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Temperature Sensitivity of Avocado Fruit in Relation to C_2H_4 Treatment

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ABSTRACT. Avocado fruit *(Persea americana* Mill. 'Fuerte') were stored in air with or without C_2H_4 treatment (100 ppm) at 6°, 9°, 12°, 14°, 16°, 20°, 24°, 27°, 30°, and 34°C. During the storage period, respiration was measured by an automated continuous gas flow system. Fruit stored in air for more than 20 days at 6° developed chilling injury as indicated by gray discoloration of the mesocarp tissue. At high temperatures (30°, 34°), avocado fruit ripened abnormally, showed considerable surface pitting, and had poor flavor. When fruit were stored with 100 ppm C_2H_4, tissue discoloration was severe below 12°, which implied that chilling sensitivity of avocado fruit increased with C_2H_4 treatment. Fruit, whether stored with C_2H_4 or not, showed breaking points around the same temperature region on an Arrhenius plot, suggesting possible involvement of other mechanisms in addition to phase changes of membrane lipid components.

Each biological system has its optimum temperature range. If temperatures are significantly increased or reduced, physiological disorders may occur which cause injury to cells. In the storage and transportation of fruit and vegetables, the extent of injury is a function of the magnitude of temperature extremes and the duration of damaging temperatures (8). Generally, high temperature injury occurs above 30°C, and a low temperature disorder, or chilling injury (CI), appears below about 12°, depending upon the commodity.

Symptoms of high temperature injury of avocado fruit include surface pitting, necrosis, improper ripening, susceptibility to decay, and undesirable flavor. The most significant symptom at low temperature is the internal graying of mesocarp tissue, and the darkening of vascular strands. Fruit also ripen abnormally and are susceptible to decay.

CI is believed to be due to a phase change of membrane lipid components below a critical temperature (19). Many investigators have demonstrated the phase transition of

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lipids, showing a break or breaks on Arrhenius plots of various biological activities such as succinate oxidation (10, 11, 22), ethylene production (6, 16), and respiration (2, 18). The break on Arrhenius plots has been attributed to a change in the physical state (a phase change) of membrane components (13). The phase change is thought to be a factor in increasing permeability and causing conformational alteration of membrane-associated enzymes which shift activation energy due to inhibition. This shift results in dysfunction, imbalance in metabolism, and the accumulation of metabolites which cause injury to cells (14).

Therefore, fatty acid components play an important role in chilling sensitivity. In general, the ratio of unsaturated to saturated fatty acids in membrane lipids is much higher for the chilling-resistant species than for the chilling-sensitive ones, because unsaturated fatty acids solidify at a lower temperature than saturated fatty acids (5, 15). Intermittent warming processes and the treatment of free radical scavengers, such as sodium benzoate and ethoxyquin, were reported to increase fatty acid unsaturation and thus to alleviate the chilling symptoms in cucumber and peppers (21).

Temperature effects on intact fruit respiration in connection with C_2H_4 treatment have not been studied extensively. To obtain more information on temperature and C_2H_4 effects during storage, we studied the respiratory climacteric pattern and ripening of avocado fruit with or without C_2H_4 treatment. Arrhenius plots of the respiration of intact fruit were made in an attempt to relate the phase change of membranes with evidence of CI.

Materials and Methods

Mature 'Fuerte' avocado fruit of uniform size were harvested from the Univ. of California South Coast Field Station and stored at different temperatures over the range of 6° to 34°C. Each individual fruit was placed in a respiration jar, and humidified air was provided continuously at about 70 ml/min by bubbling through distilled water. Throughout the storage time, respiration rates and ethylene production rates were measured by an automated continuous gas flow system. The system consisted of an infrared gas analyzer (Beckman, Model 215B) for CO_2 measurement, a gas chromatograph (Varian Aerograph Series 1400), a custom-built system controller, and a microcomputer (HP 9825) for data processing.

Half of the sample lots were treated during storage with 100 ppm C_2H_4 regulated by flow meters. External and internal conditions of fruit were examined when fruit became soft. Results are the average of 4 replications for each treatment.

Results and Discussion

The respiration rate curves in air at various temperatures are shown in Fig. 1. Typical climacteric patterns occurred between 12° and 27°C. Over this temperature range, the respiratory rate at the climacteric peak increased as temperatures increased. Also, the respiration rates for initial and preclimacteric minima were increased at high temperatures. At 30°, there was a preclimacteric minimum and no climacteric peak. Fruit did not ripen properly, and the experiment was discontinued at day 5 because fruit were obviously injured. Zaubermann et al. (24) also observed similar symptoms of high temperature injuries around 30°.

A slight increase in respiration rates occurred at 9°C, and fruit softened after about 4 weeks. The exact time of the climacteric peak was not obvious because the slight increase in respiration rates remained until fruit softened. At 6°, fruit remained firm even for 6 weeks after which the experiment was terminated. These fruit began to show typical CI after 3 to 4 weeks of storage but did not soften.

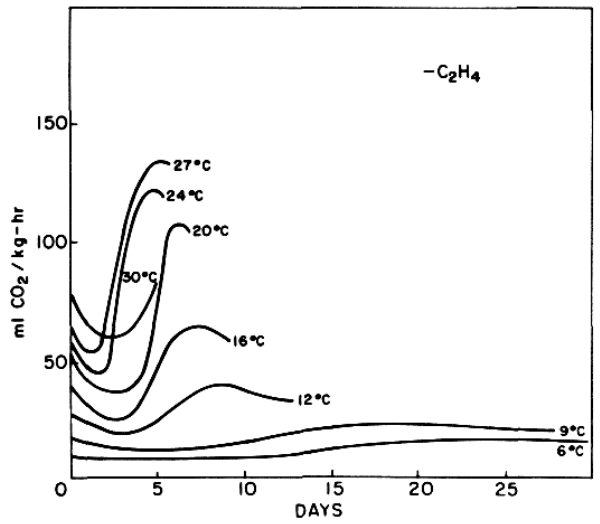

Fig. 1. Respiration rates of 'Fuerte' avocado fruit during storage in air at different temperatures. Each curve represents respiration rates of a single fruit out of 4 replications.

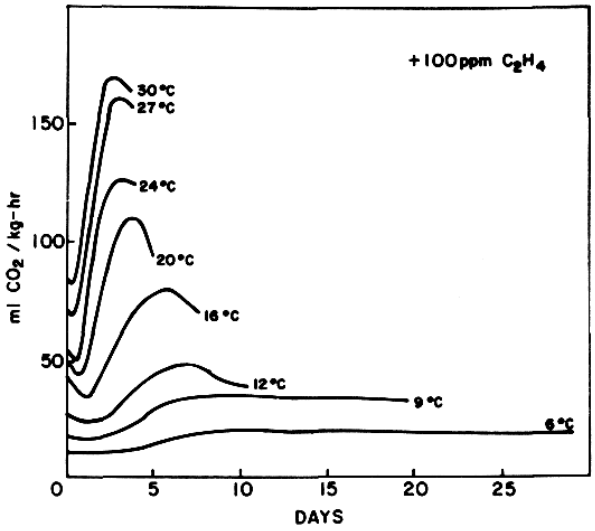

Fig. 2. Respiration rates of 'Fuerte' avocado fruit treated with 100 ppm C_2H_4 during storage at different temperatures. Each curve represents respiration rates of a single fruit out of 4 replications.

Respiration rates under C_2H_4 treatment at various temperature conditions are shown in Fig. 2. Climacteric peaks were obvious in the 12° through 30°C range. At 34°, fruit ripened abnormally, produced a putrid odor, and were invaded rapidly by fungi. Fruit softened over the range of 9° through 30°; however, CI was apparent below 12° and was severe at 9° after only 2 weeks.

Table 1. Climacteric and ripening conditions of 'Fuerte' avocado fruit at various temperatures with or without 100 ppm C_2H_4 treatment during storage.

Temperature (°C)	C_2H_4 treatment	Occurrence of climacteric peak	Ripening condition
34	−	No	No ripening
	+	No	No ripening
30	−	No	No ripening
	+	Yes	Fair
27	−	Yes	Fair
	+	Yes	Good
24 ~ 14	−	Yes	Excellent
	+	Yes	Excellent
12	−	Yes	Good
	+	Yes	Slight CI[z]
9	−	No	Good
	+	No	Severe CI
6	−	No	No ripening, severe CI
	+	No	No ripening, severe CI

[z]CI = chilling injury.

The occurrence of the climacteric and ripening responses in air or air plus C_2H_4 at different temperatures are summarized in Table 1. It is clear that 100 ppm C_2H_4 increased CI sensitivity. Since ethylene generally is present at variable concentrations in fruit storage units, the variation in critical chilling temperatures reported in the literature logically could be attributed, at least in part, to C_2H_4. Such a conclusion is supported by early studies (4).

Campbell and Hatton (3) reported that CI was variable and could occur in avocados within 2 weeks at temperatures as high as 10°C. While they concluded that preharvest factors might contribute to susceptibility, postharvest factors such as gas components and C_2H_4 contamination around storage units could

have additional significant effects on CI. Removal of C_2H_4 from the atmosphere maintained avocado fruit quality (9, 23) and also reduced the incidence of core browning of apple fruit (7). Hypobaric conditions which accelerate diffusion of C_2H_4 reduce O_2 levels, extended the storage life of produce and minimized the effect of C_2H_4 (1, 17). Controlled atmosphere storages also may reduce CI, because low O_2 and high CO_2 partial pressures retarded respiration and C_2H_4 production (20).

The effect of temperature on respiration rates also is shown on an Arrhenius plot (Fig. 3). The climacteric peak and the preclimacteric minimum of respiration rates are convenient points for studying the response of fruit to temperature. The Arrhenius plot of climacteric peak rates shows 2 transition temperatures at around 20° and 12°C whether or not fruit are treated with C_2H_4. Fruit treated with C_2H_4 have higher rates at climacteric peaks than fruit in air. In contrast, the plot for the preclimacteric minimum shows 1 transition temperature around 12°. The preclimacteric minimum of the treated fruit was not included in the plot because this point is not physiologically significant.

Table 2. Activation energy of intact fruit respiration rates at 3 temperature ranges from 'Fuerte' avocado fruit at 2 climacteric stages.

Temperature (°C) range	Activation energy (kcal/mole)		
	Preclimacteric minimum	Climacteric peak	
	$-C_2H_4$	$-C_2H_4$	$+C_2H_4$
High (20–30)	13.4	6.8	8.4
Middle (12–20)	13.4	18.1	14.7
Low (6–12)	19.6	26.8	26.0

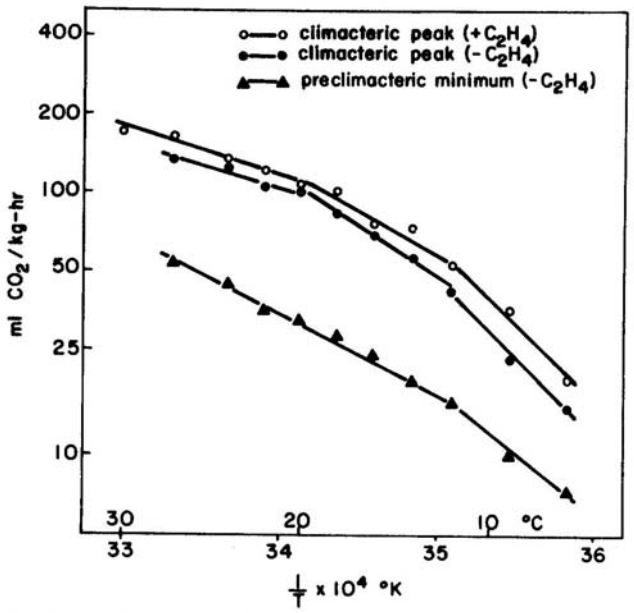

Fig. 3. Arrhenius plot of respiration rates at 2 stages of ripening (climacteric peak and preclimacteric minimum) of 'Fuerte' avocado fruit. Transition points were determined by linear regression analysis. LSD (5%): climacteric peak ($+C_2H_4$); 15.5, climacteric peak ($-C_2H_4$); 11.9, preclimacteric minimum ($-C_2H_4$); 4.5.

Changes in the activation energy (Ea) calculated from the slopes of the figure is shown in Table 2. The Ea in the low temperature range (6° ~ 12°C) is higher (6 kcal/mole for the preclimacteric minimum and 18 ~ 20 kcal/mole for the climacteric peak) than the Ea in the high temperature range (20° ~ 30°).

Kosiyachinda and Young (11) also observed a breaking point for the preclimacteric minimum and 2 breaks for the climacteric peak of membrane-associated succinoxidase activity of avocado mitochondria. They interpreted the high temperature transition (20°) as a change from a "liquid state" to a "solid disordered state"; and the low temperature transition (12°) as a change from a "solid disordered state" to a "solid ordered state" of membrane lipids. Yamaki and Uritani (22) also reported that succinoxidase activity of sweet potato roots showed 2 transition temperatures on an Arrhenius plot. They suggested that the transition induced breakage of hydrophobic interactions between membrane lipid and protein complex and resulted in "cold denaturation."

Time from picking to the climacteric respiratory peak is influenced strongly by temperature. The days from picking to the climacteric peak of fruit treated with 100 ppm C_2H_4 during storage at various temperatures is shown in Fig. 4. Results took the form of 2 linear lines with dramatically different slopes above and below 20°C. This change in slope coincides with the 20° break on the Arrhenius plot of the climacteric peak respiration and is consistent with the idea that temperatures affect respiratory rates and softening by causing phase changes in membrane components.

We have found that C_2H_4 increased the threshold temperature of CI and high temperature injury and that the Arrhenius plot of climacteric peak rates of intact fruit showed 2 transition temperatures. Under C_2H_4 treatment, there was a change in the ripening rate around 20° when the time for the climacteric peak was compared at different temperatures. We also suggest that removal of C_2H_4 from old storage atmosphere may be effective in reducing CI. Fruit stored in air plus C_2H_4 at low temperatures had more CI than fruit stored in air. Fruit, however, whether treated with C_2H_4 or not, showed breaking points on an Arrhenius plot around the same temperature (20° and 12°). This may suggest, at least in part, the possible involvement of other mechanisms in CI along with membrane lipid phase change. It would be interesting to examine soluble enzymes of fruit treated with C_2H_4 at chilling temperature. Increased metabolism of chlorogenic acid was reported at chilling temperature in peppers, sweet potatoes, and eggplant (12).

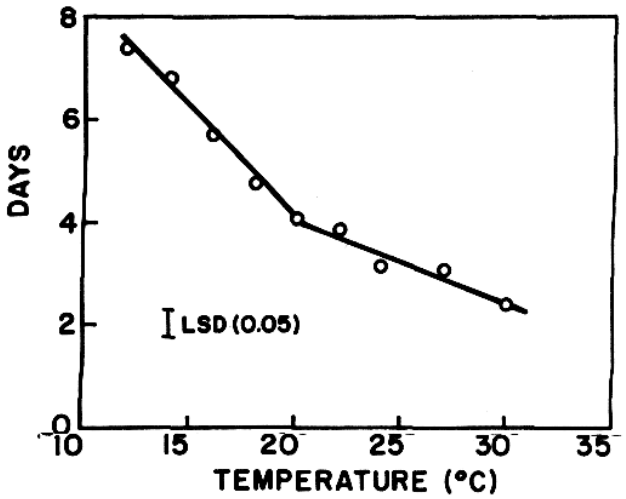

Fig. 4. Number of days to the climacteric peak of 'Fuerte' avocado fruit treated with 100 ppm C_2H_4 during storage at different temperatures.

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