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Influence of Storage Temperature and Ethylene on Firmness, Acids, and Sugars of Chilling-sensitive and Chilling-tolerant Tomato

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Abstract. **Two cultivars and 2 experimental chilling-tolerant lines of tomato (*Lycopersicon esculentum* Mill.) were harvested mature-green and stored for 15 days at 5°, 20°, and 35°C with or without the introduction of ethylene; portions of the high and low temperature samples were moved to 20° for an additional 10 days. Samples were analyzed for firmness, sugars, and acids. Fruit of the chilling-tolerant tomato lines were firmer than the commercial cultivars in all temperature treatments. Ethylene enhanced softening in the chilling-sensitive cultivars only at 20°, while the chilling-tolerant lines showed an effect only at 35°. The chilling-tolerant lines appeared to be more heat-tolerant than the sensitive cultivars. Sugar and organic acid analyses were not as clear-cut, often revealing a tendency for the cherry-sized fruit to behave similarly to each other and different from the normal-sized fruit. The chilling-tolerant lines held at 5° or moved from 5° to 20° had lower monosaccharide levels than the corresponding sensitive cultivars. This also was true when fruit were moved from 35° to 20°. 'New Yorker' tomato had low levels of malate after exposure to 35°, which resulted in a high citrate/malate ratio not evident in the other 3 cultivars. Phosphoric acid levels were higher in the chilling-tolerant tomato fruit and increased with increasing storage temperature. Line 281 deviated from the other 3 cultivars in that, in general, acids increased and sugars decreased with increasing storage temperature.**

Normal fruit ripening in tomato is affected by low temperatures (5, 15) and exposure to high temperatures inhibits the development of red color and softening (11, 12, 19). While the rates of softening and color development have been predicted mathematically at temperatures from 12° to 27°C, the model did not describe accurately the situation outside of this range (23). This emphasizes the limited conditions under which normal ripening will occur.

Tomato fruit ripen relatively quickly with many simultaneous changes, making it difficult to understand the effect of each change. A number of reports have concentrated on isolating various facets of ripening through studies of tomato mutants that do not ripen normally (24). Another approach has been to store tomato fruit in controlled atmospheres, where substrates of glycolysis and the citric acid cycle change as in ripening fruit while color development and some enzyme synthesis does not proceed until the fruit are transferred to a normal atmosphere (9, 10). A 3rd method has been to store tomato fruit at temperature extremes and note the effect (11, 12, 15, 17, 19).

Low temperature or chilling tolerance has been demonstrated in some tomato lines (14, 17), and there is evidence that chilling tolerance indicates an ability to endure a wide range of temperatures rather than just the low end of the scale (1, 20). To test this idea, we compared the firmness and levels of sugars and organic acids in 2 chilling-tolerant tomato lines and 2 commercial cultivars at 5°, 20°, and 35°C. Since the production of ethylene has been shown to be reduced at high temperatures (16), it was introduced as a variable.

Materials and Methods

Two chilling-tolerant breeding lines, 281 (cherry) and 79-546 (normal size), were compared with the chilling-sensitive cultivars 'Early Cherry' (cherry) and 'New Yorker' (normal size). Fruit was harvested mature-green and the 3 field replications for each line or cultivar were kept separate and used as treatment replicates. Each replicate contained 2–3 kg of fruit. The tomato fruit were held at 5° or 35°C in a flow-through air system with or without ethylene (about 50 ppm) for 15 days. Control fruit received the same treatments at 20°. Control fruit were analyzed after 15 days and samples were taken from all other treatments for analysis. The remainder of the fruit held at 5° and 35° was partitioned so that fruit that had received air during the first 15 days of storage were divided into 2 equal samples, one to be exposed to ethylene, the other not. Those that were exposed originally to ethylene were divided similarly. These fruit were moved to 20° to ripen for 10 days and then were analyzed.

Fruit firmness was determined by compression with a 500-g weight for 5 sec, using a penetrometer (GCA/Precision Scientific) modified for deformation testing (2, 18). Fruit were sliced, frozen, freeze-dried, and ground in a Wiley Mill after deformation testing.

Sugars were analyzed by extracting 0.5 g of each freeze-dried sample with hot 80% ethanol, evaporating the ethanol under

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reduced pressure, passing the resulting aqueous solution through an anion-cation exchange column (Amberlite 93 and Amberlite 252, Rohm & Haas), and derivatizing (TMS) by the method of Sweeley et al. (21). Organic acids were extracted with hot ethanol from a different 0.5-g sample of freeze-dried material and precipitated by the method of Fernandez-Flores et al. (6). Derivatization was identical to that of the sugars. Quantitation of both acids and sugars was by gas chromatography using a flame ionization detector and a 3 m × 2 mm i.d. SS column packed with 3% OV-101 on 80/100 mesh Supelcoport. Tartaric acid was used as an internal standard for organic acid analysis and B-phenyl-D-glucoside was the internal standard for sugar analysis. Both temperature programs were raised 8°/min after a 2-min initial hold. Temperature ranges were 190°C to 270° and 100° to 220° for sugars and acids, respectively.

Results

Firmness. Chilling-tolerant lines were firmer than the sensitive cultivars at all temperatures (Table 1). The data for both chilling-sensitive and -tolerant fruit showed the fruit held at 5°C to be the firmest with ethylene having no effect. Ethylene significantly decreased the firmness of chilling-sensitive fruit at 20°. Chilling-tolerant fruit at 35° were not as soft as those at 20° and the deformation readings were especially low when the fruit were not exposed to ethylene. All fruit held at 5° and 35° showed additional softening after transfer to 20° (Table 2). Both small-fruited tomatoes were firmer after being transferred to 20° from 5° than after 15 days at 20°. Increased softening of the fruit moved from 35° to 20° over the control fruit held at 20° was found in all fruit except those from line 281.

Sugars. Sugar levels were influenced primarily by temperature treatment (Table 3). Ethylene had no significant effect. Fructose levels in 'New Yorker' and 79-546 (both normal size) after 15 and 25 days were always greater than or equal to the initial level regardless of the temperature treatment. This also was true for glucose with the exception of fruit moved from 35° to 20°C.

Fructose and glucose levels in 'Early Cherry' and 281 (both cherry size) tended to fluctuate. Fructose levels in 281 fruits were all lower than the initial level with the fruit at 35°C being lowest. The same was true for 'Early Cherry' with the exception of those fruits exposed to 5°. Glucose levels in 281 tomatoes dropped with increasing temperature and/or time. 'Early Cherry' fruit held at 35° had glucose levels similar to the initial level but dropped precipitously after transfer to 20°. The lowest levels for all tomato types were found in fruit ripened at 20° after storage at 35°.

Sucrose levels were much lower than either glucose or fructose. The sucrose level in all tomato fruit after the 5°C treatment was similar to or greater than the initial level. Once these fruit were moved to 20° there was a dramatic drop in sucrose except in 281, where the decrease was not significant. All other temperature treatments in the 2 larger tomato types yielded sucrose levels similar to that of the initials. 'Early Cherry' tomato responded similarly to both high and low temperature treatments. 281 fruit moved from 35° to 20° were low in sucrose.

Acids. Citrate levels generally increased as storage temperatures increased (Table 4). Ethylene had no significant effect. There was a tendency for the larger fruit types to show a drop in citrate when the 5° or 35°C fruit were moved to 20°. There was no change when the smaller fruit were moved from 35° to

Table 1. Firmness of chilling-sensitive ('New Yorker' and 'Early Cherry') and chilling-tolerant (79-546 and 281) tomato cultivars held for 15 days at 5°, 20°, or 35°C with (+) or without (−) ethylene.

Treatment: Temp (°C)	Treatment: Ethylene	Deformation (mm/500g-5 sec): Sensitive	Deformation (mm/500g-5 sec): Tolerant
5	+	1.39 f[z]	1.01 g
	−	1.39 f	1.04 g
20	+	3.07 a	2.47 cd
	−	2.79 b	2.31 d
35	+	2.69 bc	2.06 e
	−	2.82 b	1.57 f

[z]Mean separation by Duncan's multiple range test, 5% level.

Table 2. Firmness of chilling-sensitive ('New Yorker' and 'Early Cherry') and chilling-tolerant (79-546 and 281) tomato cultivars held at different temperatures.

Temp (°C)	Deformation (mm/500g-5 sec): NY	79-546	EC	281
5	1.09 d[z]	0.83 e	1.58 d	1.16 d
5–20	4.30 a	3.40 a	2.58 c	2.17 b
20	2.88 c	2.41 c	2.97 b	2.38 a
35	2.73 c	1.35 d	2.81 b	1.99 c
35–20	3.74 b	2.78 b	3.20 a	2.30 a

[z]Mean separation in each cultivar by Duncan's multiple range test, 5% level.

Table 3. Sugar content of chilling-sensitive ('New Yorker' and 'Early Cherry') and chilling-tolerant (79-546 and 281) tomato cultivars held at different temperatures.

Temp (°C)	Sugar content (mg/g dry wt): New Yorker	79-546	Early Cherry	281
		Fructose		
Initial	260.2 b[z]	245.8 ab	298.2 ab	285.8 a
5	316.6 a	282.6 a	279.0 bc	243.4 b
5–20	324.2 a	277.8 a	301.0 a	249.4 b
20	310.6 a	288.8 a	241.2 d	230.2 b
35	242.2 b	254.2 ab	255.8 cd	185.8 c
35–20	248.8 b	237.8 b	179.4 e	169.4 c
		Glucose		
Initial	408.4 ab	409.4 ab	432.0 a	406.2 a
5	447.8 a	422.8 ab	366.0 b	324.6 b
5–20	440.6 a	399.6 b	363.2 b	295.0 c
20	359.8 bc	385.0 b	311.0 c	254.6 d
35	418.2 a	456.4 a	417.2 a	217.6 e
35–20	324.2 c	321.4 c	249.0 d	191.0 e
		Sucrose		
Initial	6.2 ab	4.2 bc	5.6 b	6.0 a
5	9.6 a	25.6 a	12.6 a	3.8 abc
5–20	0.4 c	0.8 c	3.6 b	1.2 bc
20	4.8 ab	1.6 c	7.0 b	4.2 ab
35	2.4 bc	6.0 bc	11.6 a	1.8 abc
35–20	6.6 ab	10.0 b	3.8 b	0.6 c

[z]Mean separation in each variety by Duncan's multiple range test, 5% level.

Table 4. Acid content of chilling-sensitive ('New Yorker' and 'Early Cherry') and chilling-tolerant (79-546 and 281) tomato cultivars held at different temperatures.

Temp (°C)	Acid content (mg/g dry wt)			
	New Yorker	79-546	Early Cherry	281
		Citrate		
Initial	99.2 bc[z]	95.1 bc	106.9 bc	78.8 c
5	105.0 bc	117.8 ab	129.9 b	107.5 bc
5–20	89.4 c	91.3 c	100.6 c	113.1 bc
20	96.3 c	127.4 ab	165.5 a	135.6 ab
35	133.7 a	133.0 a	154.0 a	150.6 a
35–20	115.8 ab	100.7 bc	158.8 a	160.8 a
		Malate		
Initial	46.6 a	45.8 bc	45.3 b	35.9 bcd
5	34.7 b	47.8 b	43.1 b	39.2 bc
5–20	23.8 c	29.0 d	28.0 d	33.9 cd
20	28.3 bc	31.5 cd	35.0 c	28.5 d
35	31.0 bc	67.6 a	43.4 b	44.5 b
35–20	30.9 bc	46.0 bc	56.3 a	57.1 a
		Phosphate		
Initial	6.3 bc	9.9 b	4.2 b	5.5 c
5	5.4 c	10.8 b	5.0 b	7.0 c
5–20	6.8 bc	12.0 b	4.8 b	10.0 b
20	8.2 b	15.2 a	6.3 b	10.6 b
35	10.5 a	18.2 a	8.7 a	15.1 a
35–20	11.5 a	17.3 a	10.2 a	16.8 a

[z]Mean separation in each cultivar by Duncan's multiple range test, 5% level.

20° and only 'Early Cherry' showed a decrease in citrate when moved from 5° to 20°.

Malate levels at 5°C remained similar to initial levels except for a drop in fruit of 'New Yorker' (Table 4). The malate content of all tomato types except 281 decreased when the 5°-treated fruit were moved to 20°. Both 'Early Cherry' and 281 showed an increased malate level when the fruit held at 35° were moved to 20°. Levels in the larger fruit either decreased or showed no change.

Phosphoric acid content generally increased with increased storage temperatures (Table 4). Chilling-tolerant lines had higher levels than their sensitive analogues and the larger tomatoes had higher levels than the cherry tomatoes.

Discussion

A considerable number of tomato fruit developed decay during these experiments. Losses from decay for the normal-sized fruit at 35°C were about 14% and 40% for fruit without or with exposure to ethylene, respectively. The cherry-sized fruit had higher levels of decay with 40% lost in air at 35°C. Line 281 showed losses of 75% in ethylene at 35° while 'Early Cherry' was most susceptible with losses of 90%. Some treatments, therefore, were not fully represented since decayed fruit were not included in the analyses. If the number of decayed fruit was high, the intact fruit were pooled and divided into replicates for the ripening phase of the experiment. The results were affected in that differences were not as marked as if the decayed (and apparently more sensitive) fruit had been included in the analyses.

In a comparison of the firmness of different tomato cultivars ripened at 12° and 22°C, Thomas et al. (22) found that some cultivars showed no difference in firmness while others became softer when ripened at the higher temperature. Chilling-tolerant lines in our experiments were firmer than the chilling-sensitive cultivars at all temperatures. This may be explained partially by slower ripening rates in the tolerant lines since all tomato fruit were analyzed at the same time regardless of color development. Lower decay levels in tolerant lines also may be attributed to slower ripening rates.

Both Hall (11), using high temperature (32° to 42°C) with exposure times up to 24 hr, and Ogura et al. (19) with exposure times up to 20 days, have shown reduced lycopene development and rate of softening when tomatoes were moved to 20° to ripen. While fruit held at 35° and moved to 20° never turned fully red, they did become softer and except for line 281 were significantly softer than the control fruit ripened at 20°.

Goodenough has shown that glucose and fructose levels increase under normal ripening conditions (7) which is similar to our findings in the larger tomato types. However, both sugars dropped in the cherry-type tomato fruit. The chilling-tolerant lines exposed to 5°C or moved from 5° to 20° had lower monosaccharide levels than the corresponding sensitive cultivars.

Goodenough also found that citrate levels increased with ripening in many cultivars while malate levels decreased (7). The levels in all tomato types in our experiment either remained the same as the initial levels or moved in the predicted direction.

Buescher (3) reported that tomato fruit stored at low temperatures exhibit an increase in citrate and a decrease in malate. He indicated that upon ripening these fruit continue to show a drop in malate, and citrate levels were higher when the treatment included a longer chilling time (21 days) prior to ripening. The malate levels for 'New Yorker' were as predicted. There was no decrease in malate for the other 3 tomato types after chilling and, in fact, the tolerant lines were elevated slightly from their initial levels. When the chilled fruit were ripened, all showed a drop in malate although this was not significant in line 281. Citrate levels from the 5°C treatment were all slightly higher than initial levels, agreeing with Buescher (3). On the other hand, both citrate and malate levels for all of the high temperature treatments were at least as high as the initial levels with the exception of malate levels in 'New Yorker'.

It has been reported that phosphofructokinase is a control point of respiration (4) and that it is inhibited by high concentrations of citrate (7). This would indicate that levels of citrate should result in high levels of monosaccharides. However, there was no temperature treatment under which an increase in citrate over the initial level resulted in a significant increase in either glucose or fructose.

Sucrose is found in small quantities in tomato fruit and decreases with ripening (7). Buescher (3) demonstrated that levels in the pericarp declined during low temperature storage and were very low in ripened fruit. While we did find that fruit ripened after chilling had the lowest level of sucrose, the fruit held at 5°C had higher sucrose levels in all but line 281. All tomato lines except 'Early Cherry' showed a slight decrease in sucrose levels in the normally ripened control fruit.

Phosphoric acid, although not often considered to be a major acid, was present in large enough quantities to be of interest. Chilling-tolerant lines contained higher quantities of this acid than their sensitive counterparts under all temperature treatments, which may point to a role of phosphoric acid in chilling tolerance. Yet this acid was found in cabbage and increased with

Table 5. Acid ratio of chilling-sensitive ('New Yorker' and 'Early Cherry') and chilling-tolerant (79-546 and 281) tomato cultivars held at different temperatures.

Temp (°C)	Ratio (citrate/malate)			
	New Yorker	79-546	Early Cherry	281
Initial	2.13 c[z]	2.07 c	2.36 d	2.20 d
5	3.06 bc	2.72 bc	3.02 cd	2.77 cd
5–20	3.78 ab	3.28 b	3.59 b	3.37 bc
20	3.39 abc	4.29 a	4.77 a	4.79 a
35	4.42 a	2.58 c	3.55 bc	3.53 b
35–20	4.35 a	2.20 c	2.86 d	2.85 cd

[z]Mean separation in each cultivar by Duncan's multiple range test, 5% level.

increasing storage time (13) which would appear to be more connected to senescence than to fruit ripening, although the 2 events are related.

The acid ratio (citrate/malate), an indicator of ripening, was highest in the 20°C control fruit in all but 'New Yorker' (Table 5). These fruit had the highest ratio when stored at 35°. All fruit exhibited a higher ratio when moved from 5° to 20° as would be expected. The results for the fruit stored at 35° were more confusing. While the ratio for the small tomato fruit dropped when they were moved from 35° to 20°, the 79-546 ratio remained the same and the 'New Yorker' ratio was high at 35° both before and after ripening at 20°. Thus, malate and citrate in fruit of 'New Yorker' shifted in a manner similar to ripening fruit. The similarity in behavior between 'Early Cherry' and 281 in the high temperature may have been due in part to the fact that 'Early Cherry' had such a limited survival rate in these treatments. Therefore, in addition to working with very limited samples it also is quite possible that the few fruit that survived had some degree of tolerance.

Equal quantities of glucose and fructose have been observed in many tomato cultivars during normal ripening (8); however, we consistently found higher glucose levels and a change in the glucose/fructose ratio under the different temperature regimes. The sugar ratios (Table 6) of the larger fruit were lowest in the control fruit ripened at 20°C and highest at 35°. The 'Early Cherry' ratio also was highest at 35° while it was lowest after transfer to 20° from 5°. The ratio of line 281 was lower than the initial ratio at all temperatures except 5°. The ratio generally dropped for all tomato types when the fruit at 5° or 35° were moved to 20°, except the 281 fruit ratio which was already low at 35°.

Table 6. Sugar ratio of chilling-sensitive ('New Yorker' and 'Early Cherry') and chilling-tolerant (79-546 and 281) tomato cultivars held at different temperatures.

Temp (°C)	Ratio (glucose/fructose)			
	New Yorker	79-546	Early Cherry	281
Initial	1.57 b[z]	1.67 b	1.45 b	1.42 a
5	1.42 c	1.50 c	1.31 c	1.33 a
5–20	1.36 d	1.44 cd	1.21 d	1.18 b
20	1.16 f	1.33 e	1.29 c	1.11 c
35	1.73 a	1.80 a	1.63 a	1.12 bc
35–20	1.30 e	1.35 de	1.38 b	1.13 bc

[z]Mean separation in each cultivar by Duncan's multiple range test, 5% level.

Chilling-tolerant fruit were firmer at all temperatures and ripened at a slower rate. Yet a comparison of the levels of sugars and acids revealed that similarities were observed between fruits of the same size rather than tolerance level. Goodenough (7) found that tomatoes stored in a controlled atmosphere exhibited sugar and acid levels similar to ripening fruit while their firmness and coloration remained unchanged. Tolerant fruit appear to respond similarly to tomatoes in controlled-atmosphere storage at both high and low temperatures, indicating a greater capacity for both chilling and heat tolerance. Between the 2 tolerant lines 281 shows the most predictable response to temperature treatments in that sugars decreased and organic acids increased with increasing temperatures.

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