Ethanol Inhibits Ripening of Tomato Fruit Harvested at Various Degrees of Ripeness without Affecting Subsequent Quality

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Additional index words. carbon dioxide production, climacteric, ethylene production, *Lycopersicon esculentum*, soluble solids concentration, titratable acidity

Abstract. Tomato fruit (*Lycopersicon esculentum* Mill., cv. Castlemart) were harvested at various degrees of ripeness and exposed to ethanol vapor at 0, 2, or 4, ml·kg⁻¹ in a 20-liter jar for 0, 2, 4, or 6 hours at 20C. Ripening was measured as changes in subjective color and in firmness and production of CO₂ and ethylene. The soluble solids concentration (percent), titratable acidity (percent), and pH were measured at the end of the storage period when the fruit were red-ripe. Ethanol's inhibition of ripening was not confined to mature-green fruit, but also inhibited reddening of breaker, turning, and pink fruits. Storage of mature-green fruit at 20, 15, or 12C after treatment with 0 or 2 ml ethanol/kg at 20C prolonged the delay in ripening for 5, 6, and 7 days, respectively, compared with controls. There was no reduction in the quality of these fruit when they were red-ripe, even though there was an 11-day difference between the time the 20C control and the 12C-treated fruit became red-ripe. An informal panel did not detect any differences in flavor between these control and ethanol-treated fruit that were red-ripe. Increasing the duration of exposure to ethanol vapors from 2 to 6 hours had a pronounced effect on ethylene and CO₂ production, but it did not significantly prolong the inhibition of ripening of mature-green fruit nor did it change their rate of softening during ripening. Increasing the temperature during exposure increased the effectiveness of ethanol, with the same level of inhibition produced by 6 hours at 20C, 4 hours at 25C, or 2 hours at 30C. Postharvest use of ethanol vapor to retard ripening may be a useful technique to extend the market life of tomato fruit.

Ethanol, a natural compound produced by plant tissue under anaerobic atmospheres, reversibly inhibits the ripening of mature-green tomatoes (Kelley and Saltveit, 1988; Saltveit, 1989; Saltveit and Mencarelli, 1988). Exposure to ethanol vapors for 4 to 6 h inhibits ethylene action and synthesis for ≥ 5 days before normal ripening continues. The biosynthesis of ethanol is a normal occurrence in the ripening of some fruit in air (Smock, 1944). Ethanol can accumulate in anaerobically stored blueberries (*Vaccinium corymbosum*) (Saltveit and Ballinger, 1983a), grapes (*Vitis rotundifolia* Mich. cv. Carlos) (Saltveit and Ballinger, 1983b), and pears (*Pyrus communis* L.) (Ke et al., 1990) without adversely affecting subsequent quality. Other alcohols also inhibit tomato fruit ripening, but ethanol is the least phytotoxic (Kelley and Saltveit, 1988). Ethanol in the holding solution of cut carnation (*Dianthus caryophyllus* L.) flowers inhibits the ethylene climacteric and delays senescence (Heins, 1980). As with tomato fruit, other alcohols were either not as effective or phytotoxic (Wu et al., 1992).

Since ethanol is an effective inhibitor of tomato fruit ripening, postharvest ethanol treatments could facilitate marketing of fruit harvested at more advanced degrees of ripeness than mature-green. In this series of experiments we investigated the inhibitory effect of ethanol on ripening of tomatoes harvested and exposed to ethanol vapors when mature-green, breaker, turning, and pink. We also studied the effects of temperature during exposure to ethanol vapors and during subsequent storage on tomato fruit ripening and quality.

Materials and Methods

'Castlemart' tomato fruit were hand-harvested at the mature-green, breaker, turning, and pink stages from plants grown at the Vegetable Crops Field Station in Davis, Calif., under normal cultural practices. Unblemished, uniformly shaped fruit with a mean fresh weight of ≈ 170 g were washed in a 0.26% sodium hypochlorite solution (1:20, v/v dilution of 5.25% commercial bleach to water), rinsed with sterile deionized water, and air-dried. Twelve fruit at each stage of maturity were weighed and placed in 20-liter glass jars at 20C.

Ethanol was applied at 0, 2, or 4 ml·kg⁻¹ fruit. Fruit were exposed to ethanol vapors by pipetting a measured amount of ethanol based on the fresh weight of the fruit in the jar onto a folded 10-cm-diameter Whatman no. 1 filter paper disk positioned in the bottom of a 100-mm-diameter petri dish. The jars were capped with a rubber stopper immediately after addition of the ethanol and left for 0, 2, 4, or 6 h. All the ethanol had evaporated from the filter paper and been absorbed by the fruit at the end of the usual 4-h treatment. At the end of the ethanol treatment, the rubber stoppers were removed and the fruit was kept either in the jars or in fiber paper trays at 20C until the fruit reached the red-ripe stage. Fruit were also treated with 0 or 2 ml ethanol/kg at 20, 15, or 12C and kept at 20, 15, or 12C. Some of the fruit treated and kept at 12C were transferred to 20C after 2 or 4 weeks. In all experiments, each treatment was replicated three times, and each experiment was repeated at least twice. Data were subjected to an analysis of variance (ANOVA) with a least significant difference calculated at $P = 0.05$ when ANOVA was significant.

Measurements of color, firmness, respiration, and ethylene production were taken every 2 to 3 days after the end of the ethanol treatment. Color development was visually scored using a scale in which mature-green = 1, breaker = 2, turning =

Received for publication 15 July 1991. Accepted for publication 13 Apr. 1992. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

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Abbreviations: SSC, soluble solids concentration; TA, titratable acidity.

3, pink = 4, light red = 5, and red-ripe = 6 (Saltveit, 1989), as based on illustrations adopted by the United Fresh Fruit and Vegetable Assn. and supplied by the John Henry Co., Lansing, Mich.

Firmness was measured as the amount of compression in millimeters when a fruit was subjected to a 500-g weight for 15 sec as described by Brady et al. (1983). Rates of CO₂ and ethylene production were calculated from measurements of the gas concentration that accumulated in closed 20-liter containers for multiple fruit samples or in 1-liter containers for single fruit after 1 h, as described by Saltveit (1989).

At the end of the storage period, i.e., when the fruit were red-ripe, the soluble solids concentration (SSC), pH, and percent titratable acidity (TA) were measured from a combined blended sample of four sections from four quartered tomatoes. SSC was read with a bench-top refractometer. TA was measured by titrating a 5-ml sample to pH 8.1 with 0.1 N NaOH. The percent acidity is expressed as citric acid.

Ethanol was assayed as described by Saltveit (1989). Pericarp tissue (≈ 1 g from each of four fruit) was weighed and homogenized for 10 sec in 5 ml of 1.8% (w/v) Ba(OH)₂/g fruit in a Sorvall homogenizer (Omni Corp., Waterbury, Conn.) at high speed. Five milliliters of 2.0% (w/v) ZnSO₄/g fruit was added and the solution homogenized again for 10 sec. The mixture was centrifuged at $3000 \times g$ for 3 min. A 30 μ l aliquot of the clear supernatant was added to a cuvette with 1.0 ml of a reaction mixture containing 1.3 mg NAD and 12.5 units alcohol dehydrogenase in 0.5 M potassium phosphate buffer (pH 9.0). Reduction of NAD was measured as changes in the absorbance of NADH at 304 nm. The ethanol concentration in the supernatant was calculated from a standard curve. The assay was sensitive to 5 μ g ethanol/g tissue. Recovery of ethanol-spiked tomato tissue was consistently >90%, and the extract did not interfere with the assay.

The rate of ethylene production vs. the maturity of fruit was measured to further study the inhibitory effect of ethanol on ripening of fruit of various degrees of ripeness. Each pair of fruit in two sets of 30 fruit each was matched on the basis of their similar rates of ethylene production. One set was treated with 4 ml ethanol/kg fruit for 4 h at 20C. Before treatment, and after 1 week, the ripeness score was visually estimated and the rate of ethylene production measured for all fruit in both sets.

Results and Discussion

Initial fruit maturity. Exposure to ethanol vapors at 2 or 4 ml ethanol/kg tissue for 4 h at 20C inhibited the ripening of mature-green (Fig. 1), breaker (Fig. 2), turning (Fig. 3), and pink (Fig. 4) tomato fruit. The highest concentration of ethanol was usually the most inhibitory. While ethanol inhibited the development of red pigmentation in all stages, it only slightly retarded the softening, as measured by deformation, of mature-green (Fig. 1) and breaker (Fig. 2) fruit, while it had no significant effect on the firmness of turning (Fig. 3) or pink (Fig. 4) fruit. Although not always statistically significant, ethylene production by fruit within a few hours of ethanol exposure was usually stimulated over the controls at all maturities. In other experiments, both ethylene and CO₂ production were stimulated during exposure of tomatoes to ethanol vapors (data not shown). In mature-green (Fig. 1) and breaker (Fig. 2) fruit, ethylene production subsequently declined below the control on day 3, and then increased 2- to 3-fold over that lowest rate by day 12. In contrast, ethylene

production by turning (Fig. 3) and pink (Fig. 4) fruit remained above the rate from untreated fruit.

The respiratory climacteric, as shown by changing rates of CO₂ production, is clearly seen during the ripening of control and treated mature-green fruit (Fig. 1) and by control breaker fruit (Fig. 2), but not by treated breaker fruit. Since the climacteric peak had already occurred, turning (Fig. 3) and pink (Fig. 4) fruit showed the postclimacteric decline in respiration. After an initial slight stimulation of CO₂ production in some of the treatments, treatment of fruit at all stages with ethanol vapors generally suppressed the CO₂ production rate. In mature-green (Fig. 1) fruit, the delayed climacteric was evident at both levels of ethanol, while in breaker fruit (Fig. 2), the 4 ml·kg⁻¹ treatment prevented even the slight climacteric rise evident in the 2 ml·kg⁻¹ treatment. In turning (Fig. 3) fruit, fruit from the 2 ml·kg⁻¹ treatment responded similarly to the control, while that in the 4 ml·kg⁻¹ treatment consistently produced less CO₂ than the control. While lower than the control for most of the time, ethanol-treated pink (Fig. 4) fruit exhibited rates of CO₂ production roughly the same as the controls at the beginning and end of the experiment.

If the inhibitory effect of ethanol on tomato fruit ripening was similar for different degrees of ripeness, e.g., for mature-green to pink fruit, then the slopes of regression equations relating color development to ethylene production should be parallel for ethanol-treated and control fruit. The initial measurements produced the regression equation: color score = $0.0 + 0.38$ (μ l ethylene/kg per h), with an r^2 of 0.79 (Fig. 5). Initial measurements were made for both groups of 30 fruit and were similar, so the values are only reported for the set that was subsequently treated with 4 ml ethanol/kg fruit. After 1 week at 20C, the control group had ripened and measurements produced the regression equation: color score = $3.1 + 0.42$ (μ l ethylene/kg per h), with an r^2 of 0.83. In contrast, the ethanol-treated fruit had not ripened as much, and measurements produced the regression equation: color score = $1.5 + 0.45$ (μ l ethylene/kg per h), with an r^2 of 0.85. The parallelism of the regression equations calculated from measurements of the fruit before treatment and the control fruit after 1 week (i.e., their similar slopes) indicates the uniformity of ripening in these fruit. Similarly, the parallelism between the regression equations for the initial measurements and the ethanol-treated fruit, and between ethanol-treated fruit and 1-week-old controls, indicates that the inhibitory effect of ethanol was similar for all stages of ripeness tested.

Treatment duration and temperatures. Increasing the time of exposure to 2 ml ethanol/kg fruit from 2 to 6 h for mature-green tomato fruit had no significant influence on the inhibitory effect of ethanol on color development or on the loss of firmness (Fig. 6). However, the duration of exposure had pronounced effects on the rates of ethylene and CO₂ production. While the rate of ethylene production was similar among the exposure times at the beginning and end of the experiment, increasing exposure times resulted in decreased rates of ethylene production between 3 to 8 days. Carbon dioxide production was initially the same for all treatments. It then decreased until day 3, then increased until day 9, after which it declined until the rates from the ethanol-treated fruit were similar at the end of the experiment. Rates of CO₂ production during these periods of depression and stimulation were roughly proportional to the length of the exposure period to ethanol.

Temperatures in the range of 20 to 30C during a 2-h exposure of mature-green tomato fruit to 2 ml ethanol/kg fruit only slightly

Figs. 1–4. Color score, firmness (millimeters deformation), and ethylene (microliters per kilogram per hour) and CO₂ (milliliters per kilogram per hour) production of mature-green (1), breaker (2), turning (3), and pink (4) tomatoes treated with 0, 2, or 4 ml ethanol/kg fruit for 4 h at 20C. Vertical bars represent 5% LSD for each measurement.

influenced the inhibitory effect of ethanol (Fig. 7). In contrast, during a 4-h exposure, the higher temperatures significantly increased the inhibitory effect of ethanol on ripening. A 6-h ex-

posure at 20C gave roughly the same inhibition of ripening as did a 4-h exposure at 25C or a 2-h exposure at 30C.

Storage temperatures. The rate of ripening was slowed for

Fig. 5. Initial and 1 week color scores vs. ethylene production of two sets of 30 fruit at various initial ripeness stages. One set was treated with 4 ml ethanol/kg fruit for 4 h at 20C.

Fig. 6. Color score, firmness (millimeters deformation), and ethylene (microliters per kilogram per hour) and CO_2 (milliliters per kilogram per hour) production of mature-green tomatoes treated with 2 ml ethanol/kg fruit for 0, 2, 4, or 6 h at 20C. Vertical bars represents 5% LSD for each measurement.

both control fruit and fruit treated with 2 ml ethanol/kg the lower the temperature (Fig. 8). It took 14, 19, and 30 days for the untreated fruit kept at 20, 15, and 12C, respectively, to become red-ripe (i.e., a score of 6), while it took 19, 25, and 37 days for the ethanol-treated fruit to become red-ripe. The difference in time for controls and ethanol-treated fruit to become red-ripe was 5, 6, and 7 days at 20, 15, and 12C, respectively. Similar differences of 5, 6, and 8 days were apparent by the time the fruit had become pink (i.e., a score of 4). In general, the rate of color development of control and ethanol-treated fruit was roughly parallel as they ripened from breaker to red-ripe (i.e., scores of 2 to 6).

Fig. 7. Color score of mature-green tomatoes not treated with ethanol at 20C or with 2 ml ethanol/kg fruit for 2 or 4 h at 20, 25, or 30C, or at 20C for 6 h. Vertical bars represent 5% LSD for each measurement.

Fig. 8. Ripening of mature-green tomatoes treated with 2 ml ethanol/kg fruit and kept at 12, 15, or 20C. See Table 1 for composition of red-ripe fruit at end of the storage period. Vertical bar represents 5% LSD for each measurement.

A similar parallelism in ripening was evident with fruit kept for 2 or 4 weeks at 12C before transfer to 20C (Fig. 9). The slow rate of ripening at 12C was similar to that shown in Fig. 8 for fruit kept at 12C. When transferred to 20C, however, both control and treated fruit rapidly ripened. At the 2- and 4-week transfers, ethanol had retarded fruit ripening by 8 and 10 days, respectively. After transfer to 20C and ripening to red-ripe, there was only a 3-day difference between the control and treated fruit for both transfer periods.

The SSC in control fruit declined from 3.2 to 3.0 to 2.6 as the storage temperature decreased from 20 to 15 to 12C (Table 1). Fruit kept at 12C and transferred to 20C after 2 or 4 weeks had a SSC similar to the control fruit kept continuously at 12C. The range of 2.7%–2.6% SSC for fruit kept at 12C was signif-

Fig. 9. Ripening of mature-green tomatoes treated with 2 ml ethanol/kg fruit and kept continuously at 12C, or for 2 or 4 weeks at 12C before transfer to 20C. The arrows indicate the time of transfer. Vertical bar represents 5% LSD for each measurement.

Table 1. Effect of ethanol and storage temperature on the composition of red-ripe tomato fruit at the end of storage.

Temp and ethanol concn (ml·kg ⁻¹)	SSC (%)	TA (%)	SSC : TA	pH
Continuous at 20C				
0	3.2 a ^z	0.45 a	7.68 bc	4.18 b
2	3.0 a	0.38 b	7.86 ab	4.27 a
Continuous at 15C				
0	3.0 a	0.45 a	6.64 c	4.15 b
2	3.0 a	0.34 b	8.74 a	4.28 a
Continuous at 12C				
0	2.6 b	0.39 ab	6.74 c	4.15 b
2	3.1 a	0.40 ab	7.92 ab	4.25 a
2 weeks at 12C then at 20C				
0	2.7 b	0.38 b	7.07 c	4.17
2	2.9 ab	0.34 b	8.36 ab	4.24 a
4 weeks at 12C then at 20C				
0	2.6 b	0.35 b	7.47 bc	4.18 b
2	2.9 ab	0.38 b	7.64 bc	4.27 a

^zMean separation within columns by LSD, $P = 0.05$.

ificantly lower than the range of 3.2%–3.0% for fruit kept at higher temperatures. In contrast, the SSC of ethanol-treated fruit was not significantly different among any of the treatments and was similar to that of the control fruit at the higher temperatures. Ethanol treatments apparently have little or no effect on SSC.

The percent TA was significantly higher in control fruit than in ethanol-treated fruit held at 20 and 15C (Table 1), but there was no difference between control and ethanol-treated fruit for any of the 12C treatments. The lower values for ethanol-treated fruit at 15 and 20C were similar to values for fruit at 12C. Application of ethanol at 15 and 20C, or use of a lower temperature (12C), appears to reduce TA. The pH of ethanol-treated fruit was significantly higher (4.26 ± 0.02) than that of control fruit (4.17 ± 0.02) for all treatments (Table 1).

The ratio of SSC : TA was always numerically, if not statistically, higher for the ethanol-treated fruit (Table 1). A statistically significant increase in the ratio of from 32% to 18% between control and ethanol-treated fruit was calculated for fruit continuously kept at 15 and 12C and for fruit transferred from 12 to 20C after 2 weeks.

High-quality tomato fruit are characterized by containing >3% SSC and 0.32% TA, and having a SSC : TA ratio >10 (Kader et al., 1978). The SSC was a little <3% in some treatments, but TA was consistently >0.32%. While the SSC : TA ratios were consistently <10 for all treatments, the ratio in ethanol-treated fruit was either similar or significantly higher than that in the control fruit. These measurements of quality all indicate that ethanol-treated fruit were of similar or higher quality than control fruit. This high quality was maintained even though ethanol-treated fruit were in 20 to 12C storage from 5 to 7 days longer than control fruit (Fig. 8).

Ethanol tissue concentration. The ethanol concentration in fruit exposed to 4 ml ethanol/kg was initially the same regardless of their stage of ripeness (Fig. 10). At 20C, ethanol content declined rapidly in all fruit, reaching background levels (<5 $\mu\text{g}\cdot\text{g}^{-1}$ fresh weight) within 4 days. The reduction in ethanol concentration was exponential in nature with a half-life of ≈ 0.5 days. Regression equations fitted to log ethanol tissue concentration vs. days after treatment had r^2 values around 0.99 (range 0.996–0.979) and similar slopes (range -0.68 to -0.71). The exponential decline in ethanol content was similar to that observed in blueberries and grapes in which high endogenous ethanol levels were induced by anaerobiosis (Saltveit and Ballinger, 1983a, 1983b). The rapid decline in ethanol content of treated fruit and the approximate week-long delay in ripening of treated fruit both combine to ensure that the ethanol used to treat fruit would have dissipated to background levels by the time the fruit became red-ripe.

Exposure of tomato fruit to 2 to 4 ml ethanol/kg as a vapor at 20C for 4 h significantly slowed reddening at initial degrees of ripeness ranging from mature-green to pink. Slowing of ripening for up to 1 week did not significantly reduce quality measured when the fruit had become red-ripe. Use of ethanol vapor as a postharvest treatment to retard tomato fruit ripening may be a viable technique to extend the market life of tomato fruit.

Fig. 10. Loss of ethanol from mature-green, breaker, turning, and red-ripe tomatoes initially treated with 4 ml ethanol/kg fruit for 4 h at 20C and then kept in air at 20C. Regression equations were calculated for initial measurements taken before treatment [color score = $0.0 + 0.38$ (μl ethylene/kg per h)] with an r^2 of 0.79, for measurements of control fruit taken after 1 week [color score = $3.1 + 0.42$ (μl ethylene/kg per h)] with an r^2 of 0.83, and for measurements of the ethanol-treated fruit taken after 1 week [color score = $1.5 + 0.45$ (μl ethylene/kg per h)] with an r^2 of 0.85. Insert shows log-transformed ethanol concentration vs. days after treatment. Vertical bar represents 5% LSD values.

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