MATURATION OF MALAYSIAN FRUITS
I. STORAGE CONDITIONS AND RIPENING OF PAPAYA
(CARICA PAPAYA L. CV. SUNRISE SOLO)

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RINGKASAN

Satu penyiasatan telah dijalankan keatas faktor-faktor yang mempengaruhi proses 'masak' buah-buah betik yang dipetik. Untuk menggalakkan proses pemasakan dan penyimpanan yang memuaskan suhu optima yang diperlukan ialah 20°C. Suhu yang lebih tinggi dari ini akan menyebabkan buah-buah ini menjadi peka terhadap serangan kulat, manakala suhu yang lebih rendah akan melambatkan pencapaian 'climacteric', dan 'kecederaan akibat kesejukan' akan diperlihatkan. Suhu bilek (28° hingga 32°C) tidak sesuai untuk penyimpanan buah. Pengasingan ethylene atau air dari suasana keliling tidak membawa apa-apa kesan terhadap percapaian 'climacteric', sedangkan pengasingan karban dioksid mempercepatkan proses tersebut. Pencapaian 'climacteric' buah lambat sedikit jika suasana kelilingnya ketepuan air. Tingkat gelukos bertambah apabila buah masak tetapi dengan penyimpanan yang lama.

Keadaan penyimpanan yang disyurkan ialah: suhu 20°C, kelembapan udara lebih kurang 50% dengan sedikit tambahan karban dioksid jika boleh. Dalam keadaan begini buah-buah yang dipetik 80 hingga 90 hari selepas mengorak (dan masih hijau) akan masak dengan memuaskan dan dapat disimpan selama tujuh hingga empat belas hari.

INTRODUCTION

Many changes occur in fleshy fruits as they ripen and finally senesce. Amongst these changes are (a) abscission of the fruit from the plant, (b) changes in the rate of gas evolution (particularly ethylene and carbon dioxide), (c) changes in tissue and organelle permeability, (d) changes in colour including destruction of chlorophyll and the synthesis of new pigments, (e) certain biochemical changes especially involving carbohydrates, organic acids, pectic substances, proteins etc., (f) changes in enzyme activity, (g) the synthesis and release of flavour volatiles, and (h) the development of wax on the skin of the fruit (see example PRATT and GOESCHL, 1968).

During the development of a fruit, from a fertilised ovum to maturity, ethylene is continuously produced albeit in very small amounts. Just before the onset of the respiratory climacteric, the concentration of ethylene rises to physiologically effective levels and initiates the whole ripening process (LYONS, McGLASSON, and PRATT, 1962; BURG and BRUG, 1962a). While many fruits respond to increased production of ethylene and ripen on the tree, the response of certain fruits (e.g. avocado – *Persea americana* Mill) to ethylene appears to be inhibited until after the fruit is harvested (BURG and BURG, 1962b; 1965a). Some fruits may produce an effective concentration of ethylene before they are fully mature. Such fruits however, only become sensitive to ethylene when they are fully developed (BURG and BURG,

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1965a and b). PRATT and GOESCHL (1968) found that physiologically active concentrations of ethylene were present in the intercellular spaces of fruit just before the climacteric. They also found that exogenous ethylene will induce autocatalytic ethylene synthesis in unripe fruits and so trigger the climacteric.

Apart from ethylene, marked rises in oxygen uptake and carbon dioxide output are characteristics of the climacteric which foretell fruit ripening. After these threshold levels have been attained, rates of respiration drop back to constant levels. PRATT and GOESCHL (1968) attributed this rise in respiration to the general changes in metabolic organization of the fruit tissues. It may also reflect an increase in energy requirements during the early, synthetic stages of ripening.

Attractive flavours, as well as the appearance, texture and vitamin contents of fruit are manifestations of their sugar content. Common sugars include glucose, fructose and sucrose which is the form in which sugar is translocated to the fruit. Also, of course, it is the carbon and energy source for biosynthesis and energy production in most fruits. During the ripening of non-climacteric fruits, there is a gradual change in sugar content, so that harvest may be spread over a relatively long period without loss of fruit quality. In climacteric fruits however, there is a rapid conversion of starch to sugars at or just after the climacteric rise in respiration.

Obviously, temperature influences the rate of ripening. The response to ethylene stimulation decreases with decreasing temperatures, but on the other hand at temperatures above 35°C, many fruits fail to produce ethylene or ripen normally (BURG and BURG, 1962a). BIALE (1946a) found that avocados stored at lower temperatures took a longer time to ripen, and that the amount of carbon dioxide produced during the climacteric was lower. Each fruit has its own particular range within which satisfactory ripening will occur, and a knowledge of this range is extremely important in prolonging storage life. Low temperatures often cause "chilling injuries" such that the fruits either cannot ripen at all, or they ripen very slowly and unevenly. Such fruits often develop physical blemishes such as browning of the peel in bananas. Conversion of starch to sugars, and the decrease in organic acid content are greatly retarded at lower temperatures so that the fruit develops a flat, acid, or astringent flavour. At high temperatures, the fruit pulp becomes very soft long before any noticeable signs of ripening. Higher temperatures, coupled with high relative humidities predispose the fruit to fungal attacks, which can cause considerable fruit loss especially in tropical conditions.

For all of these reasons, it is important to determine environmental effects of the ripening of Malaysian fruit. Only with such information can optimum storage and ripening conditions be determined. Optimal storage conditions are necessary in any consideration of fruit export, and it is equally important that the fruit be ripened satisfactorarily in the recipient country. Similar considerations apply in attempts to extend the selling season in the producing country. This is particularly important in Malaysia where many of the fruit tend to be of the climacteric type, for this poses the further problem of harvesting them at maturity but before they attain the climacteric.

Accordingly, we have initiated a programme to (a) study the effect of temperature and humidity on storage and ripening of Malaysian fruit, (b) to study the biochemical changes that accompany ripening under the conditions listed in (a) above, and (c) to determine what environmental modifications lead to the best conditions for storage and after storage ripening in Malaysian fruits. The results of the first series of experiments with papaya are listed below.

MATERIALS AND METHODS

Fruit

Green, mature but unripe papayas *(Carica papaya* cv. Sunrise Solo*)* (see *Plate 1*) were obtained from the Malaysian Agricultural Research and Development Institute, at Serdang, Selangor. The fruits used were normally gathered about 80 to 90 days after anthesis, and measured 12 to 14 cm. long, and 8 to 10 cm. across the widest portion. As soon as possible after removal from the plant, the cut-end of the fruit stalk was smeared with vaseline to minimise dehyration and fungal growth. Then the fruits were weighed and placed in a gas-tight container of the type shown in *Fig. 1*. Each series of experiments (i.e. to test one condition) were performed on a single batch of fruit harvested on the same day.
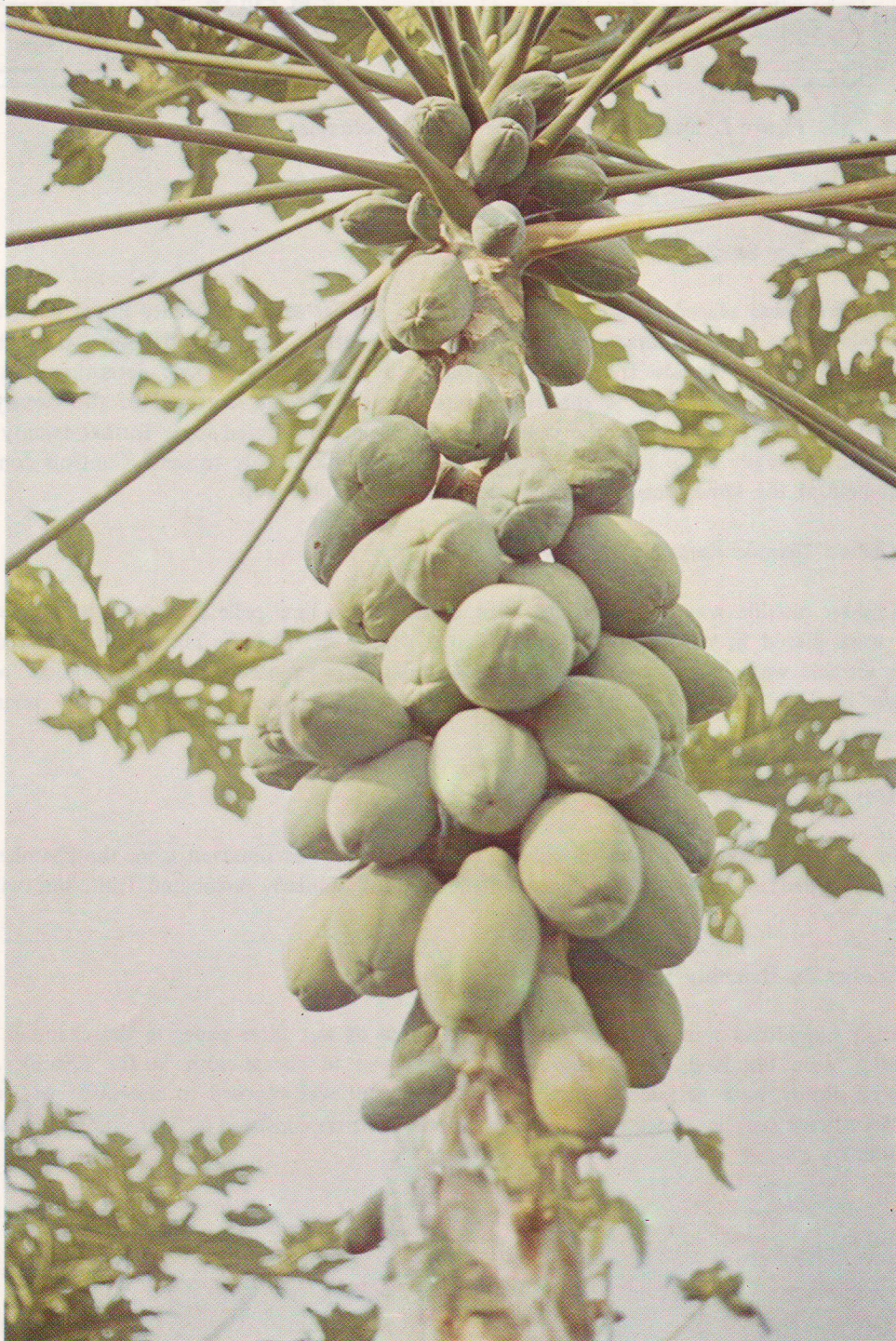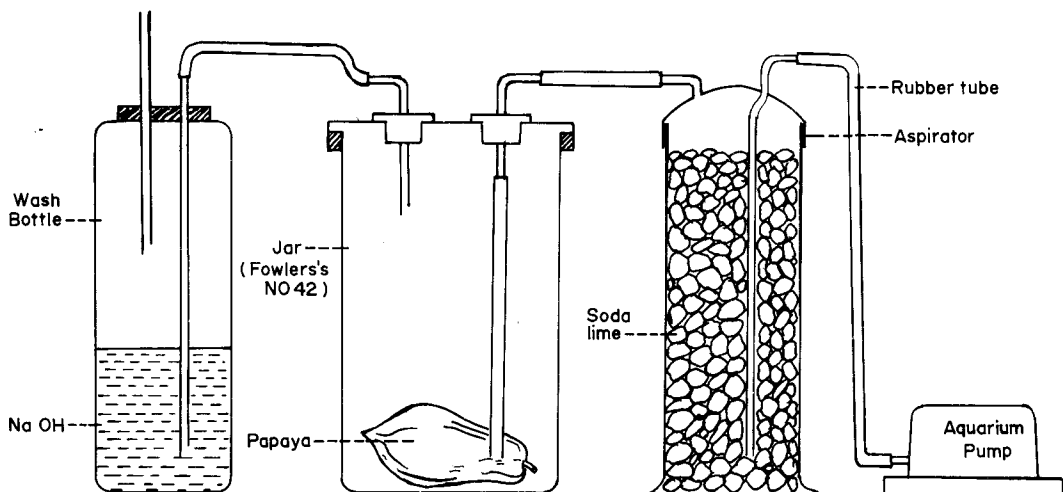
Figure 1. Diagram showing the gas-tight container used in the study.

Incubation

(a)   Temperature Studies

One fruit was placed in each gas-tight jar, and the jar sealed. Twenty hours later, a one ml. sample of gas was withdrawn for ethylene determination as described below, the chamber flushed with carbon dioxide free air during the course of carbon dioxide determinations, and left open to ordinary air (at the specified temperature) for what remained of the twenty-four hours. At the end of this time, the chamber was resealed, incubated for a further twenty hour period, and this daily cycle continued until the fruit was thoroughly ripened. Controls consisted of fruit held at the same temperature, but not enclosed in chambers.

(b)   Carbon Dioxide Removal Studies

Carbon dioxide was removed with self-indicating soda lime pellets wrapped in cheese-cloth which were placed in the chamber concomitantly with fruit. Periodic replacement ensured that carbon dioxide was always removed, and the chamber was exposed to carbon dioxide free air for four hours a day as described under (a) above. Controls consisted of similarly incubated fruit, but without soda lime.

(c)   Ethylene Removal Studies

In similar fashion to carbon dioxide removal, ethylene was removed from the chambers by $KMnO_4$ wrapped in cheese-cloth. Controls consisted of similarly incubated fruit, but without $KMnO_4$.

(d)   Studies on Humidity Modification

High humidities were achieved by placing a piece of wet filter paper in the chamber. Low humidities were obtained by placing silica gel wrapped in cheese-cloth in the chamber. The functional agents were periodically replaced, and the fruit was exposed to stream of wet or dry air for four hours per day as described under (a) above. Controls consisted of fruit similarly incubated with ordinary air.

Ethylene Measurement

One ml. sample of the gas from within the air-tight jars were withdrawn at various times with a gas-tight syringe (Hamilton Co., Reno, Nevada), and injected into a Varian Model 1440 Gas-chromatograph equipped with a flame ionization detector. Chromatograph operating conditions were: column temperature 100°C, injector and detector temperatures 130°C, nitrogen-carrier gas flow-rate 30 ml.min^{-1} on a 150cm. x 0.25cm. diameter column of "Porapak T" (Waters Assoc. Inc., Massachusetts). Under these conditions ethylene eluted in 20 sec. and calibration was performed using ethylene.

Carbon Dioxide Determination

After standard incubation periods, carbon dioxide-free air was used to flush out the gaseous contents of the chamber into a known volume of 0.200 M NaOH.

Equal volumes of the reacted NaOH solution and 10% (w/v) $BaCl_2$ were then mixed together, and the excess NaOH back-titrated with 0.1 M HCl using a phenolphthalein indicator. Empty jars were taken through the same procedure to serve as controls.

Glucose Determination

Ten grams of papaya pulp (minus skin and seeds) were homogenised with a mortar and pestle in 5 ml. of 10% (w/v) trichloracetic acid. The homogenate was centrifuged at 10,000G for 20 min. (4°C), and the supernatant assayed for glucose using the glucose-oxidase-peroxidase procedure of BERGMEYER (1965).

Materials

Glucose oxidase (EC 1.1.3.4), peroxidase (EC 1.11.1.7), and o-dianisidine were obtained from the Sigma Chemical Co., St. Louis, Missouri; ethylene (99.5% pure) was obtained from E. Merck, Darmstadt; Incubation chambers — (Fowlers No. 42 Preserving Jars) were obtained from Fowlers Vacola Pty. Ltd., Hawthorn, Victoria.

RESULTS

Sunrise Solo papayas show a climacteric rise in respiration as well as a significant increase in ethylene production when they ripen *(Figures 2 and 3)*. Lowered temperatures delayed the onset of the climacteric by 20 to 22 days in going from 20°C to 10°C. Control papayas stored at the same temperatures but not enclosed in containers visibly appeared to ripen at the same time as those stored in the chambers. Room temperature (28°C to 33°C) was not satisfactory for storing papayas as the skin became soft and was very susceptible to fungal attack, well before the fruits were edible.

At all temperatures, the peak of carbon dioxide production preceded the peak of ethylene evolution by one to three days (of *Figs. 2 and 3*, and *Table 1*), with the difference being less at lower temperatures. Whether or not there is any significance in these data remain unclear, for if ethylene really is the fruit ripening hormone as suggested by KIDD and WEST (1933, 1945) one would have expected ethylene production to precede the respiratory climacteric. Fortunately, other data shed some light on this problem. Removal of carbon dioxide from the storage chamber brings the time of peak ethylene production forward by six days *(Fig. 4)*. Yet removal of ethylene has no effect on the respiratory climacteric *(Fig. 5)*.
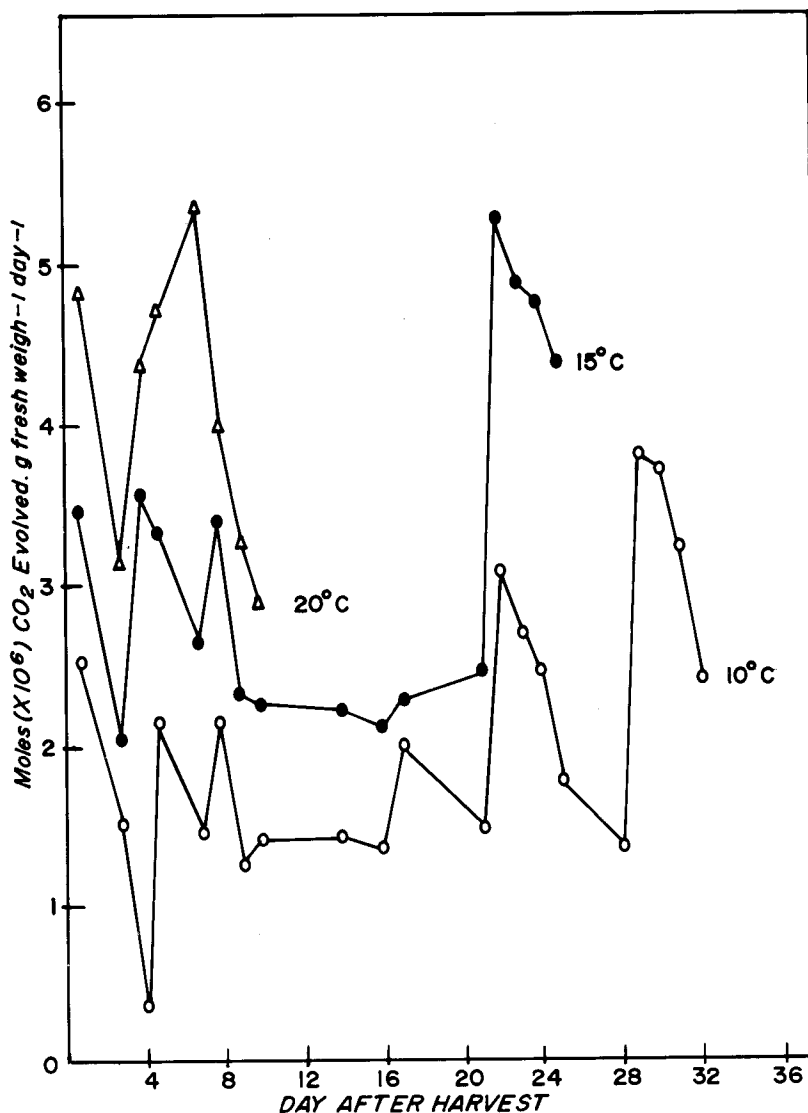
Figure 2. The effect of temperature on respiration in papaya during ripening.

This suggests that exogenous ethylene has little effect on the ripening process, but that removal of the respiratory product, carbon dioxide does. Perhaps the papaya skin allows only a sort of selective directional permeability such that certain gases more readily diffuse out than in. A mechanism of this sort would allow concentration dependent phenomena such as carbon dioxide production and ethylene evolution to manifest themselves at different rates and so explain these apparent differences.

There seems to be little doubt that carbon dioxide accumulation delays ripening. What is surprising however, is that the magnitude of the ethylene peak is the same whether or not carbon dioxide was removed from the chamber. This situation is similar to that obtained by YOUNG, ROMANY, and BIALE (1962). They found that carbon dioxide delayed the induction of the climacteric without affecting the rate of respiration in bananas. On the other hand, the opposite occurred with avocados. In the present context, the significance of this result, lies in
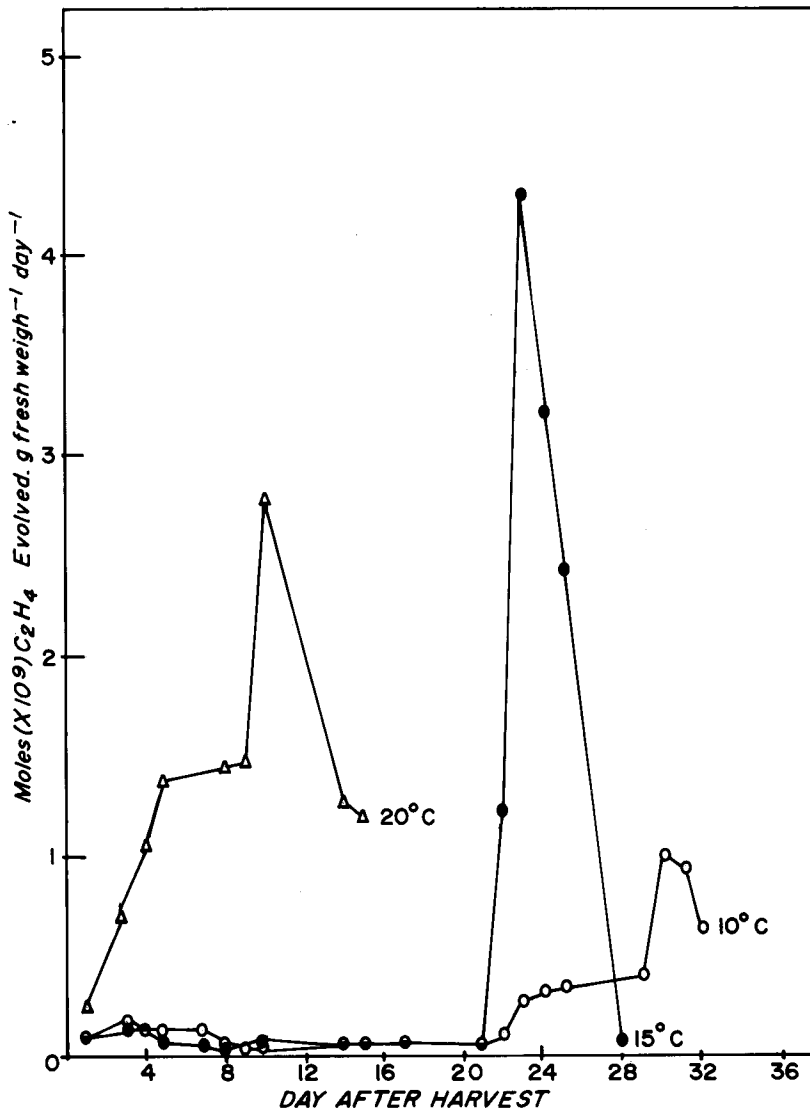
Figure 3. The effect of temperature on ethylene evolution in papaya during ripening.

the fact that the fruit can be stored in an atmosphere enriched with carbon dioxide (or with reduced oxygen) for extended periods of time without sacrificing fruit quality.

A dry environment slightly hastens the ripening process *(Fig. 6)*. Humidities in the respiration chamber would normally approach 100%, and all that adding water-saturated filter paper does is to ensure this. Therefore our experiments really only show the difference between saturated water vapour and no water vapour, but they do show that peak ethylene production was lower in the dry treatment than in the wet. A plausible explanation for this phenomenon was advanced by WILKINSON (1970). He reasoned that since the permeability of fruit skin to gaseous exchange decreases rapidly when evaporation takes place, the effect of early water loss may be a physical one resulting in restricted ventilation of the intercellular spaces, and it might induce a sluggish metabolism. Nevertheless, the fruit in the dry environment ripened satisfactorily, whilst those in the wet condition were overcome by fungus (see *Table 1*). During the experimental period (12 to 15 days), papayas maintained in a dry condition lost 17.5% of

TABLE 1. CHARACTERISTICS OF SUNRISE SOLO PAPAYAS STORED UNDER DIFFERENT CONDITIONS

Condition	Time of Climacteric (day) as measured by —		Maximum Glucose Content Obtained (% fresh weight)	Fruit Appearance and Taste at the Climacteric	See also
	CO_2	C_2H_4			
30°C	—	—	—	Fungal attack, fruit rotten	—
20°C	7	10	4.1	Greenish-yellow (partially ripe)	*Figs. 2,3 & 7*
15°C	22	23	4.3	Green with yellow patches (partially ripe)	*Figs. 2,3 & 7*
10°C	29	30	4.1	Yellowish-green (partially ripe)	*Figs. 2,3 & 7*
4°C	No climacteric after 52 days		—	Green	—
CO_2– removed (20°C)	—	4	—	Greenish-yellow, taste satisfactory (10 d later)	*Fig. 4*
C_2H_4– removed (20°C)	7	—	—	Yellowish-green, taste satisfactory (5 d later)	*Fig. 5*
100% Humidity (20°C)	—	10	—	Yellow, with fungus (4 d later)	*Fig. 6*
0% Humidity (20°C)	—	7	—	Yellow, taste satisfactory (7 d later)	*Fig. 6*

their original fresh weight, while these in a humid environment lost only 2.8% (w/v). Normally incubated papaya lost amounts similar to those in the humid treatment.

Unlike the analyses mentioned above, determination of glucose is a destructive procedure. For this reason, simple logistics precluded the use of the normal storage chambers in this study. Accordingly, we developed a storage technique using plastic bags sealed at the top, but with small ventilation holes in the side. Many of these were incubated at each of 10°C, 15°C, and 20°C and the glucose content determined on the days shown in *Fig. 7*. Initially, those papayas stored at 20°C had the highest glucose content when compared to those stored at lower temperatures. Glucose contents continued to rise until about the tenth day, after which they began to fall at 20°C, and 15°C.
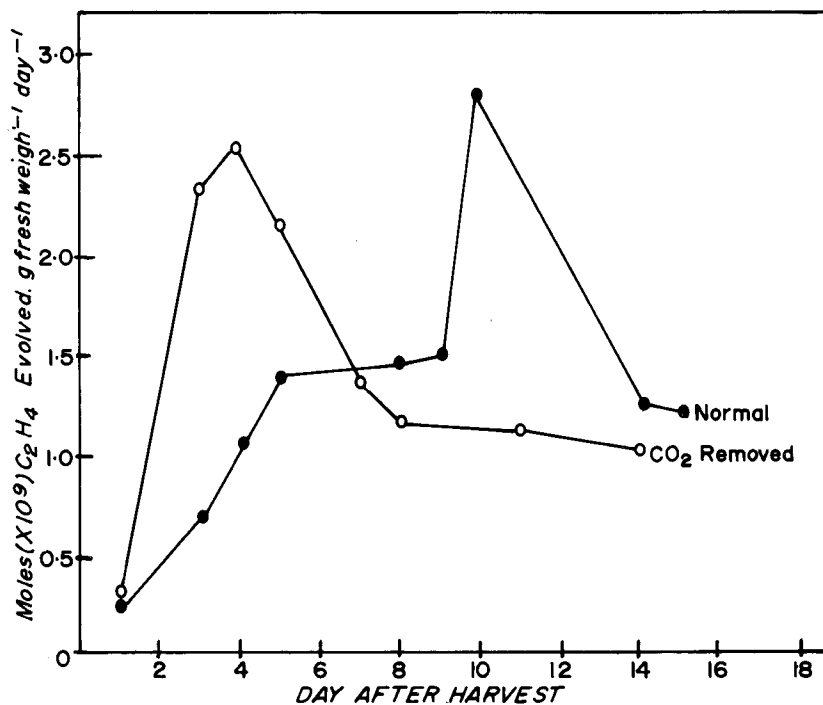
Figure 4. The effect of removing CO_2 from the storage chamber on ethylene evolution in papaya during ripening.

DISCUSSION

The results of the various treatments on the Sunrise Solo papayas showed that its behavioural pattern to the different storage conditions was quite similar to that of most fruits However there are also distinct differences. BIALE, YOUNG and OLMSTEAD (1954) thought that in most fruits, the ethylene peak occurred prior to the respiration peak. In the case of the Sunrise Solo papaya, the carbon dioxide peak preceded the ethylene peak (see *Table 1*). NAZEEB (1976) reported a reverse in the trend for the Bentong and Taiping variety of papayas subjected to similar storage conditions.

The optimum ripening temperature (i.e. the range of temperatures within the detached fruit ripens to a state similar to that ripened normally on the tree) for the Sunrise Solo papayas was found to be around 20°C. At this temperature the fruits take about ten to twelve days to ripen. AKAMINE (1966) however found that "Solo" papayas did not ripen normally when stored at 22.8°C (73°F) but those stored at 25°C (77°F) did. At temperatures above 25°C, the ripening time was very much shortened and so was storage life. The storage life was much longer at temperatures below 15°C. Unfortunately no transfer experiments were done to determine whether the fruits stored at temperatures below 15°C could be made to ripen normally or not. However NAZEEB (1976) reported that the Taiping and Bentong varieties of the papaya became damaged by 'Chilling' at temperatures below 15°C. An even higher chilling temperature for "Solo" papayas (22.8°C) was reported by AKAMINE (1966).

Removal of carbon dioxide ·from the storage chamber hastened the onset of the climacteric and ripening to a greater extent than in the case where carbon dioxide was allowed to accumulate (see *Table 1*). On the other hand the removal of ethylene had no apparent effect
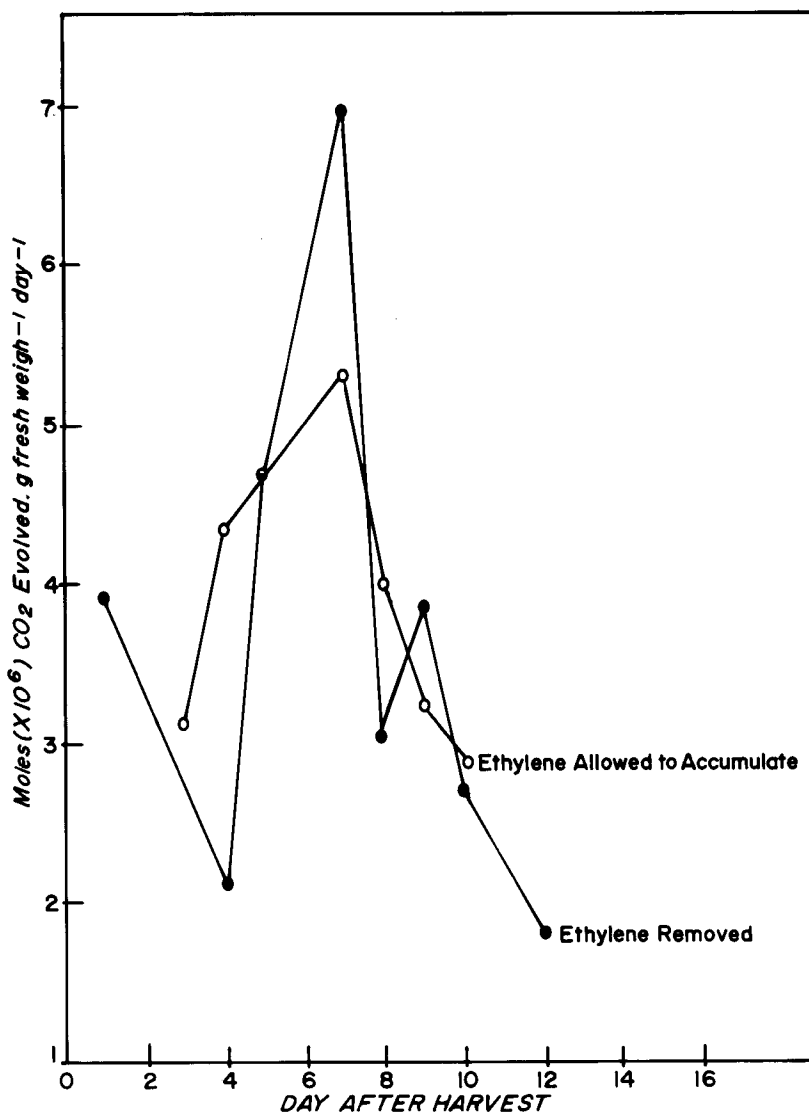
Figure 5. The effect of removing C_2H_4 from the storage chamber on respiration in papaya during ripening.

on the onset of the respiratory climacteric (see *Fig. 5*) and ripening. This behaviour is again peculiar since many fruit physiologists have shown that most fruits remain unripe for a much longer period when ethylene in the storage environment is removed. FORSYTH, EAVES, and LIGHTFOOT (1969) stored McIntosh Apples *(Malus pumilo)* for 189 days at 33°C without any loss in firmness and quality by removing ethylene from the storage chamber. SCOTT, BLAKE, STRACHAN, TUGWELL and McGLASSON (1971) found that by storing bananas in polyethylene bags (which absorb ethylene), the fruits could be made to remain green for eight to eighteen days at room temperature whereas the control fruits had ripened by then.

As for the effect of humidity, it was found that the climacteric was induced earlier in a drier environment (see *Fig. 6*). The fruits stored at high relative humidities took slightly longer to ripen but became easily infected with fungus. Fruits stored at very low relative humidities
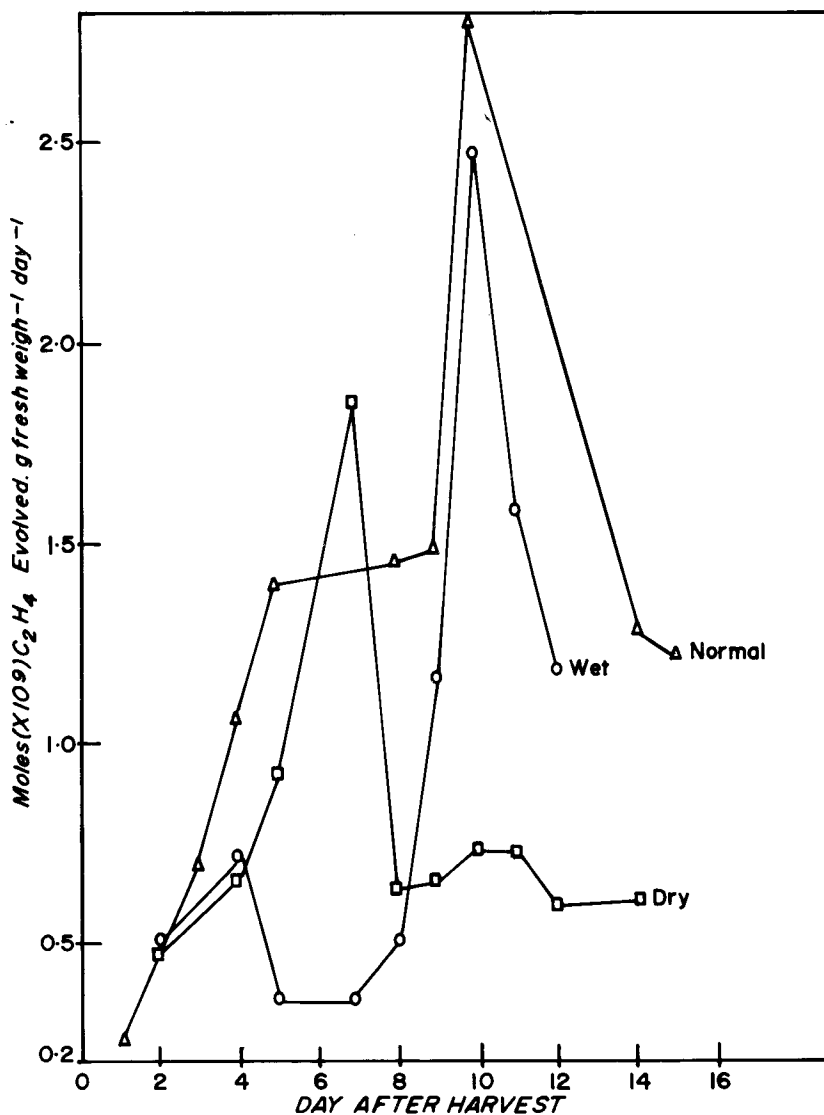
Figure 6. The effect of relative humidity on ethylene evolution in papaya during ripening.

were more resistant to fungal infection. However in both cases, the storage life was found to be less than fourteen days (see *Table 1*).

Monitoring the changes in glucose levels of Sunrise Solo papayas with storage times gave the trend as depicted in *Fig. 7* and *Table 1*. At all temperatures studied, the level of glucose steadily increased with time corresponding to an increasing state of ripening and then fell (due to overripeness and fungal attack). Maximum glucose levels were reached after ten days in storage for fruits stored at 15°C and 20°C while those stored at 10°C took a very much longer time. These changes in glucose levels exhibited by ripening Sunrise Solo papayas are similar to most fruits as BRADY, O'CONNELL, SMYDZUK, and WADE (1970) found for bananas and KRISHNAMURTHY and SUBRAMANYAM (1973) found in mangoes.
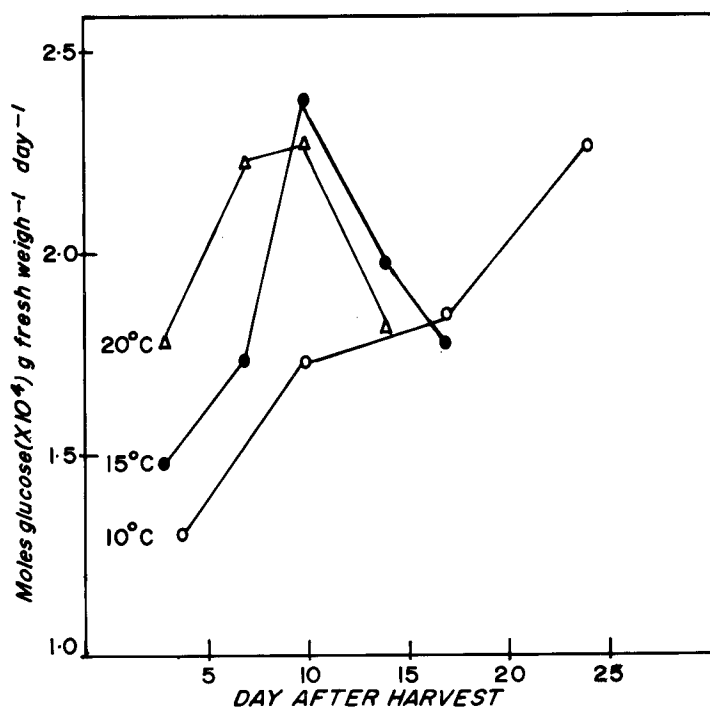
Figure 7. The effect of temperature on glucose content of papaya during ripening.

ACKNOWLEDGEMENTS

SUMMARY

An investigation was made into factors affecting the ripening of harvested papaya fruits. A temperature of about 20°C was optimal both for inducing ripening and for satisfactory storage of the fruits. Temperatures above this made the fruit susceptible to fungal attack, while at lower temperatures the onset of the climacteric was delayed, but "Chilling injuries" were manifested. Room temperature (28°C to 32°C) was particularly unsuitable for fruit storage. Removal of ethylene or water from the storage atmosphere had no apparent effect on the onset of the climacteric, while removal of carbon dioxide hastened the process. The climacteric of fruits in water saturated atmospheres was only slightly delayed. Glucose levels increased as the fruits ripened. but dropped with prolonged storage.

Recommended storage conditions are: temperature 20°C, humidity about 50% and with slightly elevated carbon dioxide levels if possible. Under these conditions fruit harvested 80 to 90 days after anthesis (and still very green) will ripen satisfactorily and can be kept for seven to fourteen days.

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