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# Biochemical Changes, Use of Polyethylene Bags, and Chilling Injury of Carambola (*Averrhoa carambola* L.) Stored at Various Temperatures

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Key Words: **pH, Titratable acid; sugar content; Soluble solids;** *Averrhoa carambola;* **polyethylene, chilling injury, disease; storage; temperature.**

## RINGKASAN

*Perubahan pH, jumlah asid titratable dan pepejal terlarut dalam buah belimbing Segi yang muda dan masak, yang disimpan pada suhu-suhu 5°. 10°, 15° dan 20°C tidak mempunyai perbezaan yang jelas. Begitu juga halnya bagi jumlah kandungan gula dalam buah. Bahan kanji tidak didapati dalam buah muda dan juga yang masak. Buah muda yang disimpan dalam beg-beg yang bertebuk dan tidak bertebuk pada suhu 20°C menjadi masak apabila beg-beg dibuka. Warna kulit buah matang menjadi kuning seluruhnya. Peratus buah yang berkulat dalam beg-beg tidak bertebuk adalah rendah dibandingkan dengan buah-buah kawalan yang disimpan dalam beg-beg bertebuk selepas 1 hingga 3 minggu dalam simpanan. Buah-buah muda yang mempunyai kurang dari pada 25% warna kuning pada kulit mengalami kecederaan ringan selepas 5 minggu disimpan pada 5°C. Kecederaan akan bertambah jika ia disimpan lebih lama lagi. Gangguan fisiologi kelihatan sebagai tompok-tompok berwarna hijau tua, tetulang menjadi kecut dan hitam dan buah-buah tidak bertukar ke warna kuning setelah dipindahkan pada suhu 20°C.*

## SUMMARY

*The effects of storage temperature and the use of polyethylene film on chilling injury and biochemical changes in carambola* (Averrhoa carambola *L.) fruits were studied. The pH, total soluble solid changes were not significantly different in ripe and unripe fruits stored at 5°, 10°, 15° and 20°C. No starch was detected in either ripe or unripe fruits, and no significant changes occurred in the total sugar content. Unripe fruits pacted in sealed perforated and nonperforated polyethylene bags stored at 20°C those in perforated bags or the control after 1 and 3 weeks in storage. Chilling injury of unripe carambola fruits, less than 25% full yellow in colour, occurred after 5 weeks in storage. The degree of injury was slight but became more severe as storage time lengthened. The ribs of the fruits turned black and dark brown patches appeared on the surface of the skin. Unripe fruits having less than 25% yellow colour on the skin surface suffered chilling injury after 5 weeks storage at 5°C. The severity increased with storage time. The physiological disorder appeared as dark green brown patches on the skin, shrivelled and darkened ribs, and failure of the fruits to turn yellow when transferred to 20°C.*

## INTRODUCTION

An important chemical composition of a fruit that determines its sweetness at maturity is the amount of sugar present. Chan and Heu (1975) identified the major sugars in carambola as fructose, glucose, and sucrose. Pratt *et al.* (1977) found that the sugar content of muskmelon does not increase after harvest because the fruit does not contain starch. In fruits that contain starch at the unripe stage, such as mangoes (Subramanyam *et al.*, 1975) and bananas (Von Leosecke, 1949), starch content decreases and sugar content increases during ripening.

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Vines and Grierson (1966) have measured seven organic acids in carambola fruits. Oxalic and tartaric were the major acids in mature-green fruits. As the fruit reached the mature-yellow stage the tartaric acid decreased, while the oxalic and $\alpha$ -ketoglutaric acid content increased.

Plastic films are commonly used to achieve modified atmospheres in the postharvest storage of fruits. The storage life of bananas is extended from 10 to 50 days when they are stored in sealed polyethylene bags (Scott and Gandanegara, 1974). The bag is a *dyamic* system, allowing uptake of oxygen ($O_2$), and evolution of carbon dioxide ($CO_2$), ethylene ($C_2H_4$), and water. At the same time, permeation of gases through the plastic film is restricted.

Storage at low temperatures extends the storage life of the produce as the rate of respiration and metabolism is reduced. (Biale, 1960). However, tropical fruits exhibit chilling injury as a result of exposure to low temperatures above freezing point for a certain duration of time. The severity of chilling injury is difficult to determine quantitatively, but can be estimated by visual symptoms (Lyons, 973).

The critical temperature at which chilling injury occurs varies with species. The green skin of the durian turns brown and then black when stored at 12°C, and fruits kept at 5°C failed to ripen when transferred to ambient temperature (Lam and Lye, 1982). Mandoza *et al.* (1972) reported that the skin of rambutan darkens when stored at 7°C.

This paper reports on effects of storage temperature and the use of polyethylene film on chilling injury and biochemical changes of Carambola *(Averrhoa carambola* L.) fruits.

## MATERIALS AND METHODS

Mature carambola fruits of clone B.10 were obtained from an orchard in Serdang: The fruits were classified visually as unripe or ripe based on the percent yellow colour present on the surface of the skin. Fruits that were less than 25% full yellow were classified as unripe and those having more than 25% as ripe.

The fruits were kept in 300 x 300 mm polyethylene bags of 0.04 mm thickness with a 1.5% perforation and stored at 5°, 10°, 15° and 20°C. Analysis of pH, total soluble solids, total titratable acid, and total sugars were carried out at intervals of 2 weeks until the fruits began to rot. Acidity (pH) of the juice was determined, after blending a whole fruit in a Beckman digital pH meter (model 3500). In the determination of total titratable acid, a known weight of the blended fruit sample was titrated to pH 8.1 with 01. N NaOH, and the results expressed as percent oxalic acid, which is the predominant acid. A Kyowa HR-1A (0 – 32%) hand refractometer was used to determine total soluble solids. The total sugar content of the juice samples was determined according to the method described in AOAC (1975). However, the step using ethanol for extraction was omitted from the normal procedure because no starch was detected in earlier determinations.

The effects of polyethylene film on the storage life of the fruits was studied using bags of similar gauge and size as in the previous experiment. The bags containing fruits were sealed by tying the open ends, held in storage at 20°C, and removed after 1 to 2 week intervals. On removal, the fruits were exposed to normal atmosphere at 20°C. Both skin colour and incidence of disease were scored. Changes in skin colour were scored visually from 1 to 8 with 1 = green, 6 = yellow, and 8 = brown. The number of diseased fruits was determined for each storage temperature and expressed on a percentage basis.

Using another batch of fruits, each fruit was sealed in a nonperforated 150 x 230 mm polyethylene bag 0.04 mm in thickness for $O_2$, $CO_2$ and $C_2H_4$ determinations. One ml gas samples were withdrawn with a syringe through a rubber tubing affixed to one corner of the bag. The determinations were done daily until infection appeared on the fruits. $O_2$ and $CO_2$ were determined on a Varian 1420 gas chromatograph fitted with a thermal conductivity detector. $C_2H_4$ evolution was measured using a Varian 1440 gas chromatograph equipped with a flame ionisation detector.

Only fruits stored at 5°C were used to evaluate chilling injury. The fruits were removed from storage at intervals of 2 weeks until the ninth week of storage, and left at 20°C. Observations were made at intervals of 3 and 7 days.

All treatments consisted of 4 replicates of 5 fruits each unless stated otherwise. The results were subjected to analysis of variance tests where applicable.

## RESULTS

Fruits, ripe and unripe, could be held at 5°C for a period of 12 weeks with no signs of decay setting in. However, fruits held at 10°, 15° and 20°C began to rot after 5 weeks in storage. Thus, only data on biochemical changes occurring in the fruits up to 5 weeks in storage will be presented.

Changes in pH, oxalic acid, total soluble solids (TSS), and total sugar content of unripe fruits are shown in Table 1.

The pH ranged from 3.6 to 4.1 during storage at the various temperatures, with no significant changes being detected.

The oxalic acid varied from15% to 29%, and decreased significantly after one week in storage for all the temperatures tested. Thereafter, no significant changes were observed to have occurred in the fruits.

The TSS of the fruits at all storage temperatures remained rather consistent. It fluctuated within a narrow range of 8.6 to 9.1 at all storage intervals.

The values for total sugar content decreased slightly during storage, but was insignificant, regardless of storage temperature.

TABLE 1
Effect of storage temperature on the pH, oxalic acid, total soluble solids, and total sugar content of unripe carambola fruits.

| Temp. | Storage period (wk) | | | | |
|---|---|---|---|---|---|
| (°C) | 0 | 1 | 3 | 5 | 7 |
| | | $pH^{z}$ | | | |
| 5 | $3.6^{bc}$ | $4.0^{bc}$ | $4.0^{ab}$ | $3.6^{c}$ | $3.8^{bc}$ |
| 10 | $3.6^{a}$ | $4.1^{a}$ | $4.0^{a}$ | $3.7^{a}$ | – |
| 15 | $3.6^{b}$ | $4.1^{a}$ | $3.9^{ab}$ | $3.7^{ab}$ | – |
| 20 | $3.6^{a}$ | $4.1^{a}$ | 4.1 | – | – |
| | | Oxalic acid (% x $100)^{z}$ | | | |
| 5 | $29^{a}$ | $18^{a}$ | $20^{b}$ | $21^{b}$ | $22^{b}$ |
| 10 | $29^{a}$ | $17^{b}$ | $20^{b}$ | $21^{ab}$ | – |
| 15 | $29^{a}$ | $17^{b}$ | $20^{b}$ | $22^{ab}$ | – |
| 20 | $29^{a}$ | $17^{b}$ | $18^{b}$ | – | – |
| | | Total soluble solids $(\%)^{z}$ | | | |
| 5 | $8.6^{a}$ | $8.7^{a}$ | $8.6^{a}$ | $8.6^{a}$ | $8.4^{a}$ |
| 10 | $8.6^{a}$ | $8.0^{a}$ | $8.7^{a}$ | $8.5^{a}$ | – |
| 15 | $8.6^{a}$ | $8.5^{a}$ | $9.1^{a}$ | $8.6^{a}$ | – |
| 20 | $8.6^{a}$ | $8.2^{a}$ | $8.6^{a}$ | – | – |
| | | Total Sugar content $(\%)^{z}$ | | | |
| 5 | 7.1a | 7.0a | 6.7a | 6.5a | $6.7^{a}$ |
| 10 | $7.1^{a}$ | $6.5^{b}$ | $6.9^{a}$ | $6.7^{b}$ | – |
| 15 | $7.1^{a}$ | $7.0^{a}$ | $6.7^{a}$ | $6.6^{a}$ | – |
| 20 | $7.1^{a}$ | $6.6^{b}$ | $6.1^{b}$ | – | – |

$^{z}$Mean separation across columns by DMRT at 5% level. Means with the same letter are not significantly different from one another.

The pH, oxalic acid, TSS and total sugar content values of ripe fruits obtained indicated that no significant changes occurred during storage for all the temperatures studied (data omitted) Additionally, the parameters measured above were not significantly different from the unripe fruits.

Sealed bags were effective in retaining the green colour of the carambola fruit. The unripe fruits remained green, whereas the control (fruits without any packaging) and fruits in perforated bags turned yellow after 1 week in storage at 20°C. The fruits in sealed bags tasted normal. The unripe fruits turned completely yellow after 9 days when the sealed bags were left open at 20°C in normal atmosphere, thus indicating that ripening was not permanently inhibited. There were no major differences in colour change between the control and fruits in perforated bags.

Ripe fruits stored in perforated bags showed the greatest percentage of disease infection after 1 and 3 weeks in storage (Table 2). There was no significant difference between the control and fruits in sealed bags after 1 week in storage. However, after 3 weeks the percentage of diseased fruits in control increased significantly compared to that of sealed polyethylene bags. The fruits in sealed bags, both ripe and unripe, were less infected than the rest after 3 weeks in storage.

TABLE 2
Percentage of diseased fruits in sealed and perforated bags at 20°C.

| Storage Period (Weeks) | Treatment | Diseased Fruits (%)[z] Ripe | Unripe |
|---|---|---|---|
| 1 | Control | 13 c | 6 b |
| | Sealed | 19 c | 0 b |
| | Perforated | 56 b | 6 b |
| 3 | Control | 100 a | 100 a |
| | Sealed | 56 b | 25 b |
| | Perforated | 100 a | 100 a |

[z]Mean separation within columns and sets by DMRT at 5% level. Means with the same letter are not significantly different.. NA = not available as fruits decayed.

Unripe fruits could be kept for 1 to 3 weeks in sealed bags, and than for another 1 week when the bags were opened. Ripe fruits in sealed bags, on the other hand, when opened also remained good for 1 week, but the percentage of diseased fruits was much higher. The unripe and ripe fruits changed to an orange colour when exposed to normal atmosphere indicating that they would ripen on removal from storage.

The $CO_2$ concentration of the atmosphere in the sealed bags containing the unripe and ripe fruits was observed to be markedly different as storage time progressed (Fig. 1).

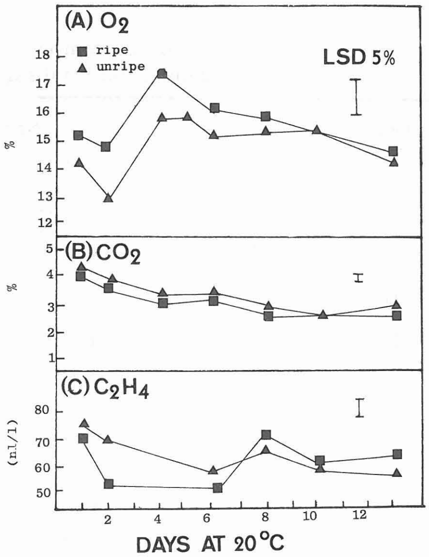


*Fig. 1 Percent $O_2$, $CO_2$, and nl/l $C_2H_4$ in sealed nonperforated polyethylene bags containing ripe and unripe fruits.*

The $CO_2$ concentration deceased from 4% to approximately 2% for unripe fruits, and from 4.5% to 2% for ripe ones on the 13th day at which time the experiment was terminated due to decay of the fruits. There was no observable difference between $CO_2$ production and fruit ripeness. No sharp change in $CO_2$ evolution was detected.

The concentration of $O_2$ and $C_2H_4$ in the sealed bags containing the unripe and ripe fruits was found not to be markedly affected by time or stage of ripeness. The $O_2$ content initially increased and then gradually decreased. The increase in $O_2$ content observed could probably be due to a high respiration rate of the fruit initially. This created a difference in concentration inside the bag and that of the outside atmosphere. As a result, $O_2$ diffused into the polybag from the outside atmosphere. The level of $C_2H_4$ showed a fluctating pattern with no distinct peaks being observed.

All the fruits, ripe and unripe, remained physically good, well coloured and firm with no visible signs of diseases up to 7 weeks in storage at 5°C (Table 3). Unripe fruits stored for 1 and 3 weeks at 5°C continued to ripen when they were transferred to 20°C for another 7 days

Unripe fruits could be stored for 3 weeks at 5°C, followed by 1 week at 20°C, making a total postharvest storage life of 4 weeks. No chilling injury symptoms were observed in either the ripe or unripe fruits. The ribs of unripe fruits stored for 5 weeks at 5°C were dark brown and black. *Cercospora* rot were commonly seen in both ripe and unripe fruits when stored for another 7 days at 20°C. The occurrence of fungi attack was aggravated by the presence of dried calyxes at the stem-end. These should be removed to minimise diseases developing at the stem-end. Chilling injury though minor, began to develop in the unripe fruits, but not in the ripe fruits.

Chilling injury was distinct in the unripe fruits after 7 weeks at 5°C. All the ribs of the fruits were black and there were dark brown patches on the skin. These brown patches were shown as discolorations and were present only on the surface of the skin. They were not diseases as such as seen at higher temperatures. The fruits were dull green or turned to a dull yellow colour on further storage for 3 days at 20°C. The ripe fruits after 3 days storage at 20°C were still good and firm without any chilling injury being seen. Some diseased dark spots caused by *Cercospora species* were present. Also, some ribs of the fruits turned black due to shrivelling.

TABLE 3

Percentage of diseased fruits, and colour index changes of ripe and unripe fruits stored at 5°C.

| Storage Period (wk) | Duration at 20°C (day) | Diseased fruits (%)[Y] | | Color· index[YZ] | |
|---|---|---|---|---|---|
| | | Ripe | Unripe | Ripe | Unripe |
| 1 | 0 | 0 | 0 c | 6 bc | 2 de |
| | 7 | 15 cd | 0 c | 7 a | 5 bc |
| | 14 | — | 100 a | — | 7 a |
| 3 | 0 | 0 d | 0 c | 6 c | 3 d |
| | 7 | 30 bcd | 5 c | 7 ab | 6 b |
| 5 | 0 | 0 d | 0 c | 5 c | 2 de |
| | 7 | 100 a | 95 a | 7 a | 7 a |
| 7 | 0 | 0 d | 0 c | 5 a | 1 de |
| | 3 | 60 b | 15 c | 6 c | 3 d |
| 9 | 0 | 30 bcd | 15 c | 6 c | 2 de |
| | 3 | 50 bc | 30 b | 7 ab | 4 c |

[Y]Means of treatment with the same letter within each column is not significantly different from one another at the 5% level according to DMRT.

[Z]Colour index score : 1 = green, 2 = green with trace of yellow; 3 = more green than yellow; 4 = more yellow than green; 5 = yellow with green rib; 6 = yellow; 7 = yellow with some brown flecks; 8 = brown.

No chilling injury developed in ripe fruits after 9 weeks storage at 5°C. Generally, the ribs of fruits were shrivelled and brown in colour. There were some diseased dark spots present on the fruits. Diseases became more severe on further storage of 3 days at 20°C although the fruits remained firm. Unripe fruits exhibited chilling injury. The symptoms were similar to those shown in fruits stored for 7 weeks at 5°C. Fruits were firm and were either dull green or brownish green in colour.

## DISCUSSION

Vines and Grierson (1966) reported that the pH in carambola rose from 2.3 in the green fruits to 3.8 in the ripe fruits. In this study, no significant increase in pH was observed at the various storage intervals regardless of the temperature the unripe fruits were stored in (Table 1). This, perhaps, was due to the fact that the unripe fruits used had already begun to ripen since they had passed the green stage.

Total titratable acid decreased during ripening which is similar to other fruits such as 'calamansi' citrus (Data *et al.*, 1975), mango (Morga *et al.*, 1979), and papaya (Broughton, *et al.*, 1977). This is similar to the observation made by Grierson and Vines (1965). The decrease in total titratable acid was probably due to it being metabolized in the Krebs-tricarboxylic Acid Cycle as reported by Vines and Grierson (1966).

The TSS content did not change significantly when the fruits ripened from the green to the orange stage during storage at all temperatures. In contrast, mango exhibits a large change in TSS from 7 to 19% (Morga *et al.*, 1979). The mature green carambola fruit, therefore, did not show a significant increase in TSS during ripening after harvest. This was probably because of the absence of starch in the fruit. In this respect it resembles melons. Pratt *et al.* (1977) reported that the soluble solids in mature melons did not increase because sugar accumulation from the hydrolysis of starch was not possible. Data *et al.* (1975) showed that TSS increased with storage time in small and medium green mature 'calamansi' citrus fruits. In the citrus the stored starch was converted to sugars during storage.

Total sugar content did not change significantly regardless of temperature. The percentage of sugar in carambola is rather low when compared to that of bananas. Bananas (CSIRO, 1972) have approximately 1% in the mature green stage and 16% in the ripened stage. The marked increase is due to the hydrolysis of starch to sugars as the green mature bananas have about 22% starch. The total sugars found by Chan and Heu (1975) in carambola added up to 3.19% of the fresh weight of the fruit. The total sugars ranging from 6.1 tp 7.5%, of the cultivar used in this study is, therefore, twice that reported by the above researchers.

Ripe and unripe fruits in perforated bags and controls senesced at the same rate. Both were 100% diseased after 3 weeks in storage (Table 2). This was significantly higher than the fruits stored in sealed bags. Only 56% of the ripe fruits in sealed bags and 25% of the unripe fruits were diseased. Furthermore, the green colour of the fruits was maintained when stored in sealed polyethylene bags indicating that their storage life could be prolonged. Sealed bags have also prolonged the storage life of bananas (Scott *et al.*, 1970; 1971) and avocados (Chaplin and Hawson, 1981) by retarding ripening due to alteration in the atmosphere around them.

Initially the $CO_2$ content in the sealed bags was high and when the respiration of the fruit reached a steady state, the $CO_2$ content decreased to a constant level. (Fig. 1). Normal atmospheric air contains about 0.03% $CO_2$, and the concentration in the bag was found to vary from 2.5 to 4.5%. The $CO_2$ concentration was, therefore, approximately 80 to 150 times that found in normal atmosphere. This range in $CO_2$ concentration lies between the range of 3 to 9% as reported by Duvekot (1961) and Moiseyeva (1960) in the internal atmosphere of apple packages. Similarly, they found that packaged apples have a longer shelf life than unpackaged apples.

The $O_2$ content fell to approximately 15%. This is 6% lower than the noral atmospheric concentration of 21%. The modified gaseous composition of 2.5 to 4.5% $CO_2$ and 15% $O_2$ in the polyethylene bags would reduce the respiration rate of the unripe fruits. It also maintained the chlorophyll content of the skin of the fruits for 2 to 3 weeks at 20°C.

The small deviations in $CO_2$ and $O_2$ concentrations at equilibrium was similar to the results of Chaplin and Hawson (1981). They found that the levels of gases in bags of avocados at ambient temperature were not different whether stored for 5 or 10 days. On the other hand, Pantastico *et al.* (1970) found that 1% $O_2$ or 10% and 15% $CO_2$ produced irreparable peel injury and impaired the ripening process in bananas. But the levels of these two gases in the bags of carambola fruits in this study were within the range reported by Pantasico *et al.* (1970). They also reported that ripening was induced earlier in 10% and 15% $CO_2$

than in 5% $CO_2$ level probably because of $CO_2$ injury

Dull *et al.* (1967) showed that decreased $O_2$ and increased $CO_2$ concentrations had no obvious effect on pineapple quality. They recommended that there was no advantage on quality maintenance by manipulating the concentration of these two atmospheric gases. However, we found that it could extend the storage life of the carambola fruits.

Chilling injury of unripe carambola fruits occurred after 5 weeks in storage at 5°C (Table 3). The degree of injury was slight but became more severe as storage time lengthened. This is similar to bananas as reported by Pantasico *et al.* (1967). They showed that the portion near the blossom end, which was less mature than the bigger stem end, was more susceptible to chilling injury. Ripe carambola fruits are not effected by chilling injury at 5°C. Carambola fruits which are unripe could only be stored for 5 weeks or less at 5°C. If a longer storage period at 5°C is desired, then only ripe fruits should be used.

The development of chilling injury symptoms was similar to those exhibited by avocado fruits (Eaks, 1976). Eaks (1976) demonstrated that avocado fruits do not soften or show symptoms of chilling injury when they are stored for 5 weeks at 5° or 0°C. However, after transfer to 20°C for I week or longer they developed chilling injury and the sensitivity increased as the exposure period was lengthened.

## CONCLUSION

The pH, titratable acid, total soluble solids, and total sugar content changes were not significantly different during storage at the temperatures studied. No starch was detected in the mature ripe fruits. Carambola should, therefore, be harvested at the ripe stage and then stored. Ripe fruits will give a better quality and colour than the stored unripe fruits.

There was no difference in the storage life of fruits packed in perforated polyethylene bags and control. Fruits, particularly the unripe ones, could be stored longer in sealed polyethylene bags. Additionally, the green colour of the unripe fruits could be maintained longer. The fruits ripened and turned completely yellow in colour when the bags were opened.

Unripe fruits are susceptible to chilling injury when stored for 5 weeks at 5°C. The severity is increased as storage time is prolonged at this temperature. The fruit will ripen when they are removed before 5 weeks in storage at 5°C and placed at 20°C.

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