Respiratory activity and compositional changes in Eksotika papaya fruit following storage in low-oxygen atmosphere

(Aktiviti pernafasan dan perubahan komposisi buah betik Eksotika selepas penyimpanan dalam atmosfera beroksigen rendah)

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Key words: papaya fruit cv. Eksotika, low- O_2 atmosphere, CO_2 and ethylene, soluble solids, sugar, ethanol, respiratory quotient

Abstrak

Betik Eksotika telah disimpan selama 4 minggu dalam atmosfera yang mengandungi 2% dan 5% $\rm O_2,$ dan atmosfera udara biasa (21% $\rm O_2$) pada suhu 12 °C. Kadar pengeluaran gas CO2 dan etilena oleh buah yang disimpan dalam 2% dan 5% O2 berada pada aras yang rendah dan meningkat setelah buah dipindah kepada udara biasa pada suhu 20 °C. Walau bagaimanapun, kadar pengeluaran gas etilena daripada buah yang disimpan dalam atmosfera beroksigen rendah meningkat lebih perlahan berbanding dengan buah yang disimpan dalam udara biasa. Atmosfera yang mengandungi 2% O2 lebih berkesan daripada 5% O2 bagi mengawal kadar pengeluaran gas etilena. Kesan sampingan penyimpanan dalam atmosfera beroksigen rendah lebih ketara pada kadar pengeluaran gas etilena daripada pengeluaran gas CO2. Buah yang disimpan dalam 2% dan 5% O2 selama 4 minggu mencapai puncak pengeluaran CO2 selepas 3 hari, manakala buah yang disimpan dalam udara biasa mencapai puncak tersebut setelah 2 hari didedahkan kepada udara biasa. Nilai hasil bahagi pernafasan sebanyak satu dan ketiadaan etanol daripada buah yang telah disimpan dalam 2% dan 5% O_2 selama 4 minggu menunjukkan bahawa metabolisma anaerobik tidak berlaku. Penyimpanan dalam 2% O2 selama 4 minggu tidak memberi kesan pada kandungan pepejal larut dan gula setelah buah masak. Melanjutkan tempoh simpan dalam atmosfera beroksigen rendah daripada 1 hingga 4 minggu menyebabkan sedikit penurunan nilai a* (merah) isi buah, manakala nilai b* (kuning) tidak berbeza.

Abstract

Papaya cv. Eksotika fruit were stored for 4 weeks in 2% and 5% O₂ atmosphere, and air (21% O₂) at 12 °C. The CO₂ and ethylene production rates of fruit stored in 2% and 5% O₂ were suppressed during storage but recovered after transfer to air at 20 °C. However, the ethylene production recovery was slower in the low-O₂ stored fruit than the air-stored fruit. The 2% O₂ atmosphere was more effective than 5% O₂ in suppressing the ethylene production. The residual effect of low-O₂ storage was more apparent on the ethylene than CO₂ production rate. Fruit stored in 2% and 5% O₂ for 4 weeks attained peak production of CO₂ after 3 days,

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while peak CO₂ production was attained in air-stored fruit after 2 days following transfer to air. The respiratory quotient value of about 1 and the absence of ethanol in the headspace gas of fruit that had been stored in 2% and 5% O₂ for 4 weeks indicated that anaerobic metabolism was non-existent. The soluble solids and sugar contents of ripe fruit stored in 2% O₂ were unaffected even after 4 weeks of storage. Extending the storage period from 1 to 4 weeks in low-O₂ atmosphere resulted in a slight reduction in the *a** (red) value of the fruit pulp, while the *b** (yellow) value was insignificantly different between the low O₂ and the air-stored fruit.

Introduction

The papaya (Carica papaya L.) cv. Eksotika, formerly known as the MARDI Backcross Solo, is characterized by attractive orangyred coloured pulp, sweet taste and distinctive pleasant aroma. This variety has gained popularity in the domestic markets and has great potential for export. However, the short storage life limits the export of Eksotika fruit to distant markets by refrigerated sea containers. The ripe fruit soften rapidly and are easily infested by diseases. The fruit are easily chill injured as a result of exposure to temperature lower than 10 °C (Abd. Shukor A. R., Rohani, M. Y. and Che Omar, D., Study on handling and transportation of Eksotika papaya to Dubai by ship, MARDI, Serdang, not published 1995). Improper handling and storage can drastically shorten the storage life and result in ripe fruit of inferior quality.

Controlled atmosphere (CA) and modified atmosphere (MA) are possible methods of storage that can be employed to extend the storage life of Eksotika papaya. Controlled atmosphere or MA storage involves storing fruits or vegetables in an atmosphere containing a low level of O₂ and/or an elevated level of CO₂. It is well documented that reduced O2 and/or elevated CO2 in CA environment cause significant reductions in respiration and C_2H_4 production rates, reduce tissue sensitivity to C_2H_4 , delay ripening and softening, and decelerate biochemical changes associated with ripening and senescence (Kader 1986). Kennedy et al. (1992) have shown that storage under extremely low levels of O2

can induce anaerobiosis resulting in the accumulation of ethanol and other toxic products which can be harmful to the plant tissues. Additionally, anaerobiosis can also induce an appreciable increase in the respiratory quotient, resulting in an increase in CO_2 production relative to O_2 consumption (Hole et al. 1992). Although a lot of research have been directed toward determination of the optimum CA conditions for a wide range of temperate fruits and vegetables, limited studies are being done on tropical fruits. This study aims to elucidate the respiratory characteristic and quality changes in Eksotika papaya fruit exposed to low levels of O_2 .

Materials and methods Fruit material

Papaya fruit were harvested at colour index 2 (green with trace of yellow) from a commercial farm in Chui Chak, Perak. The fruit were packed in a 40-kg plastic container and transported to the Horticulture Research Centre, MARDI in Serdang, Selangor. Upon arrival, the fruit were sorted for uniformity of peel colour, shape, size and absence of defects.

Disease treatment

The fruit underwent recommended packing house operations comprising washing, hot water treatment at 46 °C for 10 min, cooling in tap water (25–27 °C) for 20 min and fungicide treatment, i.e. dipping in 300 ppm *Tilt* (propiconazole) suspension (25–27 °C), for 5 min (Abd. Shukor, A. R., Rohani, M. Y. and Che Omar, D., Study on handling and transportation of Eksotika papaya to Dubai by ship, MARDI, Serdang, not published 1995). The fruit were then arranged on a rack to air dry.

Low-O₂ treatment

The fruit were sealed individually in 2.8-L glass jars containing 2% and 5% O2 in N2 respectively for 4 weeks at 12 °C. The levels of O_2 were regulated precisely using the Oxystat 2 system (David Bishop Instruments Ltd., London). The control comprised fruit exposed to air (21% O₂) at 12 °C. Earlier studies have shown that exposure of Eksotika papaya fruit to 10% or 15% O_2 resulted in no significant difference in respiratory activity as compared with the control (21% O₂) (Abd. Shukor, A. R., Controlled atmosphere storage of fruits and vegetables, MARDI, Serdang, unpublished 1995). The humidified O_2 mixture and air were introduced by a continuous flowthrough system and the flow rates were adjusted to keep the CO_2 levels below 0.3%(Abd. Shukor et al. 1993). Four fruit from each gas treatment were removed weekly and transferred to air, and ventilated with humidified air at 20 °C. Carbon dioxide and C_2H_4 production rates, oxygen consumption rates and ethanol content from the headspace gas were determined daily during exposure to air until the fruit attained colour index 5 (yellow with trace of green).

In a similar experiment, 16 fruit in three plastic cabinets (48 L) were exposed to humidified mixture of 2%, 5% O_2 and air respectively for 4 weeks at 12 °C. Four fruit from each gas treatment were removed and transferred to air, and ventilated with humidified air at 20 °C. The fruit were allowed to ripen to colour index 5 and analysed for total sugar content, soluble solids content and pulp colour (Hunter a^* and b^* values).

Gas measurements

The rates of CO_2 and C_2H_4 production as well as O_2 uptake were measured 6 h after transfer of fruit to air from the various O_2

mixtures. The pulp temperature was equilibrated to 20 °C prior to gas measurements. Subsequent measurements were taken daily following transfer of the fruit to air. For determining CO₂ production and O₂ uptake, 0.5 mL of the headspace gas (2.8-L glass jars sealed for 1 h) was injected into a Varian 1420 gas chromatography equipped with a thermal conductivity detector. The stainless steel column was packed with Porapak R (80/100 mesh) for CO_2 and molecular sieve type 5A (45/60 mesh) for O_2 measurements. The detector temperature was set at 50 °C while the flow rates of the carrier gas (helium) was maintained at 25 mL/min for CO₂ and 20 mL/min for O₂ measurements. Ethylene in 0.5-mL headspace gas samples was measured using a Varian 1400 gas chromatography equipped with a flame ionization detector and a stainless steel column packed with Porapak T (100/120 mesh). The column temperature was set at 100 °C and the flow rate of the carrier gas (N_2) was maintained at 30 mL/min. The gas measurements for all treatments were replicated four times.

Ethanol was determined using the Kentville method (Lidster et al. 1985) following transfer of fruit to air, by injecting 0.2 mL of headspace gas into a gas chromatography equipped with a flame ionization detector and a 5% carbowax (80/ 120 mesh) column operated at 80 °C. The detector and injector were set at 100 °C. Peak heights were compared with a standard curve prepared from the headspace of a series of ethanol dilutions in distilled water kept at 20 °C. This method was able to detect ethanol concentrations as low as 0.01% in the headspace gas.

The respiratory quotient (RQ) values were measured by dividing moles of CO_2 produced by moles of O_2 consumed. Under optimum conditions, the RQ values of plant tissues are equivalent to 1, 0.36 or 1.33, with glucose (carbohydrate), palmitic acid (lipid) or malic acid (organic acid) respectively as the respiratory substrates (Kays 1991).

Chemical composition

The total sugar content (TSC) and soluble solids content (SSC) of the fruit which had attained colour index 5, were analysed. The analysis of TSC was done following the methods of AOAC (1975). Each analysis was replicated four times. The SSC was determined with a refractometer using the supernatant of the blended sample.

Pulp colour

Pulp colour was determined by macerating the fruit tissue at colour index 5 using a waring blender. The macerated tissue was placed in a 50-mL plastic cup and the colour was evaluated with a chromameter and expressed as numerical values of a^* or b^* . The a^* value represented a colour range from green (-60) to red (+60) while the b^* value from blue (-60) to yellow (+60). Higher values of a^* or b^* indicated a greater intensity of red or yellow colour respectively.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and the least significant difference (LSD) values were expressed at p = 0.05. The results of all treatments were based on the average values of four similar experiments conducted over a period of 2 years. The treatments in a cold room with uniform temperature, humidity and air circulation were completely randomized.

Results

The magnitude of reduction in CO_2 production 6 h after transfer to air for papaya fruit stored in 2% and 5% O_2 at 12 °C for 1 week was only significantly (*p* <0.05) greater than that of fruit stored continuously in air (*Figure 1*). Thereafter, the CO₂ production rate of fruit stored in low O₂ (2% and 5% O₂) and air increased gradually without any significant difference and attained peak values after 2 days in air at 20 °C. At the climacteric peak of respiration, the magnitude of CO₂ production was not significantly different between fruit stored in $low-O_2$ atmosphere and fruit stored in air. Thereafter, the CO₂ production rates decreased as the exposure period in air was extended to 4 days, where fruit attained colour index 5.

Fruit stored in low O₂ and air for 2 weeks showed a similar trend in CO_2 production as fruit stored for 1 week (*Figure 1*). After 6 h in air, the CO_2 production rate of low-O₂ stored fruit was significantly (p < 0.05) lower than that of the air-stored fruit. The magnitude in CO_2 reduction after transfer to air was significantly (p < 0.05) higher in fruit that had been stored in low O₂ compared with the air-stored fruit. The peak in CO_2 production of the low-O₂ and air-stored fruit were attained after 2 days in air. A similar trend in CO₂ production was also observed in fruit stored for 3 weeks in 2% and 5% O_2 atmosphere (Figure 1). The magnitude of CO_2 production at the climacteric peak of respiration was significantly (p < 0.05)higher in the air-stored fruit compared with fruit stored in 2% and 5% O2. Similarly, the peak of CO₂ production of the low-O₂ and air-stored fruit were attained after 2 days in air. The pattern of CO_2 production rate of fruit following storage in 2% O₂ for 4 weeks was not as distinct as the air-stored fruit (*Figure 1*). The CO_2 production rate after transfer to air was significantly (p < 0.05)lower in the low-O2 stored fruit compared with the air-stored fruit. Fruit stored in 5% O_2 for 4 weeks attained the peak CO_2 production after 3 days while the air-stored fruit after 2 days in air. However, peak CO₂ production of fruit stored in 2% O₂ was attained after 1 and 3 days in air. The magnitude in CO₂ reduction after transfer of fruit to air was significantly (p < 0.05) higher in fruit that had been stored in 2% O₂ compared with 5% O2 and air-stored fruit.

Fruit stored for 1 week in 2% and 5% O_2 and transferred to air for 1 day showed a significantly (p < 0.05) lower level of C_2H_4 production than fruit stored continuously in air (*Figure 2*). The C_2H_4 production rate of the low- O_2 and air-stored fruit continued to



Figure 1. Carbon dioxide production rates of Eksotika papaya in air at 20 °C following storage in 2, 5 and 21% O_2 for 1, 2, 3 and 4 weeks at 12 °C

increase and attained peak values after 2 days in air. The magnitude in C2H4 production of the low-O₂ stored fruit following transfer to air was significantly (p < 0.05) lower than that of the air-stored fruit. A similar trend in C_2H_4 production was observed in fruit stored for 2 weeks and 3 weeks following transfer from the low-O₂ atmosphere to air (Figure 2). However, this trend was not distinct in fruit stored for 4 weeks. Additionally, the magnitude in C_2H_4 production of fruit stored for 2, 3 and 4 weeks in 2% O₂ and transferred to air was significantly (p < 0.05) lower than fruit stored in 5% O₂ and air. In contrast to the other storage periods, fruit stored for 4 weeks in low O_2 or air showed a rapid increase in C_2H_4 production after transfer to air.

Measurements taken 6 h after transfer of fruit to air demonstrated that the RQ values of fruit stored for 4 weeks in 2% and 5% O_2 were significantly (p < 0.05) higher than the air-stored fruit (*Table 1*). The highest RQ value of 1.83 was observed in fruit stored in 2% O_2 . Thereafter, the RQ decreased and attained values closed to one, with no significant difference between the low- O_2 and air-stored fruit.

Ethanol was not detected in the headspace gas obtained from fruit after storage from 1 to 4 weeks in 2% and 5% O_2 atmospheres. It was also not detected 24 h after transfer from the low- O_2 atmosphere to air.

Ripe fruit at colour index 5 from 2% O_2 atmosphere showed no significant difference in their SSC throughout the storage period (*Table 2*). However, fruit stored in 5% O_2 for 4 weeks had a significantly (p < 0.05) lower SSC than fruit stored for 3 weeks. Reduction in SSC values with prolonged storage also occurred in fruit



Figure 2. Ethylene production rates of Eksotika papaya in air at 20 °C following storage in 2, 5 and 21% O_2 for 1, 2, 3, and 4 weeks at 12 °C

Table 1. Respiratory quotient of Eksotika papaya fruit after storage for 4 weeks in 2, 5 and 21% oxygen, and following transfer to air for 1, 2 and 3 days

Oxygen level (%)	Respiratory quotient after transfer from low O ₂ to air				
	0 day	1 day	2 days	3 days	
2	1.83a	1.06c	0.91c	0.97c	
5	1.34b	0.96c	0.90c	1.03c	
21	1.02c	0.96c	0.96c	0.97c	

Mean values with the same letters for both row and column are not significantly different from each other at 5% level by LSD

stored continuously in air. The SSC of fruit stored in 5% O_2 for 4 weeks was significantly (p < 0.05) lower than the SSC of fruit stored in 2% O_2 atmosphere.

In contrast to SSC, the TSC of ripe fruit after storage in low- O_2 atmosphere and air remained similar throughout the storage

Table 2. Soluble solids content of Eksotika papaya fruit at colour index 5 after 4 storage periods in 2, 5 and 21% oxygen atmospheres

Storage period (week)	Soluble soli	Soluble solids content (Brix)			
	2% O ₂	5% O ₂	21% O ₂		
1	10.87ab	10.23cd	11.17a		
2	10.43bcd	10.00de	10.53bc		
3	10.43bcd	10.63bc	9.73e		
4	10.43bcd	9.60e	_		

Mean values with the same letters for both row and column are not significantly different from each other at 5% level by LSD

period (*Table 3*). There was also no significant difference in TSC between fruit stored in low- O_2 atmosphere and air throughout the storage period.

Extending the storage period from 1 to 4 weeks under 2% and 5% O_2 atmospheres resulted in a decreasing trend in the a^*

values of the fruit pulp measured at colour index 5 (*Table 4*). The *a** values of fruit stored continuously in air remained fairly uniform and were not significantly different throughout the storage period.

There was also no significant difference in the a^* values between fruit stored in 2% and 5% O₂ throughout the storage period. The pulp colour value of fruit stored for 3 weeks in air was significantly (p < 0.05) larger relative to fruit stored in the low-O₂ atmosphere.

The b^* values of the pulp obtained from fruit stored in 2% and 5% O₂ were not significantly different throughout the storage period (*Table 4*). With the exception of 3-week storage, the difference in b^* values between fruit stored in low-O₂ atmosphere and air was insignificant.

Discussion

This study examines the physiological basis of low- O_2 atmosphere (2% and 5%) on the

Table 3. Total sugar content of Eksotika papaya fruit at colour index 5 after four storage periods in 2, 5 and 21% oxygen atmospheres

Storage period (week)	Total sugar	Total sugar content (%)			
	2% O ₂	5% O ₂	21% O ₂		
1	6.99a	6.64abc	6.57abc		
2	6.81abc	6.28bc	6.25c		
3	6.86abc	6.43abc	6.81abc		
4	6.41abc	6.98ab	-		

Mean values with the same letters for both row and column are not significantly different from each other at 5% level by LSD subsequent respiratory activity, C_2H_4 production and some compositional changes in Eksotika papaya fruit. The results demonstrate that the respiration of papaya fruit is affected by prior storage under reduced O₂ level. Ke et al. (1990) indicated that the beneficial effects of exposure to O2-reduced atmosphere included reduction of respiration and C2H4 production rates as well as retardation of skin yellowing and pulp softening. The rapid recovery of CO₂ production rate following transfer from low-O₂ atmosphere to air indicates that there is no residual effect on the respiratory activity of the fruit as a consequence of prior exposure to low O2. A residual effect of low O_2 on the respiration and C_2H_4 production rates has been demonstrated in Selva strawberries (Li and Kader 1989), and in bell pepper (Abd. Shukor et al. 1993; Abd. Shukor, A. R., Rohani, M. Y. and Che Omar, D., Study on handling and transportation of Eksotika papaya to Dubai by ship, MARDI, Serdang, not published 1995; Rahman et al. 1995). The pattern of recovery in respiration following storage in low-O₂ atmosphere is similar for fruit stored in 2% and 5% O2. A slight suppression in the CO₂ production rate after storage for 4 weeks in 2% O2 indicates the presence of a slight residual effect as a consequence of prior exposure to low O2. The respiratory climacteric pattern of the fruit is not affected by prior exposure to low-O₂ atmosphere for up to 3 weeks. This is evidenced by the occurrence of the climacteric peak in CO₂

Table 4. Pulp colour of Eksotika papaya fruit at colour index 5 after four storage periods in 2, 5 and 21% oxygen atmospheres

Storage period (week)	a^* (red) value			<i>b</i> * (yellow) value		
	2% O ₂	5% O ₂	21% O ₂	2% O ₂	5% O ₂	21% O ₂
1	18.6abc	18.4a–d	19.1ab	21.6ab	21.6ab	22.8ab
2	17.1de	17.8bcd	18.7abc	20.7b	23.0ab	22.3ab
3	16.1ef	14.7f	19.3a	20.0b	20.6b	25.1a
4	17.5cde	17.4cde	_	22.8ab	23.4ab	_

Mean values with the same letters for both row and column are not significantly different from each other at 5% level by LSD

production after 2 days in air. The attainment of maximum CO_2 production after 3 days in air for the 4-week stored fruit in 2% and 5% O_2 indicates that the preclimacteric stage is extended with prolonged storage period.

In contrast to CO_2 production, the C_2H_4 production rate is markedly affected by prior storage of fruit in low-O₂ atmosphere. This indicates that there is a greater residual effect of low O2 on C2H4 production rates. The recovery in C_2H_4 production after transfer to air is slower relative to fruit stored continuously in air. Yoshida et al. (1986) reported that storage of Bartlett pears in 1% O_2 for 4 months reduced C_2H_4 production rate and retained higher levels of organic acids. Evidently, the 2% O_2 atmosphere is more effective than the 5% O_2 or air in suppressing the C_2H_4 production rate. This is apparent in fruit that have been stored for more than 2 weeks.

The RQ value of 1.83 after storage of fruit for 4 weeks in 2% O2 indicates the occurrence of a slight incident of anaerobic metabolism. It has been shown by Hole et al. (1992) that anaerobiosis can induce an appreciable increase in the RQ, indicating an increase in CO_2 production relative to O_2 consumption. After a day in air, the respiratory activity returns to normal as indicated by the RQ value of about one. Under optimum conditions, the RQ value of plant tissues is equivalent to one with glucose (carbohydrate) as the respiratory substrate (Kays 1991). This study indicates that anaerobiosis is not occurring in papaya fruit even after 4-week storage in low-O₂ atmosphere.

Storage of fresh fruits and vegetables under excessively low levels of O_2 may induce the accumulation of ethanol which results in off-flavour (Ke et al. 1990). The absence of detectable levels (<0.01%) of ethanol in the headspace gas from papaya fruit stored in 2% and 5% O_2 for 4 weeks indicates that anaerobiosis is not occurring in the fruit tissues. While ethanol evaporates in the headspace gas, a considerable fraction may be retained in the fruit tissues. Bartlett pears stored in 0.5% or 1.0% O_2 for 10 days at 0, 5 or 10 °C had almost the same levels of ethanol as the pears stored continuously in air, while 0.25% O_2 caused a slight increase (Ke et al. 1990).

The SSC of ripe fruit is not affected even after storage for 4 weeks in 2% O_2 atmosphere. This indicates that optimum SSC is maintained during storage under reduced O_2 level. The sweetness as indicated by the TSC is also maintained during storage under reduced O_2 level. Studies conducted by Ke et al. (1990) indicated that low O_2 or high CO_2 treatment did not significantly affect either the SSC or total titratable acidity in Bartlett pears.

The intensity of the red colour of fruit pulp is slightly reduced as a consequence of prolong storage in low- O_2 atmosphere. In contrast, the yellow colour is not affected by storage of fruit in low- O_2 atmosphere.

Conclusion

The CO_2 and C_2H_4 production rates of Eksotika papaya fruit during low-O2 storage were suppressed and recovered gradually upon transfer to air at 20 °C. There is a slight residual effect or post-storage reduction in the respiratory activity of the fruit as a consequence of prior exposure to low O_2 . Extending the storage period from 1 to 4 weeks resulted in a slight residual effect on the CO_2 production rate as a consequence of prior storage to low O2. However, slow recovery in C₂H₄ production was observed in fruit that had been stored for more than 2 weeks in low O2. This indicates that the residual effect of low-O2 storage is more apparent on the C_2H_4 production rates. The absence of ethanol and RQ value of about one indicate that the low-O₂ atmospheres are not injurious to fruit tissue. Additionally, SSC and TSC of ripe fruit are not affected, while the intensity of red colour is slightly affected by prior storage of fruit in 2% O₂.

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