Quantitative Analysis of Flavor Parameters in Six Florida Tomato Cultivars (*Lycopersicon esculentum* Mill)

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Mature green tomato fruits from six Florida-grown tomato cultivars, Duke, Sunny, Solar Set, Bonita, Olympic, and IFAS 7209, were harvested and allowed to ripen at 21 °C. Ripe fruits were homogenized and analyzed for sugar and acid content, and significant differences were found for glucose, fructose, and citric acid. By use of a headspace analysis technique, 17 volatile components were detected and quantified in tomato homogenate. Nine volatile components (hexanal, *trans*-2-hexenal, *cis*-3-hexenal, geranylacetone, acetone, 6-methyl-5-hepten-2-one, 1-penten-3-one, methanol, and 2-isobutylthiazole), some of which are reported to be important for fresh tomato flavor, showed significant differences between cultivars. Of the six cultivars tested, Solar Set generally showed the highest volatile levels, while Olympic showed the lowest.

Little quantitative information is available on the sensory parameter of flavor in fruits and vegetables. Plant breeders, therefore, often have difficulty in efficiently selecting for this characteristic. For tomatoes, selection for fresh market has emphasized yield, fruit size, lack of defects, and disease resistance, which has resulted in cultivars that sometimes have mediocre flavor.

Internal factors such as genetic control and resulting metabolic regulation (ethylene and respiration) influence flavor formation in fruits. Cultivar differences markedly influence aroma character as in the case of apples (Dirinck et al., 1989). For the tomato, some attention has been directed toward cultivar evaluation of flavor from the standpoint of sugar/acid ratios (Stevens et al. 1977; Kader et al., 1978; Jones and Scott, 1984). However, little is known about the approximately 400 volatile compounds that have been identified in the tomato (Petro-Turza, 1987) and how they are affected by plant culture, harvest maturity, postharvest treatments, and genetic control (cultivar) (Dirinck et al., 1989).

Of the many volatiles identified, only a few have been singled out as having an important role in fresh tomato aroma, while others are abundant but of unknown or disputed importance to flavor (Buttery et al., 1971, 1987, 1988; Ho and Ichimura, 1982; Petro-Turzak, 1987). Classification of tomato cultivars based on objective analysis of aroma composition and sugar and acid levels would be helpful for cultivar selection and for detection of relationships between cultivars.

Tomato fruits contain a complex mixture of volatile components at low concentrations that makes quantitative analysis difficult. Due to the dynamic nature of these volatiles, changes in volatile levels occur as enzymes and substrates are mixed in homogenized tissue, and these changes are enhanced by extraction. Extraction of volatiles is time-consuming and results in variability with different methods (Buttery et al., 1987, 1988). By use of a new GC headspace analysis technique, volatiles were quantified directly from tomato homogenate, thereby dispensing with the need for extraction.

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EXPERIMENTAL PROCEDURES

Materials. Cultivars Duke, Sunny, Solar Set, Bonita, and Olympic plus experimental hybrid IFAS 7209 were grown in a randomized block design with four blocks in a field trial at the Gulf Coast Research and Education Center, Bradington, FL, during May of 1989. These cultivars accounted for about 85% of the acreage grown in Florida in the 1989–1990 season. IFAS 7209 is a cross of the more flavorful parent of Solar Set with another breeding line. Mature green tomatoes were harvested two times, one week apart, 30 fruits per cultivar per harvest. The fruit were washed and allowed to ripen in storage chambers at 21 °C over a 2-week period. The first 18 fruits per harvest date to ripen were used in this study. Six samples, consisting of three ripe tomato halves from each harvest date, were homogenized for 30 s; 2 mL was then immediately pipetted into 6-mL vials with crimp-top caps and seals, frozen, and stored at −20 °C prior to volatile analysis. This resulted in 6 composite samples per harvest date for a total of 12 samples. The rest of the composite homogenates were also frozen for subsequent sugar and acid determinations.

Analysis of Color, Sugars, and Acids. Fresh homogenate color was analyzed for *L*, *a*, and *b* values by using a scale of lightness, red, and green on a Minolta CR-200 Chroma Meter (Goodenough and Thomas, 1981).

For sugar and acid analysis, 40 g of fruit homogenate was boiled in 70 mL of 80% ethanol, cooled, and filtered. The resulting extract was passed through a C-18 Sep-Pak (Waters/Millipore, Milford, MA) and a 0.45-μm Millipore filter. Sugars were analyzed on a Waters Sugar-Pak column at 90 °C with a mobile phase of 0.0001 M ethylenediaminetetraacetic acid disodium–calcium salt (CaEDTA) at a flow rate of 2.0 mL/min. Acids were separated on an Interaction Ion 300 column (Mountain View, CA) at 65 °C with a mobile phase of 0.0008 N sulfuric acid at a flow rate of 0.4 mL/min. A Perkin-Elmer LC-25 refractive index detector and LC-85B spectrophotometric detector at 210 nm were used to measure sugars and acids, respectively. Filtered analytical grade reagents were used for standard solution preparation to establish HPLC retention times and calibration. Determination of peak purity was accomplished by absorbance index (all wavelengths monitored simultaneously) on a Perkin-Elmer LC-235 diode array detector. Twelve composite homogenates per cultivar (six from each harvest date) were analyzed.

Analysis of Volatile Flavor Components. Tomato volatiles were quantified by using a method developed for citrus fruit and juice products (Nisperos-Carriedo and Shaw, 1990) that was modified for tomato. Two milliliters of tomato homogenate (three tomato halves, homogenized for 30 s, frozen for storage and later thawed within 1 min under cool tap water) was subjected to head-

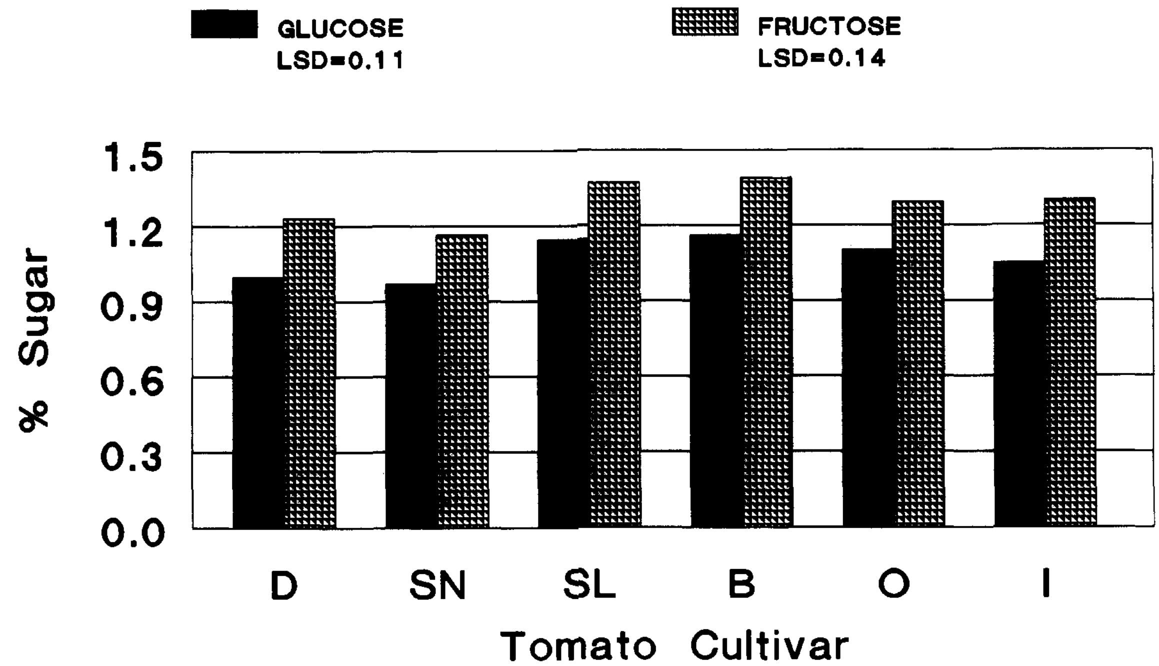

Figure 1. Sugar levels in fruits from six Florida tomato cultivars harvested at the mature green stage and ripened at 21 °C; Duke (D), Sunny (SN), Solar Set (SL), Bonita (B), Olympic (O), and IFAS 7209 (I). Data are means of 12 replicate composites of three fruits each.

space analysis using a Perkin-Elmer Model 8500 gas chromatograph equipped with a Model HS-6 headspace sampler, a 0.53 mm × 30 m polar Durowax column of 1.0-μm film thickness (J&W scientific, Folsom, CA), and a flame ionization detector. The samples were rapidly heated to 80 °C in the HS-6 headspace sampler heating block and incubated at this temperature for 15 min under pressure prior to injection. The different components were identified by comparison of retention times with those of standards and by enrichment of tomato homogenate with authentic samples. The tomato volatiles quantified in this study had also been previously identified for Sunny and Solar Set cultivars by using GC–MS with a 50-m wide-bore (0.31–0.32 mm) fused silica column of cross-linked 5% phenylmethyl silicone (Baldwin et al., 1990). Concentrations were calculated by using regression equations, determined by injecting five different concentrations of each standard to obtain a peak height calibration curve. The standard solutions were prepared by addition of 4 μL of an aqueous ethanolic solution of standards to 2 mL of bland tomato homogenate from which most volatile components had been removed by distillation at 60 °C. Determinations were made on six samples per cultivar (three from each harvest date) chosen at random. Sixteen flavor volatiles were quantified: hexanal, *trans*-2-hexenal, *cis*-3-hexenal, acetaldehyde, *trans*-2,*trans*-4-decadienal, geranylacetone, 6-methyl-5-hepten-2-one, 1-penten-3-one, acetone, ethanol, *cis*-3-hexenol, octanol, eugenol, methanol, 2-isobutylthiazole, and ethyl hexanoate.

Statistics. Data for the different components were analyzed by analysis of variance using the general linear model (GLM) procedure, a package program of the Statistical Analysis System (SAS Institute Inc., Cary, NC). The design used was a randomized complete block design (RCBD) with cultivars as treatments and harvest dates as blocks. Specific differences were determined by least significant difference (LSD). All comparisons were made at a 5% level of significance.

RESULTS AND DISCUSSION

Sugar, Acid, and Color Determinations. Differences between cultivars for sugars and acids were small but significant. Cultivars Bonita and Solar Set showed the highest levels and Sunny the lowest of both glucose and fructose. Bonita and Solar Set had significantly higher levels of glucose compared to Duke and Sunny, while Olympic showed significantly higher levels compared to Sunny as well (Figure 1). No significant differences were measured between Bonita and Solar Set in relation to fructose content. Fructose levels were significantly higher in Bonita compared to those in Duke and Sunny and significantly higher in Solar Set than in Sunny. There were no significant differences for harvest date.

Citric and malic acids were measured, but only citric acid levels showed significant differences between cultivars (Figure 2). Sunny tomatoes had significantly higher levels of citric acid compared to Bonita, IFAS 7209, Duke, and Olympic. Solar Set, in turn, showed significantly higher levels of citric acid compared to Olympic. Tomato flavor is partially due to the contribution of sugars and acids which may differ from one variety to the next (Stevens et al., 1977; Kader et al., 1978). Sugar–acid ratios may, therefore, correlate with taste differences. It would appear that Sunny fruit, being relatively high in acid and low in sugar, would not be a sweet tomato. Solar Set, on the other hand, being relatively high in both sugar and acid (since citric is the major acid in tomatoes), would be expected to have a tart but sweet taste. Fruit from the earlier harvest exhibited significantly higher citric acid levels than fruit harvested 1 week later (0.74 and 0.65%, respectively, LSD = 0.057).

There were no significant cultivar differences for color (L, a, and b values); however, there were significant differences for harvest date. Fruits from the first harvest date developed more red color than fruits harvested a week later.

Volatile Determinations. Sixteen tomato volatiles were identified and quantified by headspace analysis on the GC. A representative chromatogram of fresh tomato homogenate from a ripe Sunny tomato is shown in Figure 3. Data are presented for the nine volatile compounds that showed significant differences between cultivars. The different flavor components were grouped and presented as follows: Figure 4, aldehydes; Figure 5, ketones; and Figure 6, miscellaneous volatiles.

Hexanal, one of the major aldehydes in tomatoes, is considered to be important for fresh tomato flavor (Petro-Turza, 1987) and is reported to be a major contributor to tomato odor on the basis of calculated odor units (ratio

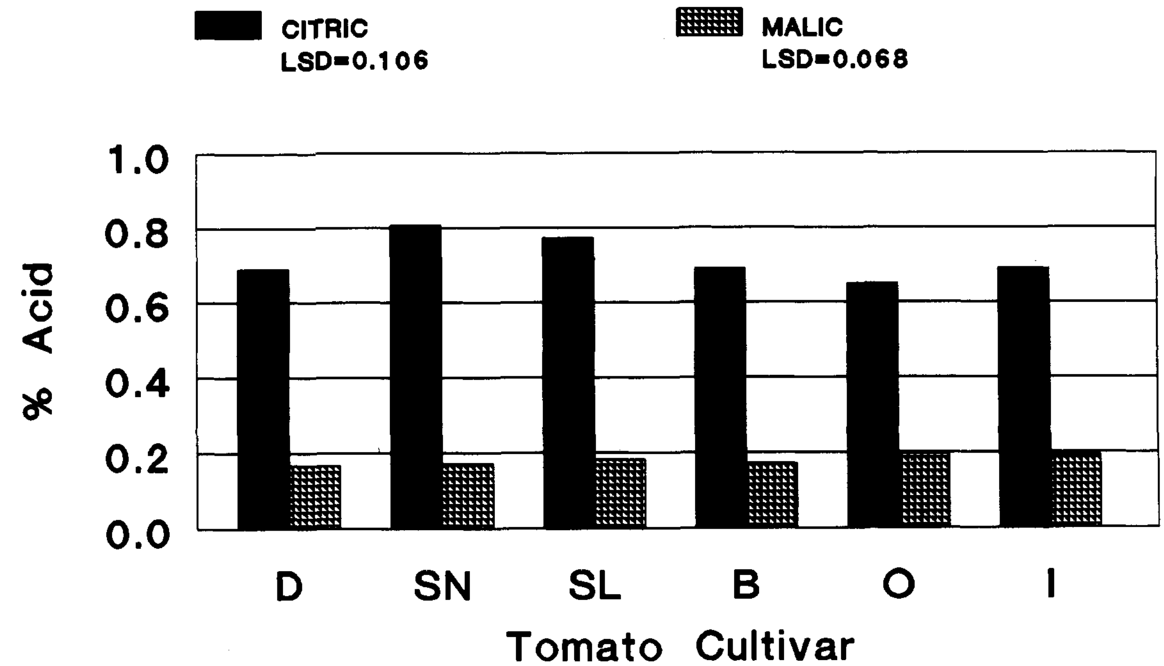

Figure 2. Organic acid levels in fruits from six Florida tomato cultivars harvested at the mature green stage and ripened at 21 °C: Duke (D), Sunny (SN), Solar Set (SL), Bonita (B), Olympic (O), and IFAS 7209 (I). Data are means of 12 replicate composites of three fruits each.

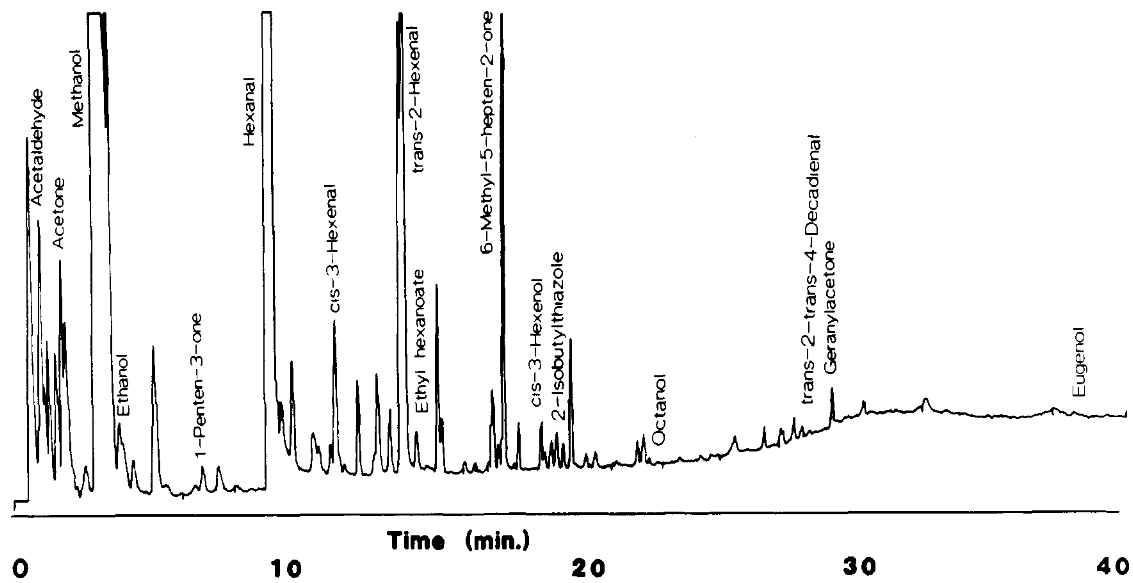

Figure 3. Capillary GC analysis of headspace volatiles from ripe Sunny tomato homogenate. For GC conditions see text.

of the concentration of the compound divided by its threshold concentration) (Buttery et al., 1987). It is reported to impart what has been described as a green fatty flavor. *trans*-2-Hexenal and *cis*-3-hexenal, having high odor unit values (Buttery et al., 1987), are also important for tomato flavor, contributing a green leafy herbaceous aroma (Heath, 1978). The levels of hexanal and *trans*-2-hexenal were significantly higher in Solar Set fruits compared to the levels in all other varieties (Figure 4). *trans*-2-Hexenal was the only aldehyde that showed a significant difference for harvest date, with fruits from the second harvest exhibiting higher levels of this volatile than fruits harvested earlier (9.31 and 8.02, respectively, LSD = 1.2). Solar Set and Duke tomatoes had significantly higher levels of *cis*-3-hexenal compared to IFAS 7209, Sunny, and Olympic. Solar Set also had significantly higher levels than Bonita (Figure 4). Another major volatile aldehyde, acetaldehyde, did not show considerable variation among cultivars. This component is considered to be important for fresh orange flavor but is of unknown importance to tomato (Nisperos-Carriedo and Shaw, 1990). There were no cultivar differences found for another important flavor component, *trans*-2,*trans*-4-decadienal.

Ketone volatiles also showed considerable variation among cultivars. Geranylacetone, 6-methyl-5-hepten-2-one, and 1-penten-3-one were significantly higher in Solar Set fruits than in the other cultivars (Figure 5). The latter two volatiles are reported to be important for tomato flavor and have high odor unit values (Buttery et al., 1971, 1987, 1988; Ho and Ichimura, 1982; Petro-Turzak, 1987). Ketones generally contribute a fruity aroma (Heath, 1978). IFAS 7209 and Solar Set fruit showed significantly higher levels of acetone compared to Bonita and Duke tomatoes, while IFAS 7209 showed higher levels compared to Olympic and Sunny as well. Ketone volatiles were not significantly different in fruits from different harvest dates.

There were no significant differences between cultivars for the alcohols ethanol, *cis*-3-hexenol, octanol, and eugenol, although ethanol, octanol, and eugenol showed significant differences for harvest date. Ethanol levels

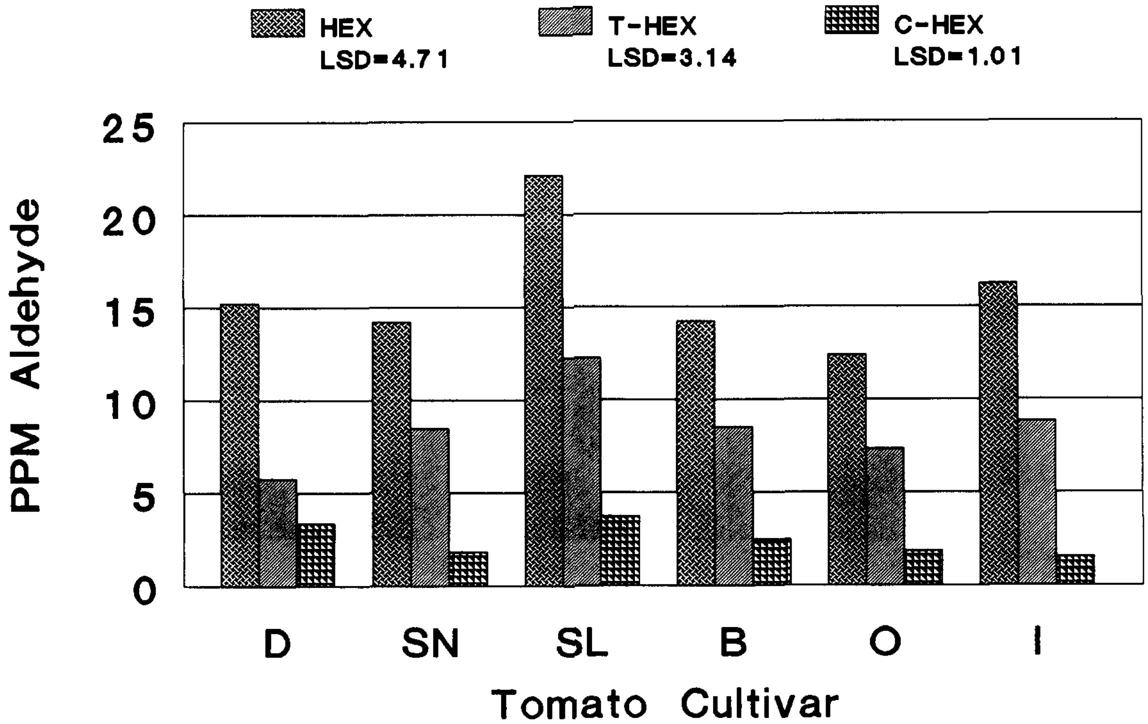

Figure 4. Aldehyde volatile levels in fruits from six Florida tomato cultivars harvested at the mature green stage and ripened at 21 °C: Duke (D), Sunny (SN), Solar Set (SL), Bonita (B), Olympic (O), and IFAS 7209 (I). Three aldehyde volatiles were quantified: hexanal (HEX), *trans*-2-hexenal (T-HEX), and *cis*-3-hexenal (C-HEX). Data are means of six replicate composites of three fruits each.

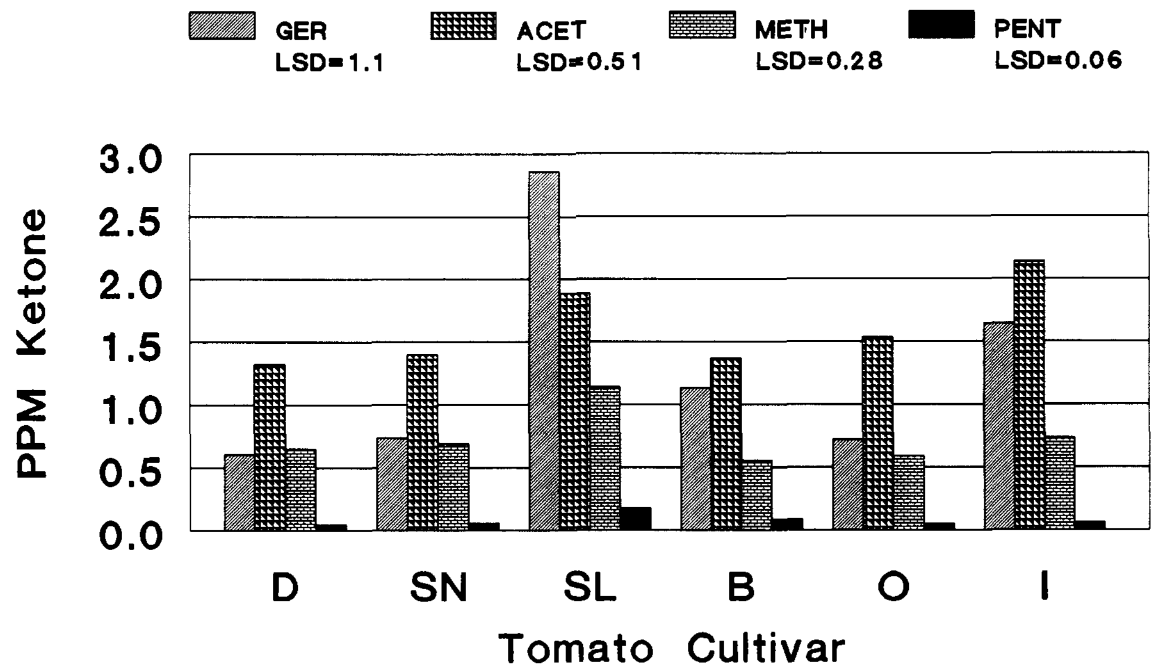

Figure 5. Ketone volatile levels in fruits from six Florida tomato cultivars harvested at the mature green stage and ripened at 21 °C: Duke (D), Sunny (SN), Solar Set (SL), Bonita (B), Olympic (O), and IFAS 7209 (I). Four ketone volatiles were quantified: geranylacetone (GER), acetone (ACET), 6-methyl-5-hepten-2-one (METH), and 1-penten-3-one (PENT). Data are means of six replicate composites of three fruits each.

were higher in fruits from the second harvest date, while octanol and eugenol levels were higher in fruits harvested 1 week later (data not shown). Methanol was significantly higher in Solar Set tomatoes than in Sunny, Olympic, and Duke (Figure 6). 2-Isobutylthiazole, an important sulfur-containing volatile with a grassy sweet fruity aroma (Petro-Turza, 1987) and high odor unit value (Buttery et al., 1987), was also significantly higher in Solar Set tomatoes compared to the other cultivars (Figure 6). IFAS 7209 had significantly higher levels of this component compared to Olympic and Duke tomatoes. Ethyl hexanoate was the only ester quantified, but no cultivar differences were found. None of these volatiles showed differences for harvest date.

Comparison of the quantitative results of volatile levels presented here to those reported by other workers using different techniques of analysis show some differences. Levels for hexanal and *trans*-2-hexenal were high in our fruits compared to levels in previous studies (12–22 compared to 2–4 ppm and 6–12 compared to 1–2 ppm for hexenal and *trans*-2-hexenal, respectively), while *cis*-3-hexenal was low (2–4 compared to 9–16 ppm) (Buttery et

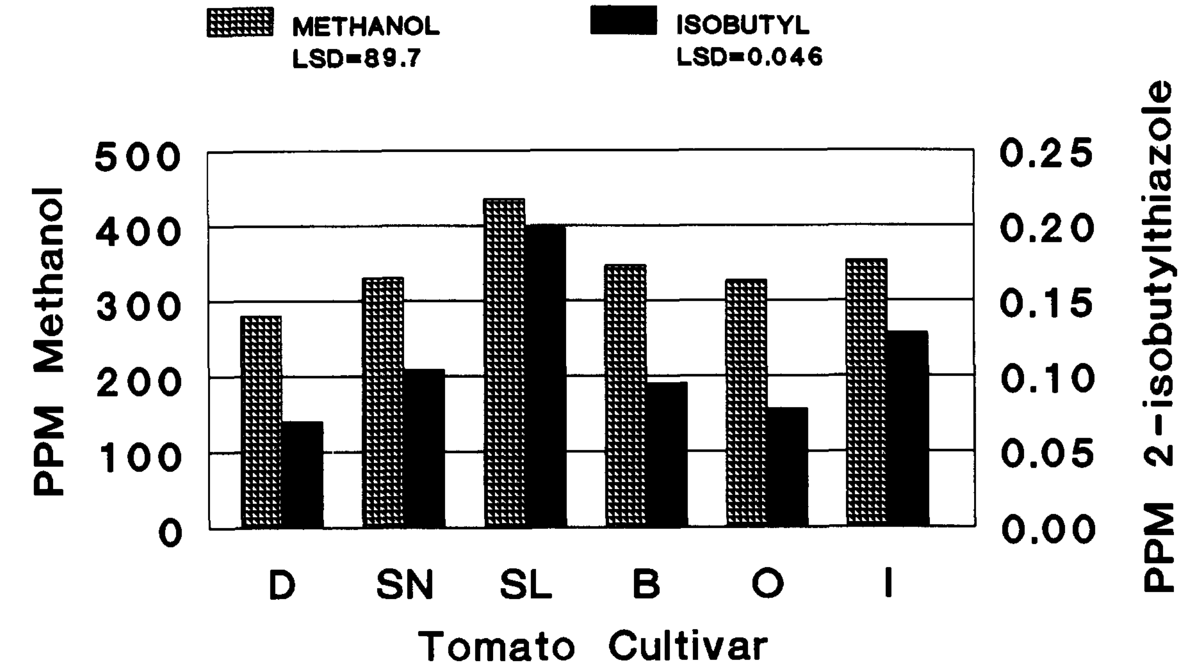

Figure 6. Methanol (METHANOL) and 2-isobutylthiazole (ISOBUTYL) volatile levels in fruits from six Florida tomato cultivars harvested at the mature green stage and ripened at 21 °C: Duke (D), Sunny (SN), Solar Set (SL), Bonita (B), Olympic (O), and IFAS 7209 (I). Data are means of six replicate composites of three fruits each.

al., 1987, 1988). Unfortunately, the blending–bottling–heating/equilibration–sampling time cycle still induced some changes in the volatile profile. When model systems were run through the process, some cis–trans isomerization occurred during preparation and storage of samples, resulting in the lower levels of *cis*-3-hexenal and higher levels of *trans*-2-hexenal. In addition, levels of hexanal and geranylacetone increased 1–2-fold during freezing and thawing. Methanol levels were high in all cultivars. High levels of methanol were also observed in freshly homogenized samples for which headspace sampling was conducted immediately, without heating or pressurization, and in samples prepared with calcium chloride (Buttery et al., 1987) to curtail enzyme activity (methanol levels were high enough to be detected by these methods, whereas the other volatile levels were not). The high levels of methanol may have been due to the presence of pectin methylesterase, an enzyme in tomatoes that liberates methyl groups from pectin in cell walls. If this is the case, the reaction must proceed very quickly upon homogenation. About a one-third increase in methanol was observed during freezing and thawing. The relative differences between varieties for the above-mentioned volatile changes, however, would have remained the same. Improved techniques for processing the tomato homogenate are under investigation.

Volatile compounds that characterize fresh tomato flavor are reported to be derived from fatty and amino acid metabolism and breakdown of carotenoids (Buttery et al., 1971, 1988; Heath and Reineccius, 1986; Petro-Turza, 1987). Many volatiles increase during ripening along with increased synthesis of lycopene, the pigment responsible for red color in tomatoes (Baldwin et al., 1991). The differences in levels of volatile components between cultivars noted in this study, however, could not be attributed to color variation since the were no significant cultivar differences for *L*, *a*, or *b* color values at the red ripe stage, at which time volatiles were analyzed.

In conclusion, according to the objective sugar, acid, and flavor volatile analysis presented here, it would appear that Solar Set tomatoes have generally better flavor due to relatively higher sugar, acid, and volatile contents compared to the other cultivars tested. Conversely, Olympic fruits would be expected to have poor flavor due to relatively low volatile content. Such a conclusion would have to be verified by sensory evaluation, however. In one taste panel test, Solar Set scored favorably relative to Sunny and Duke for overall acceptability (Scott et al., 1989). Good flavor may also be due to the proper balance in individual volatile, sugar, and acid levels. In the event that sensory studies concur with such objective analysis of fruit flavor parameters, studies of this nature would be of benefit to tomato breeders who are interested in selecting for taste as well as producitvity.

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Registry No. Glucose, 50-99-7; fructose, 57-48-7; citric acid, 77-92-9; malic acid, 6915-15-7; hexanal, 66-25-1; *trans*-2-hexanal, 6728-26-3; *cis*-3-hexanal, 6789-80-6; acetaldehyde, 75-07-0; *trans*-2,*trans*-4-decadienal, 25152-84-5; geranylacetone, 3796-70-1; 6-methyl-5-hepten-2-one, 110-93-0; 1-penten-3-one, 1629-58-9; acetone, 67-64-1; ethanol, 64-17-5; *cis*-3-hexenol, 928-96-1; octanol, 111-87-5; eugenol, 97-53-0; methanol, 67-56-1; 2-isobutylthiazole, 18640-74-9; ethyl hexanoate, 123-66-0.