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Ethanol effects on the ripening of climacteric fruit

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Abstract

The ability of ethanol to inhibit the ripening of eight species of climacteric fruit was tested. Exposure to ethanol vapors at ≤ 6 ml kg^{-1} fruit for up to 6 h, a treatment that inhibited the ripening of mature-green tomatoes by 7 days, failed to inhibit the ripening of whole banana, honeydew, muskmelon, nectarine, pear, peach, and plum fruit. In contrast, ethanol directly injected into the seed cavity of muskmelon or honeydew melons resulted in firmer ripened fruit, but the effect was inconsistent. Exposure to ethanol vapors at ≤ 2 ml kg^{-1} fruit for 3–6 h significantly inhibited the climacteric and delayed softening of mesocarp plugs excised from honeydew melons. In comparison to air controls, avocado fruit exposed to a flow of 80% ethanol-saturated air softened more slowly and had a delayed respiratory and ethylene climacteric, but exhibited skin and flesh browning upon ripening. © 1997 Elsevier Science B.V.

Keywords: Avocado; Banana; Cantaloupe; Ethylene production; Honeydew; Melons; Muskmelon; Nectarine; Pear; Peach; Plum; Respiration

1. Introduction

Climacteric fruit are those which undergo a 'ripening phase' associated with increased respiration and ethylene production, softening, composition changes, color changes, and aroma production. The management and control of fruit ripening is important for the successful transport and marketing of fresh fruit and vegetables. Fruit that ripen too early are easily damaged during transport and produce ethylene which can adversely effect other commodities. The techniques used to delay and/or manage ripening include: cold storage, controlled atmosphere storage, ethylene addition and/or removal, and inhibition of ethylene action through chemical means.

Previous research presents conflicting reports on the ability of ethanol to inhibit fruit ripening and senescence. Exogenously applied ethanol (Saltveit and Mencarelli, 1988), or endogenously synthesized ethanol (Kelly and Saltveit, 1988) inhibited the ripening of whole tomato fruit at

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various maturity stages without affecting subsequent quality (Saltveit and Sharaf, 1992). Ethanol also delayed the senescence of oat leaves and carnation flowers (Satler and Thimann, 1980; Heins, 1980; Wu et al., 1992). Mencarelli et al., 1991 reported that ethanol vapors had no effect on kiwifruit flesh softening, while Agravante et al., 1990, 1991) found that ethanol accelerated ripening of bananas. Depending on the maturity of the fruit and the amount of ethanol applied, exposure to ethanol vapors either promotes or inhibits tomato fruit ripening (Beaulieu and Saltveit, 1997). Research on tomatoes (Saltveit, 1989) and carnations (Wu et al., 1992) suggests that ethanol not only reduces ethylene production but also noncompetitively inhibits ethylene action. These reports suggest that ethanol may inhibit the ripening of a wide range of climacteric fruit. Experiments reported in this paper were undertaken to test whether ethanol treatments can inhibit the ripening of a range of climacteric fruits.

2. Materials and methods

2.1. Plant material

Whole avocado (*Persea americana* Mill., cv. Hass), banana (*Musa* AAA, Cavendish subgroup), honeydew (*Cucumis melo* (L) var. Inodorus Naud), muskmelon (*Cucumis melo* (L) var. Reticulatus Naud.), nectarine (*Prunus persica* (L) Batsch var. nectarina (Ait) maxim, cv. Fantasia), pear (*Pyrus communis* L., cv. Bartlett), peach (*Prunus persica* (L) Batsch, cv. Elegant Lady), and plum (*Prunus domestica* L., cv. Santa Rosa) fruit were obtained from a local warehouse, transported to the Mann Laboratory at the University of California, Davis and immediately placed in storage at 10°C. Preclimacteric fruit were used in all cases based on respiration and ethylene production rates (data not shown) and none of the fruit were treated with ethylene to induce ripening.

2.2. Product preparation

Prior to excision of tissue plugs, honeydew fruit were washed in a aqueous solution of commercial bleach (1:20 dilution), rinsed in sterile deionized water and dried in a laminar flow hood. To obtain mesocarp plugs, approximately 4 cm transverse slices were taken from the equatorial region of preclimacteric honeydew melons (size 8). From these slices, 1.5 cm diameter plugs were excised using a cork borer. All operations were conducted in a laminar flow hood under aseptic conditions.

2.3. Ethanol treatments

Before ethanol treatments, the fruit were warmed to 20°C, and surface moisture was allowed to dry. Individual fruits were weighed and placed inside 20 l glass jars or 35 l metal boxes to form a single layer. Melon plugs were placed inside sterilized 5 l jars. To facilitate ethanol evaporation, reagent-grade ethanol (95%) was pipetted onto a folded 9 cm diameter filter paper positioned on a Petri plate inside each container. The containers were immediately sealed for up to 6 h. In addition, melons were injected with up to 6 ml/kg ethanol directly into the seed cavity using a sterile syringe and 7 cm hypodermic needle. When quality was evaluated at the end of the experiment, no tissue damage was observed in the seed cavity of melons injected with ethanol. Avocados were exposed for up to 4 days to a flow of 20% air plus 80% air bubbled through 95% ethanol. Flows were adjusted to maintain CO_2 levels below 0.2%.

After ethanol exposure, the containers were opened and allowed to ventilate for several hours. If no respiration or ethylene production measurements were to be taken, the fruit were allowed to ripen in the open containers; otherwise, the containers were resealed and sufficient flows of humidified air were established through the container to maintain CO_2 concentrations below 0.2%. Whole bananas, honeydews, muskmelons, nectarines, pears, peaches, and plums were ripened at 20°C, avocados were ripened at 15°C, and honeydew melon plugs were ripened at 12.5°C.

2.4. Carbon dioxide and ethylene measurements

Carbon dioxide and ethylene production were measured as previously described (Saltveit, 1982; Saltveit and Yang, 1987; Saltveit and Strike, 1989) by injecting gas samples taken from the inlet and outlet from each sample jar into an infrared CO_2 analyzer (Horiba, Irvine, CA) or a gas chromatograph with a flame ionization detector (EG&G Chandler, Tulsa, OK), respectively. Respiration and ethylene production rates were calculated from fruit weights, air flow rates, and differences in the inlet and outlet gas concentrations.

2.5. Quality evaluation

All fruit quality evaluations were conducted after tissue had reached room temperature. Flesh firmness was measured using a U.C. firmness tester with an 11 mm diameter tip for the melons, and an 8 mm tip for all other fruit (Western Industrial Supply, San Francisco, CA). For the melons, an approximately 3 cm thick transverse section was cut from the equatorial region of each fruit, laid flat on the platform of the firmness tester, and four readings of the mesocarp tissue were taken at equidistant intervals around the section. Melon plugs were placed inside snug-fitting plastic cylinders for support so that the firmness readings were similar to those of whole fruit. Preliminary tests confirmed that there was no significant difference between firmness measurements from the 3 cm thick melon equatorial sections and melon plugs measured inside the plastic cylinders (data not shown). For all other fruit, firmness measurements were taken from opposite sides of each fruit after the skin was removed.

Soluble solids (SSC) were measured from extracted juice using a temperature compensated refractometer. Differences in surface color development were measured using a Minolta colorimeter.

2.6. Statistical analysis

All experiments were repeated at least once with consistent results. A completely randomized design was used in most experiments, but in some experiments replicates were blocked by size or ripeness. Data were subjected to analysis of variance.

3. Results

3.1. Whole fruit

There was no significant inhibition of ripening of whole bananas, honeydews, muskmelons, nectarines peaches, pears and plums when exposed to ethanol vapors under the described conditions (Table 1). All fruit ripened normally after treatment and there was no apparent injury from the ethanol treatments. Therefore, treatments which inhibited ripening of whole tomato fruit (Kelly and Saltveit, 1988; Saltveit and Sharaf, 1992) were ineffective at inhibiting the ripening of these fruits. Agravante et al., 1991 observed accelerated ripening when he exposed banana fingers to ethanol at roughly 3 ml/kg for 24 h. Ripening of tomato pericarp discs was also promoted by a 4 h exposure to ethanol at concentrations below 4 ml/kg (Beaulieu and Saltveit, 1997). However, compared with control fruit, we observed neither a promotion nor an inhibition of ripening in fruit exposed to ethanol vapors.

3.2. Ethanol injected into the seed cavity of melons

Although there was no delayed ripening of whole melons exposed to ethanol vapors, injecting ethanol directly into the seed cavity inhibited the softening of both muskmelon and honeydew melons (Fig. 1). Muskmelon fruit firmness was better maintained at higher ethanol levels. Muskmelon flesh was about 80% firmer than control fruit if injected with 2 or 4 ml/kg ethanol and over 2.3-fold firmer if treated with 6 ml/kg ethanol. Honeydew melons injected with as little as 1 ml/kg ethanol were about 50% firmer than control fruit, but greater amounts of ethanol did not increase firmness retention.

Table 1
Summary of the effect of ethanol on the ripening of various climacteric fruit

Commodity	Treatment	Ripening parameters	Results
Avocados	Exposed 4 days to flow of 80% ethanol saturated air	Firmness, CO_2 and ethylene production	Delayed ripening
Bananas	Exposed 6 h to ethanol vapor ($\leq$6 ml/kg)	Firmness, color, CO_2 and ethylene production	No effect
Honeydews	Exposed 6 h to ethanol vapor ($\leq$6 ml/kg)	Firmness	No effect
	Ethanol injected into seed cavity ($\leq$4 ml/kg)	Firmness	Delayed ripening
	Mesocarp plugs, 6 h to ethanol vapor ($\leq$6 ml/kg)	Firmness, CO_2 and ethylene production	Delayed ripening
Muskmelons	Exposed 6 h to ethanol vapor ($\leq$6 ml/kg)	Firmness, CO_2 and ethylene production	No effect
	Ethanol injected into seed cavity ($\leq$6 ml/kg)	Firmness	Delayed ripening
Nectarines	Exposed 6 h to ethanol vapor ($\leq$6 ml/kg)	Firmness, SSC, CO_2 and ethylene production	No effect
Pears	Exposed 6 h to ethanol vapor ($\leq$6 ml/kg)	Firmness	No effect
Peaches	Exposed 6 h to ethanol vapor ($\leq$6 ml/kg)	Firmness, SSC, CO_2 and ethylene production	No effect
Plums	Exposed 6 h to ethanol vapor ($\leq$6 ml/kg)	Firmness, SSC	No effect

Whole bananas, honeydews, muskmelons, nectarines, pears, peaches, and plums were ripened at 20°C, avocados were ripened at 15°C, and honeydew melon plugs were ripened at 12.5°C.
Unless indicated, all experiments were done with whole fruit.
Ripening parameters measured included flesh firmness, color, rates of CO_2 and ethylene production, and concentration of soluble solids (SSC).

3.3. Honeydew melon plugs exposed to ethanol vapors

There was a significant delay in ripening when honeydew melon plugs were exposed to ethanol vapors for either 3 or 6 h. Control fruit underwent typical respiratory and ethylene climacterics but no distinct climacteric occurred in ethanol-treated tissue (Fig. 2). The respiration of control fruit rose from baseline levels and peaked after 3 or 4 days before slowly declining (Fig. 2a). The respiration of ethanol treated tissue also rose from baseline levels but leveled out after 2 days. Tissue exposed to ethanol for 3 h showed an increase in respiration, again after 6 days, while tissue treated for 6 h remained relatively unchanged.

Ethylene production rose rapidly in the control and peaked after 3 days before falling again to near initial levels by day 6 (Fig. 2b). Ethanol treated tissue gradually increased in ethylene production throughout the experiment. After 6 days, tissue was firmer if either treated at higher levels (2 versus 6 ml/kg) or for longer periods (6 versus 3 h). Therefore, it appears that both the amount and duration of ethanol exposure significantly affected ethylene production during the ripening of honeydew melon plugs. After 6 days at 12.5°C, tissue treated with 2 ml/kg ethanol for 3 h or 6 ml/kg ethanol for 6 h had respectively, about 60 and 30% of the peak ethylene production of control fruit.

After 3 days at 12.5°C, melon plugs exposed to ethanol vapors were firmer than control fruit (Fig. 3). There was no significant increase in firmness of tissue exposed to ethanol vapors for 6 h in comparison to 3 h. However, both control and treated fruit tended to be firmer if sealed in jars for 6 h than for 3 h. After 3 or 6 h inside sealed jars, CO_2 concentrations rose to around 0.3% and 0.6% respectively. Wounding stimulates ethylene production in many different tissues (Yang and Pratt, 1978) and the melon plugs likely had elevated ethylene production while in the jars. Carbon dioxide is known to inhibit ethylene action (Burg and Burg, 1965); fruit exposed to 1 μl/l ethylene

in air are 14 and 30% less responsive to the applied ethylene if the atmosphere contains 0.3 and 0.6% CO_2, respectively. An additional 3 h of depressed ethylene sensitivity in the higher CO_2 atmosphere may explain why fruit sealed in the jars for 6 h tended to be firmer than 3 h stored fruit or controls.

Exposure to greater concentrations of ethanol vapor resulted in firmer fruit after 3 days at 12.5°C. Both 2 and 6 ml/kg ethanol exposure for 6 h significantly delayed softening compared with the control, while fruit treated for 3 h were significantly firmer only if treated with 6 ml ethanol/kg. There was no significant difference in SSC after 3 days at 12.5°C or in firmness or SSC after 6 days. Even though ethanol-treated fruit never underwent a distinctive climacteric, after 6 days the fruit had softened normally and were similar to control fruit.

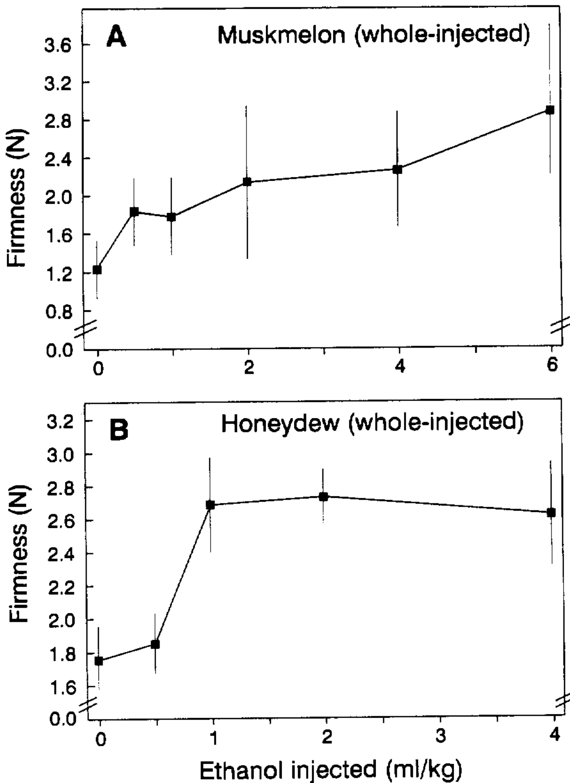

Fig. 1. Firmness of whole muskmelon (a) and honeydew melons (b) after injecting ethanol into their seed cavities and ripening at 20°C. Vertical bars represent standard error.

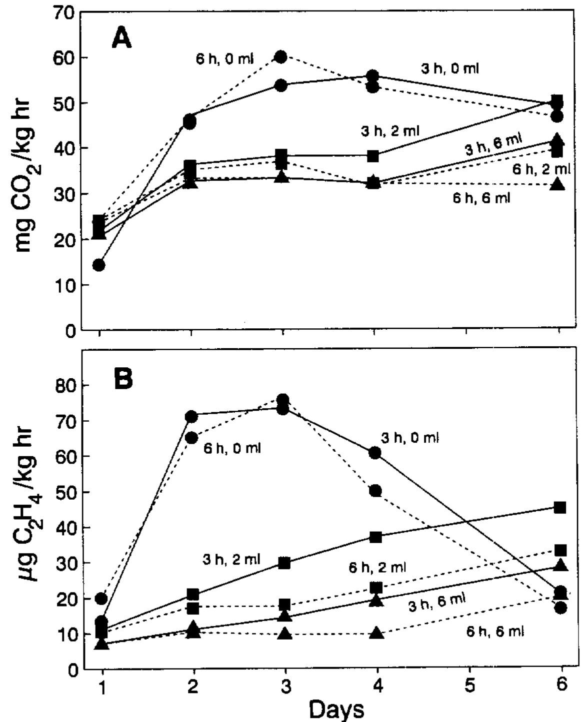

Fig. 2. Respiration (a) and ethylene production (b) of honeydew melon plugs after a 3 or 6 h exposure to vapors from 0, 2 or 6 ml of ethanol.

3.4. Avocado

Exposing whole avocado fruit to 80% ethanol-saturated air altered both respiration and ethylene production rates compared with the control (Fig. 4). The respiration of control fruit rose rapidly and peaked after 2 or 3 days before declining slightly and then leveling off (Fig. 4a). The respiration of avocado fruit treated for either 1 or 2 days began to rise 1 day after ethanol exposure was discontinued and then leveled off after about 4 or 5 days at levels similar to the peak of control fruit. Respiration declined in fruit treated for 4 days until ethanol exposure was discontinued and then recovered slightly but still remained markedly lower than the other treatments.

Control avocado fruit underwent a normal rise and fall in ethylene production associated with the

climacteric which peaked on day 3 (Fig. 4b). Exposure to 80% ethanol-saturated air for as little as 1 day eliminated an ethylene peak during the 7 day observation period. Ethylene production increased after ethanol exposure was discontinued in fruit treated for 1 or 2 days. Fruit treated with ethanol for 4 days had low ethylene production which dropped to undetectable levels after ethanol exposure was discontinued. Ethanol exposure for as little as 1 day appeared to prevent avocado fruit from undergoing a normal climacteric.

Longer exposures to 80% ethanol-saturated air produced progressively firmer fruit after 7 days of ripening (Fig. 5). By this time control fruit had softened to around 4 N while fruit treated for as little as 1 day was about four-fold firmer. Treating avocados with ethanol for 2 or 4 days inhibited flesh softening, so the fruit were about ten- and 15-fold firmer than the controls, respectively, after 7 day of ripening. After 14 days of ripening, only fruit treated with 80% ethanol-saturated air for 4 days were significantly firmer than the control.

Although melon plugs treated with ethanol vapors appeared to ripen normally, whole avocado fruit treated with 80% ethanol-saturated air did not. After 4 days of ethanol exposure at 15°C, black patches began appearing on the skin of the fruits. After 7 days, control fruit were dark reddish-green, fruit treated with ethanol for 1 day were mostly green with a few dark brown/black areas, fruit treated with ethanol for 2 days had a mixture of green and dark brown/black areas, and fruit treated with ethanol for 4 days were almost entirely black with some green spots. After 7 days of ripening, the flesh began to brown around the vascular bundles of fruit exposed to as little as 2 days of ethanol. Fruit exposed to 4 days of ethanol had severe flesh browning. After 14 days, flesh browning was severe in all ethanol exposed fruit.

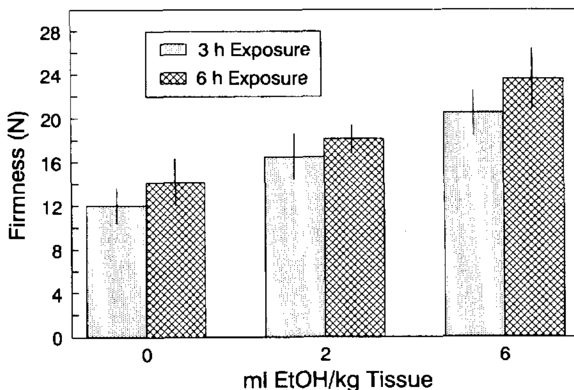

Fig. 3. Firmness of melon plugs after ethanol treatments and storage for 3 days at 12.5°C. The melon plugs were exposed to vapor from 0, 2 or 6 ml ethanol for 3 or 6 h. The vertical bars represent standard error.

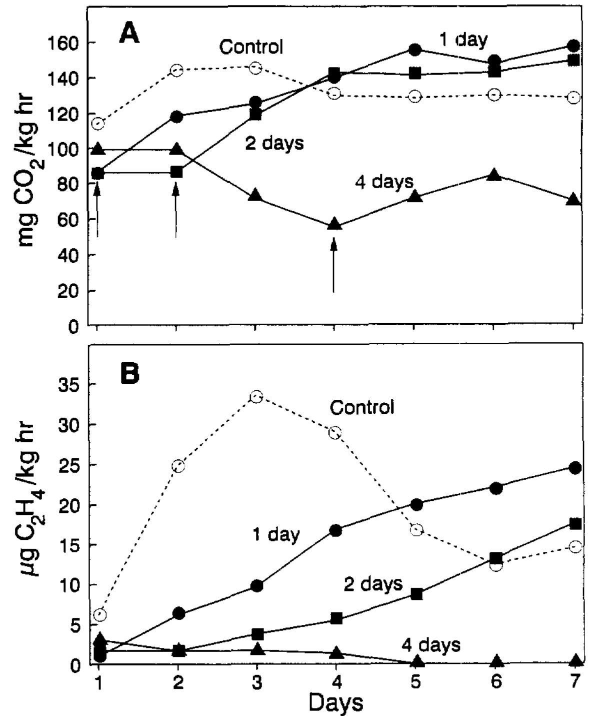

Fig. 4. Respiration (a) and ethylene production (b) of composite samples of avocado fruit during and after exposure to 80% ethanol saturated air. The arrows represent the times that ethanol exposure was halted for each treatment.

4. Discussion

Since acetaldehyde, and not ethanol, appears to be the active component in promoting or inhibiting ripening (Beaulieu et al., 1997), the conversion of ethanol to acetaldehyde by the tissue may be the critical factor in determining whether a certain

level of ethanol exposure promotes or inhibits ripening. Some fruit tissues may have insufficient constitutive alcohol dehydrogenase activity to metabolize enough ethanol to produce a physiologically active level of acetaldehyde.

Tissue responsiveness to applied ethanol also varies with maturity (Beaulieu and Saltveit, 1997). As tomato fruit ripen, they become more sensitive to the inhibitory effect of ethanol. A concentration of ethanol that promotes the ripening of mature-green fruit inhibits the ripening of breaker fruit. Since it is common for ripe fruit to contain small amounts of ethanol and acetaldehyde as part of their aroma constituents, riper fruit may also have higher levels of alcohol dehydrogenase activity and therefore be able to convert more of the applied ethanol into the physiologically active acetaldehyde.

Physical barriers to gas diffusion in whole fruit (e.g. layers of suberized epidermal cells, and rind tissue) may also have excluded or reduced ethanol diffusion into the internal tissue and contributed to the lack of a response to applied ethanol. Resistance of the external physical barriers was circumvented by injecting ethanol directly into the fruit and by exposing excised tissue to ethanol vapors. Ripening was inhibited when ethanol was injected into honeydew and muskmelon fruit or when excised mesocarp plugs of honeydew were exposed to ethanol vapors. The differential effect of ethanol on whole and segmented fruit clearly shows the importance of barriers to its diffusion into the fruit.

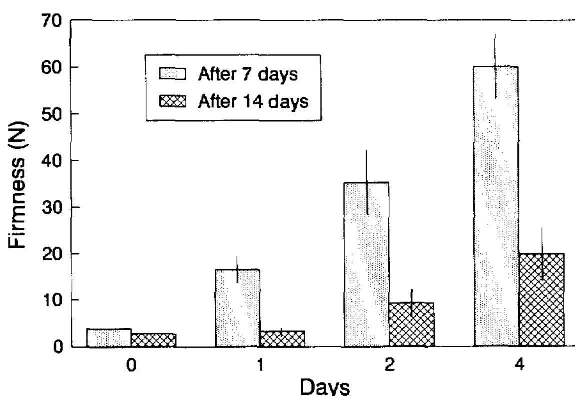

Fig. 5. Firmness of avocado fruit after ethanol treatments and storage at 15°C for 7 or 14 days. Fruit were treated for 0, 1, 2, or 4 days to a flow of 80% ethanol-saturated air. The vertical bars represent standard error.

It appears that by injecting directly into the seed cavity, ethanol was better able to penetrate the tissue. It is also possible that by injecting ethanol into the seed cavity, it was in contact with the tissue for longer than the 6 h exposure used in the vapor treatment experiments. The ethanol would be present until either metabolized or volatilized from the fruit.

The ability of ethanol to promote or inhibit the ripening of climacteric fruit seems to be dependent on a number of factors which probably include species, cultivar, maturity, applied concentration, mode of application, and duration of exposure. Tomato fruit appear to be exceptionally sensitive to the ripening promotive and inhibitory effects of ethanol since they respond at all stages of maturity to a wide range of concentrations, durations of exposure, and modes of applications. We have shown that other fruit do not exhibit this same level of sensitivity. Whether this lack of sensitivity is the result of an inherent inability of the fruit to respond to ethanol, or to one of the other factors listed above warrants further investigation.

References

Agravante, J.U., Matsui, T., Kitagawa, H., 1990. Effect of ethanol on ripening and amylase activity of banana. Nippon Shokuhin Kogyo Gakkaishi 37 (3), 235–238.

Agravante, J.U., Matsui, T., Kitagawa, H., 1991. Changes in pectinmethylesterase, polygalacturonase and pectic substances of ethanol-and ethylene-treated bananas during ripening. Nippon Shokuhin Kogyo Gakkaishi 38 (6), 527–532.

Beaulieu, J.C., Peiser, G., Saltveit, M.E., 1997. Acetaldehyde is a causal agent responsible for ethanol-induced ripening inhibition in tomato fruit. Plant Physiol. 113, 431–439.

Beaulieu, J.C., Saltveit, M.E., 1997. Inhibition or promotion of tomato fruit ripening by acetaldehyde and ethanol is concentration-dependent and varies with initial fruit maturity. J. Am. Soc. Hort. Sci. 122, 392–398.

Burg, S.P., Burg, E.A., 1965. Ethylene action and the ripening of fruits. Science 148, 1190–1196.

Heins, R.D., 1980. Inhibition of ethylene synthesis and senescence in carnation by ethanol. J. Am. Soc. Hort. Sci. 105, 141–144.

Kelly, M.O., Saltveit, M.E., 1988. Effect of endogenously synthesized and exogenously applied ethanol on tomato fruit ripening. Plant Physiol. 88, 143–147.

Mencarelli, F., Savarese, P., Saltveit, M.E., 1991. Ripening of kiwifruit exposed to ethanol and acetaldehyde vapors. HortScience 26 (5), 566–569.

Saltveit, M.E., 1982. Procedures for extracting and analyzing internal gas samples from plant tissues by gas chromatography. HortScience 17 (6), 878–881.

Saltveit, M.E., 1989. Effect of alcohols and their interaction with ethylene on the ripening of epidermal pericarp discs of tomato fruit. Plant Physiol. 90, 167–174.

Saltveit, M.E., Mencarelli, F., 1988. Inhibition of ethylene synthesis and action in ripening tomato fruit by ethanol vapors. J. Am. Soc. Hort. Sci. 113 (4), 572–576.

Saltveit, M.E., Sharaf, A.R., 1992. Ethanol inhibits ripening of tomato fruit harvested at various degrees of ripeness without affecting subsequent quality. J. Am. Soc. Hort. Sci. 117, 793–798.

Saltveit, M.E., Strike, T., 1989. A rapid method for accurately measuring oxygen concentrations in ml gas samples. HortScience 24 (1), 145–147.

Saltveit, M.E., Yang, S.F., 1987. Ethylene. In: Crozier, A. (Ed.), The Principles and Practice of Plant Hormone Analysis. Academic Press, pp. 367–401.

Satler, S.O., Thimann, K.V., 1980. The influence of aliphatic alcohols on leaf senescence. Plant Physiol. 66, 395–399.

Wu, M.J., Zacarias, L., Saltveit, M.E., Reid, M.S., 1992. Alcohols and carnation senescence. HortScience 27, 136–138.

Yang, S.F., Pratt, H.K., 1978. The physiology of ethylene in wounded plant tissue. In: Kahl, G. (Ed.), Biochemistry of wounded plant tissues. Walter de Gruyter, Berlin, pp. 595–622.