

# The Effect of Tonicity and Metabolic Inhibitors on Respiration and Ripening of Avocado Fruit Slices<sup>1</sup>

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## ABSTRACT

The ripening of avocado (*Persea americana* Mill.) fruit slices was inhibited whether they were floated in water or in buffered aqueous 0.3 M mannitol, 0.25 M KCl, and sucrose. There was no evidence to support the contention that ripening occurred when the tonicity of the bathing medium was increased. Decreased gaseous exchange is considered to be a major cause of this inhibition because by utilizing a technique that afforded better aeration, slices could be water infiltrated and still ripen normally. Metabolic studies on the ripening of slices using this method indicated that several metabolic inhibitors, malonate, cyanide, acetaldehyde, dinitrophenol, and fluoride did not prevent ripening, but that their effect on the respiration pattern was marked. This technique provides a suitable way to study control of ripening at the tissue level.

Despite inherent problems and weaknesses of tissue slice studies (21-23) this technique has been recommended as the best means to determine the events that occur during ripening of fruits (2, 19). It has been used for ripening studies in bananas (7, 18, 21), tomatoes (15, 16, 22), avocados (3, 19), and apples (8, 9). These studies usually involve incubating the fruit slice in an aqueous medium of the test substance. Floating the fruit slices in water is known to cause, among other things, decline in respiration (10, 20), impairment of ethylene production (9, 10), swelling (3, 8), and leakage of cell contents (3, 7, 22). Nowhere is the deleterious effect of water more pronounced than in avocado slices. When the slices are floated in water, they either do not ripen (23), or their ripening is markedly delayed (3). Several reasons have been proposed to account for the failure to ripen in water: it has been suggested that the failure may be due to a hydrophobic inhibitor which cannot escape readily into the water phase (23), the slower diffusion of O<sub>2</sub> (20), or the adverse effects resulting from lower tonicity of the surrounding water (3, 22). In view of this inhibitory effect of water on the ripening of the slices, it has not been possible to test the effect of soluble metabolites on the ripening process.

This paper describes a method of studying the effects of solutes on the normal ripening process and reports the effects of H<sub>2</sub>O and other aqueous media of different tonicity on ripening of avocado fruit slices. In addition, it describes the effect of

metabolic inhibitors on the normal respiration and ripening of the slices.

## MATERIALS AND METHODS

**Water and Tonicity Experiments.** In this study the incubation media whose effects were to be tested consisted of moist air, H<sub>2</sub>O, 0.3 M mannitol, 0.25 M KCl, and 0.4 M sucrose. The latter four media were adjusted to pH 6.5 with 0.15 M potassium phosphate buffer, and 25 µg/l streptomycin sulfate were added as a safeguard against microbial infection.

One avocado (*Persea americana* Mill.) fruit of the Hass variety was selected and its surface was sterilized initially by rubbing with 70% (v/v) ethanol and followed by washing for 5 min in 1:3 dilution of Clorox (sodium hypochlorite 1.9 w/v available chlorine) as outlined by Palmer *et al.* (21). With the aid of a cork borer and razor blade, slices 1 cm wide and 1 mm thick were prepared. The slices were rinsed for 2 min in sterile H<sub>2</sub>O, dried gently with filter paper, and put in groups of four (weighing 0.8-0.9 g) per Warburg flask. There were four flasks per treatment, each containing 3 ml of the treatment medium. For moist air, the flasks contained only moist filter paper. Respiration was measured at 20 C using a Gilson respirometer. The CO<sub>2</sub> produced was trapped by 3 M KOH in the center well of the Warburg flasks. All equipment used was autoclaved, and tissue slicing was done in a transfer hood under aseptic conditions.

**Slice Thickness and Infiltration Experiments.** Under the same aseptic conditions as above 2, 4, 7, and 10 mm thick rectangular slices were cut from two uniform Hass fruits. Each slice was 2.5 cm long and, while the peel was left attached, the seed coat was cut away. Sterile water was vacuum infiltrated (500 mm Hg) into half the number of slices of each size for varying periods of 12, 20, 30, and 60 sec for the 2, 4, 7, and 10 mm thick slices, respectively. A period of 60 sec was allowed for water to move in the tissue. In this way, an increased weight of about 6% was achieved for all sizes. Incubation was carried out utilizing a technique developed by Laties (personal communication) in which a continuous flow of moisture, or any substance, to a tissue is facilitated by a strip of lens paper through the wick effect. Both uninfiltrated and infiltrated slices were wrapped singly in 3 cm wide strips of lens paper and placed individually in 150-ml jars with convex bottoms. The slice was not in contact with the medium but the tapering ends of the lens paper were dipped in 2.1 ml of water (or medium). Humidified air at 60 cc/min was passed through the jar, and its rate of respiration was followed by the infrared CO<sub>2</sub> gas analyzer (Beckman Model 215A) on a range of 0 to 0.06% CO<sub>2</sub>.

**Inhibitor Experiments.** All inhibitor solutions and the water control were adjusted to pH 6.8 with 0.15 M potassium phosphate buffer. The extent of the participation of the tricarboxylic

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acid cycle in avocado slice respiration was tested by 20 mM malonate and 10 mM acetaldehyde. The latter was used on the hypothesis that it regulates the tricarboxylic acid cycle in potatoes (11, 13). The role of the glycolytic pathway was tested by 1 mM NaF, and the electron transport chain was tested by 0.1 mM dinitrophenol and 10 mM KCN.

Tissue slices 4 mm thick were prepared from a single Hass fruit. Infiltration and incubation of the slices including the determination of respiration were carried out as described previously.

## RESULTS

The respiration patterns and ripening of 2 mm thick avocado fruit slices floated in various media are shown in Figure 1. Irrespective of the type of the medium, all slices respired at a higher rate (100–120 ml CO<sub>2</sub>/Kg·hr) than intact fruits (30–40 ml CO<sub>2</sub>/Kg·hr). After a brief decline in respiration, the slices in moist air passed through a distinct climacteric rise. They softened within 3 days and attained normal ripe texture and flavor. Slices in H<sub>2</sub>O and the media of higher tonicity (mannitol, KCl, and sucrose) showed no climacteric rise in respiration, even after 12 days, while matched intact fruits ripened in 5 days. The slices in these aqueous media were still hard and showed a downward trend in respiration towards the end of the experiment. Leakage of cellular material as evidenced by darkening and turbidity of the media occurred in all treatments, but to a lesser degree in mannitol, KCl, and sucrose. The respiration pattern of the slices showed no signs of the phenomenon of induced respiration.

The respiration patterns of uninfiltated slices of various thicknesses are shown in Figure 2a. After the initial injury response, the rate of respiration for all sizes stabilized at levels bearing no definite relation to thickness. However, the rate of respiration at the climacteric was influenced by size: the thinner the slices, the higher the peak respiration. In infiltrated slices, Figure 2b, respiration was greatly reduced soon after infiltration. This was taken to be due to the presence of H<sub>2</sub>O in the intercellular spaces. After recovery from the effect of infiltration, the slices resumed respiration at a rate lower than that of uninfiltated slices of equal thickness. The rate of respiration

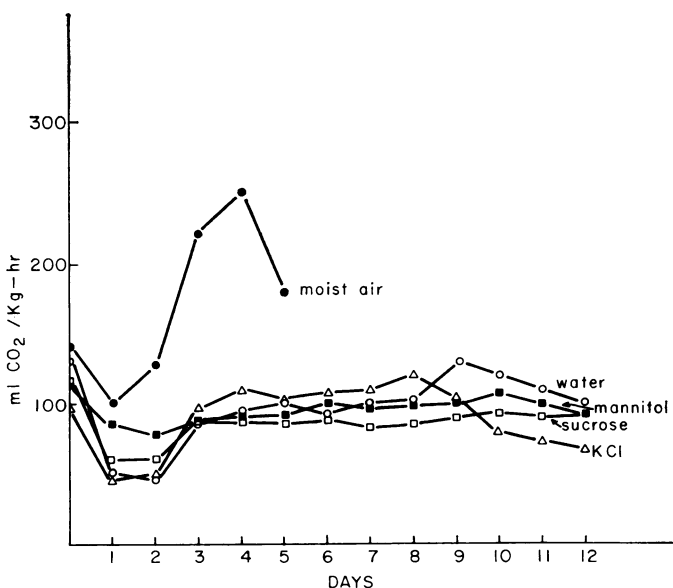


FIG. 1. Respiration pattern of 1 mm thick Hass avocado fruit slices incubated in 0.3 M mannitol, 0.4 M sucrose, or 0.25 M KCl at 20 C. Each point represents the average of 16 slices.

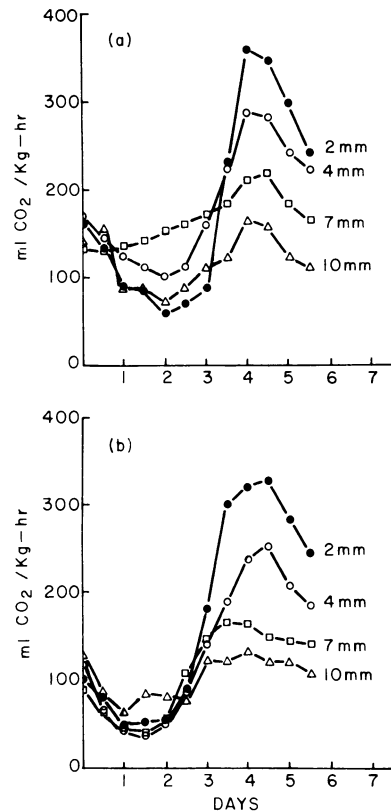


FIG. 2. Respiration pattern of (a) uninfiltated; (b) infiltrated 4 mm thick Hass avocado fruit slices at 20 C. Each point represents the average of four slices.

at the climacteric for these infiltrated slices was also inversely proportional to size, though the heights of the peaks were relatively lower than those for uninfiltated slices. Leakage of cell contents was practically negligible and within 7 days all slices had softened.

The lens paper facilitated a continuous supply of moisture through wick effect to the tissue without hampering gaseous exchange. From observations here, the 4 mm thick size was judged to be the most suitable for further slice studies because it was easy to cut uniformly and to position in the respirometer. Besides, it showed a reproducible climacteric and a single slice gave sufficient respiration to be determined with high accuracy.

The effect of applying the various metabolic inhibitors continuously to the slice is shown in Figure 3. When compared to the control, acetaldehyde and, surprisingly, malonate stimulated respiration during both preclimacteric and climacteric stages. The magnitude of respiration for these treatments during the climacteric was as high as that of uninfiltated slices (Fig. 2a). Sodium fluoride had no effect on preclimacteric respiration but stimulated climacteric respiration. It also appeared to cause a small delay in the onset of the climacteric. Dinitrophenol stimulated respiration through all stages of ripening, and KCN had no effect on preclimacteric respiration, although it depressed the climacteric respiration.

None of the inhibitors prevented softening. Within 7 days, all slices were soft to the touch, and except for the slight lag in NaF, the onset of the climacteric was uniform.

## DISCUSSION

The results of the experiments conducted here reinforce the previous view that when avocado slices are floated in H<sub>2</sub>O, their

ripening is inhibited (23). The results did not confirm the observation that ripening of avocado slices occurred in water or in aqueous media of higher tonicity as claimed by Ben Yehoshua (3). Such media of higher tonicity (0.3 M mannitol, 0.25 M KCl, and 0.4 M sucrose), as tested here, were only effective in reducing leakage but failed to bring about softening when the slices were free from microbes. In fact, the respiration pattern of the slices in these media were not significantly different from that of slices floated in water (Fig. 1). Simons and Bruinsma (22) have shown that when discs of tomato pericarp were placed in contact with water, color changes of the skin were delayed. When solutions of higher osmolarity were used, which allowed for more leaching, color formation was further delayed. In the case of the avocado slice, the inability to ripen does not appear to be influenced by changes in osmotic pressure of the surrounding medium.

While it has been claimed that reduced gaseous exchange was not the major reason for lack of ripening of the slices (3, 23), these results suggest that restricted  $O_2$  supply may still be a main cause of the failure (20). Figure 2b shows that slices can be water-infiltrated yet ripen normally; the main difference between these and direct floatation is improved aeration. The enveloping lens paper did not appear to act as a barrier to gaseous exchange. Therefore, the  $O_2$  requirement of some crucial ripening step(s) may be greater than can be obtained from an aqueous environment.

Respiration of slices in preclimacteric state is essentially unaffected by slice thickness. During the climacteric thinner slices respire at a higher rate suggesting that  $O_2$  may be limiting as was the case for "induced respiration" of potato slices (14). In contrast to other tissues, little if any, induced respiration occurred upon making slices of avocado (11, 18).

The procedure used in our experiments, which includes infiltration followed by continuous feeding of the test substances, provided a means for the studying of ripening in avocado fruit slices. At the concentrations used none of the inhibitors prevented ripening, but their effect on respiration of the slices was pronounced. We were unable to confirm the work of Ben Yehoshua (3) who reported that  $10^{-4}$  M DNP<sup>3</sup> prevented ripening of avocado slices. In a similar study with banana slices, McGlasson *et al.* (18) found that, within limits, ripening of the slices was not prevented by a number of metabolic inhibitors including DNP. Young and Biale (24) showed that ATP was formed in avocado tissue slices even in the presence of DNP and that phosphorylation actually increased with ripening. Milder *et al.* (19) found that DNP stimulated respiration in preclimacteric avocado slices in aqueous medium but not in climacteric peak slices. We find that when slices are kept in air, their respiration in both climacteric and preclimacteric state is considerably stimulated. This response to DNP confirms conclusions drawn by Young and Biale (24) and Lance *et al.* (12) that DNP does not initiate the climacteric as the respiration climacteric and associated softening occurred at the same time as untreated slices.

In aqueous media, KCN stimulated respiration of avocado slices (17). Slices in air however appear to be either unaffected or slightly inhibited in both preclimacteric and climacteric respiration. While a Cyt oxidase system is known to exist in avocado mitochondria, these data confirm that there is a cyanide insensitive oxidative pathway operating at all stages of ripening.

Likewise the tricarboxylic acid cycle is known to operate in avocado fruit mitochondria (12), and these particles are sensitive to malonate. Slices in air, however, show an accelerated

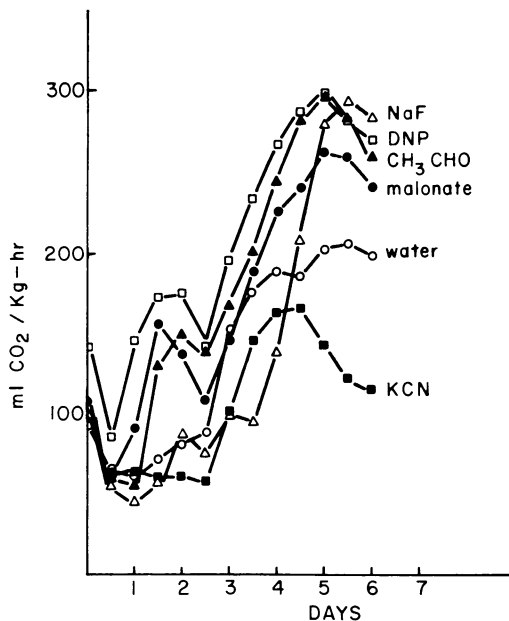


Fig. 3. Effect of metabolic inhibitors 1 mM NaF, 0.1 mM DNP, 10 mM  $CH_3CHO$ , 20 mM malonate, and 10 mM KCN on the respiration pattern of 4 mm thick Hass avocado fruit slices at 20°C. Each point represents the average of four slices.

respiration (Fig. 3) in the presence of malonate. They also show increased respiration prior to the climacteric which appeared to be like induced respiration (14). It seems however here to be different as typical induced respiration is completely inhibited by malonate. Acetaldehyde, which is presumed to be a suppressor of the tricarboxylic acid cycle (14), did not inhibit avocado slice respiration at any stage and, in fact, stimulated it considerably. The effects of both malonate and acetaldehyde suggest another oxidative system which must be active and in fact stimulated in the presence of tricarboxylic acid cycle inhibitors. A malonate-insensitive respiration in potato slices has been attributed to a fatty acid oxidative system operating independently of the tricarboxylic acid cycle through  $\alpha$ -oxidation of long chain fatty acids (11).

It is known that despite the abundance of lipids, carbohydrate and not lipid is utilized in avocado respiration (5). Therefore, the slight delay caused by NaF by inactivating enolase may reflect on the importance of the glycolytic pathway in mobilizing this reserve.

These experiments show that the deleterious effects of water on avocado slice ripening can be minimized by improved method of incubation. They also show that despite the presence of metabolic inhibitors normal respiration can occur indicating the existence of alternate oxidative pathways through all stages of the climacteric.

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<sup>3</sup> Abbreviation: DNP: dinitrophenol.

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