ^c	1.08±0.06 ^c
6	Control	60.23±0.14 ^c	8.88±0.74 ^a	5.21±0.12 ^b	0.71±0.40 ^b	40.12±1.80 ^a	2.05±0.40 ^a
	Package	69.18±2.61 ^a	6.67±0.48 ^b	5.46±0.35 ^b	0.58±0.02 ^c	29.25±3.43 ^c	1.38±0.21 ^b
9	Control	NA	NA	NA	NA	NA	NA
	Package	68.57±2.45 ^a	6.02±1.17 ^b	5.33±0.34 ^b	0.80±0.01 ^a	27.92±2.80 ^d	2.00±0.05 ^a
12	Control	NA	NA	NA	NA	NA	NA
	Package	59.21±1.62 ^c	4.38±0.42 ^c	3.12±0.91 ^d	0.88±0.12 ^a	21.54±2.88 ^c	1.95±0.06 ^a

Note: The superscript alphabets in the columns are represented the significant differences ($P < 0.05$).
NA: Not available

Antioxidant ability

Antioxidant abilities of longkong pulp are summarized in **Table 2**. Fruit stored under passive MAP storage maintained a high level of TPC as compared to the control. Fruits are rich sources of non-enzymatic antioxidants such as phenolics and they minimize the accumulation of reactive oxygen species and disease incidence under various stress conditions [20]. TPC of longkong pulp steadily increased up to 6 days of storage and then, it gradually decreased throughout storage ($P < 0.05$). The lower level of TPC in the control fruit could be due to oxidation by PPO, which converts the phenol to quinones [21,22]. Passive MAP acts as a barrier to control the reduction of TPC in longkong by reducing the co substrate (O_2) availability (**Figure 1**). Higher levels of DPPH scavenging ability and FRAP activity in fruits were observed in the control and passive MAP storage up to 6 days (**Table 2**). After that, the activities gradually decreased during storage in fruits stored under passive MAP. The increase and/or decrease in antioxidant activity in the fruits might be influenced by polyphenols [23]. Maguire and Mackay [24]

reported that the decrease in antioxidant activities in fruit were due to higher levels of CO₂ accumulation inside the package. Although, the DPPH scavenging activity in fruit pulp was at a slightly higher level than FRAP activity in the control and passive MAP during storage, it was specified that, longkong fruits have more scavenging ability than reducing power during storage. Generally, the antioxidant substances effectively delayed the reactive oxygen species which cause degenerative diseases such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the aging process [25].

Changes in pericarp PAL, PPO and POD activities

Pericarp PAL, PPO and POD activities during storage are shown in **Table 2**. PAL activity in fruit pericarp stored under passive MAP slightly decreased up to 6 days and then, it increased steadily ($P < 0.05$). Conversely, PAL activity in the control fruits rapidly increased. The induction of PAL activity is influenced by various stresses, which includes wounding, light, plant hormones and disease [16]. PAL is an essential enzyme that generates the simple phenolic compounds from L-phenylalanine via a phenyl propanoid pathway [26]. These phenolic compounds are used as a substrate for oxidoreductase enzymes such as PPO, POD to oxidize and produce the browning in various horticultural products. Pericarp PPO activity of the control fruits were continuously increased throughout the storage ($P < 0.05$). On the other hand, the PPO activity of fruit stored under passive MAP increased on the 3rd day of storage and then it continually decreased throughout the storage. This could be due to accumulation of the headspace CO₂ level in the passive MAP. Cheng and Crisosto [27] reported that, PPO activity is not a regulating factor in enzymatic browning. Meanwhile, pericarp POD activity of longkong was increased in the control and passive MAP throughout the storage ($P < 0.05$). The control fruits retained a higher POD activity during storage. Whereas, the continuous increase of pericarp POD activity in longkong under passive MAP was observed until the 9th day of storage. However, the increase of POD activity in passive MAP storage was still lower than the control. The changes in pericarp PPO and POD activities were highly correlated with an increase in the pericarp browning index during storage (**Table 1**). Overall, fruit stored under passive MAP storage had a lower level of oxidoreductase activity than the control fruits.

Conclusions

Passive MAP storage effectively controlled the respiration process by limiting the O₂ level and thus, lowering the loss of quality and peel browning. It also prolonged the shelf life of longkong up to 12 days. Pericarp PAL, PPO and POD activities were well controlled in fruits stored under passive MAP. The fruit shelf life was discontinued during 12 days of storage due to mould growth appearing on the pericarp surface. On the other hand, the control fruit could only be stored for 6 days due to severe pericarp browning. Effective fungicide treatment and passive MAP storage might prolong the shelf life of longkong for several weeks at ambient temperature with higher relative humidity storage.

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