

Quantitative Comparison of Isoamylol, Pentanol, and 3-Hexenol-1 in Tomato Juice

Varietal and Harvest Differences and Processing Effects

John Hal Johnson,¹ W. A. Gould, A. F. Badenhop,² and R. M. Johnson, Jr.

Procedures were devised to obtain a threefold concentrate of tomato juice volatiles to extract with ether which was salted out with the volatiles and to remove the solvent with minimal contamination of a small final volume. Suitable gas chromatographic conditions for analysis of the volatiles were established. The over-all separation, isolation, and analysis procedure was repeatable to within 4% of the replicate average. The quantities of iso- and active amyl alcohols (considered together), *n*-pentanol, and *cis*-3-hexenol-1 were separated and compared. The amount of a volatile compound varied with the variety and with the harvest. In fresh juice har-

vested during 1962, the amyl alcohols varied between 1.8 to 13.4 p.p.m. among varieties and as much as 4.8 p.p.m. between harvests within a variety. *n*-Pentanol ranged between 0.3 to 1.8 p.p.m. among varieties and varied as much as 1.1 p.p.m. between harvests of a variety. *cis*-3-Hexenol-1 calculated as hexanol ranged between 4.0 to 30.2 p.p.m. among varieties with differences up to 16.3 p.p.m. between harvests. The total quantity of the three volatile compounds varied from 7.4 to 40.2 p.p.m. between varieties. Losses due to processing varied with variety and with compound.

Gustafson (1934) reported and Rakitin (1945) later studied the presence of ethanol and acetaldehyde in tomatoes. Traces of formic and acetic acids, formaldehyde, and methanol also were reported. Sandor (1935) found volatile acids equivalent to 24 to 60 mg. per 100 grams in tomatoes. Jacquin and Tavernier (1955) reported 18 to 22 mg. of methanol per 100 grams. Spencer and Stanley (1954) conducted extensive research on the volatiles of tomato juice. They identified acetaldehyde, ethyl acetate, and isovaleraldehyde in the Pearson variety. Eighteen other carbonyl compounds were separated but not identified. In fresh and processed juice, 2 p.p.m. of esters, 1 and 0 p.p.m. of acids, and 33 and 43 p.p.m. of carbonyls, respectively, were found. Other fractions were separated but not identified.

Matthews (1960) reported the probable presence of acetaldehyde, furfural, and acetone while Hein and Fuller (1963) suggested the presence of diacetyl, α -pinene, citronellal, limonene, and citral. Pyne and Wick (1965) reported the following volatiles to be present in fresh tomatoes: ethyl acetate, ethanol, 2-propanol, isobutanol, butanol, isovaleraldehyde, 2-methylbutanol, 3-methylbutanol, *n*-hexanol, benzaldehyde, and methylsalicylate. Although no attempt was made at quantification, *cis*-3-hexenol-1 was reported present in large amounts. Schorrmuller and Grosch (1964, 1965) identified glyoxal, methylglyoxal, cinnamaldehyde, hydrocinnamaldehyde, 2-butanone, 2-pentanone, methylheptenone, diacetyl, and a 5-carbon dicarbonyl in a tomato extract. As noted above, most reports have indicated only in general, if at all, the quantity of volatile matter present. This study was under-

taken to determine the quantity of some volatiles in tomato juice and their variation with harvest and variety differences.

METHODS AND MATERIALS

The tomatoes were produced during 1962 on the Northwest Experiment Station, Hoytville, Ohio. The mature fruit was harvested September 6, 12, and 19. Field run tomatoes were pulped, the maturity of which was 85 to 90% USDA No. 1's, and 10 to 15% No. 2's.

The varieties were Glamour, Rutgers, Fireball, KC 135, KC 146, Libby's C 52, ES 24, Heinz 1350, Heinz 1370, and Heinz 1409. No differences due to cultural practices were believed present in the tomatoes. It was assumed that variety and harvest were the primary variables.

Juice samples were obtained after the tomatoes were washed and steamed. The volatiles extraction apparatus was patterned after that of Dimick and Makower (1951) with some modifications. A 26 \times 1/2 inch O.D. superheater tube was used. The distillate reservoir was a 4-liter flask directly below the condenser. Beyond the three cold traps, which were immersed in dry ice and alcohol, was a 3-liter vacuum reservoir. A stopcock connected the vacuum reservoir to a water aspirator. Constant steam pressure was ensured by a valve with a gage located in the steam line.

The system was evacuated to 24 inches of mercury with the steam valve set at 30 p.s.i. Thereupon, a 2-liter sample was introduced, and the vacuum was maintained between 10 and 11 inches of Hg. When the air was purged from the system, little additional aspiration was needed. After the separation, water was flushed through the system. The condensate, which ranged from 650 to 800 ml., was cooled in an ice bath to about 15° C. The condensate flask was rinsed with two 50-ml. portions and the cold fingers with a 15-ml. portion of diethyl ether (Mallinckrodt A.R.). The rinsings were dissolved in the aqueous condensate and placed in a beaker to which was added about 200 grams of (NH₄)₂SO₄ (Baker Reagent). The beaker

Ohio Agricultural Experiment Station, Department of Horticulture and Forestry, and Institute of Nutrition and Food Technology, The Ohio State University, Columbus, Ohio 43210

¹ Present address, Department of Food Science, University of Florida, Gainesville, Fla. 32601

² Present address, Department of Food Science and Technology, Cornell University, Ithaca, N. Y. 14850

was covered with foil and stirred until solution was complete. The resulting two layers were separated. The aqueous solution was extracted again with 20 ml. of ether. The combined extracts (about 110 ml.) were stored at -20°C . for later solvent removal.

Removal of the ether solvent was attempted by nitrogen gas stream, distillation in vacuo, and distillation at atmospheric pressure in a long tube. The latter method gave much better recovery. A round-bottomed tube, 14×1 inch O.D., was employed which had a female joint with a 16-inch air condenser inserted. The tube was immersed in a water bath maintained at $45^{\circ}\text{C} \pm 5^{\circ}$ with the ether level kept above that of the water bath. When the volume was reduced to about 1 ml., the ether solution was cooled and placed in a foil-lined screw cap vial. The sample was stored at -20°C . until analyzed.

A Barber Colman No. 20 gas chromatograph with a hydrogen flame detector was used. The column was an 8-foot \times $\frac{1}{8}$ -inch copper tube packed in this lab with Anakrom ABS 90- to 100-mesh support coated with 20% Carbowax 20 M (Analabs).

The recorder was a Honeywell SY153 with a chart speed of 0.5 inch per minute and equipped with a Disc Integrator (No. 201). The recorder had a range of 0 to 10 mv. and a response time of 1 second full scale.

Analysis conditions were as follows: The injector temperature was 300°C . The column pressure was increased from 10 to 17 p.s.i. after 2 minutes for a better base line. The flow rate at 17 p.s.i. was 18 ml. per minute of nitrogen. The temperature was programmed between 60° to 210°C . at the rate of approximately 6°C . per minute.

Three replications of the separation and concentration procedure gave distillates ranging from 680 to 780 ml. The extracts yielded duplications within 4% of the average for the three compounds listed below. A solution of 40 μl . of amyl alcohol in 2 liters of double distilled water gave a recovery of 42% when the entire procedure was completed. No interfering substances of any significance were detected when a procedural blank was run. Calibration curves of isobutanol were made and were linear for the range of concentrations used.

A marker of 1.0 μl . of isobutanol was added to the volatiles solution after an initial determination to indicate the volume and the relative concentration of the other components. Calculations were made from the integrator count. Since the base line was not stable, suitable base line counts were subtracted from each peak. Of the identified compounds reported in the literature, 3- and 2-methyl-1-butanol, *n*-pentanol, and *cis*-3-hexenol-1 were compared quantitatively in this study. Since 3- and 2-methyl-1-butanol emerged as one peak, they were reported together as isoamylol.

Under the conditions of analysis, the relative retention times of isobutyl, isoamyl, *n*-pentyl alcohols, and *cis*-3-hexenol-1 were 1.00, 1.34, 1.45, and 1.79, respectively. On the basis of equal weight using a flame detector, Ettre (1962) reported relative responses of 87, 100, 113, and 114 for ethanol, 2-propanol, *n*-butanol, and isobutanol, respectively. Responses of 127, 129, and 140 were calculated for *n*-pentyl, isoamyl, and *n*-hexyl alcohols, respectively. The integrator counts for isobutanol, isoamyl, *n*-pentyl, and *n*-hexyl alcohols were multiplied by factors

of 1.00, 0.898, 0.814, respectively. The factor calculated for *n*-hexanol was used for *cis*-3-hexenol-1, since it was eluted from the column next to *n*-hexanol.

RESULTS

Quantitative differences were evident not only between variety but also between harvests within a variety. Considerable difference between varieties and harvests was evident (Figure 1) in the amount of isoamyl alcohol present.

The amount of *n*-pentanol was consistently much lower than isoamylol (Figure 2). However, Heinz 1350, ES 24, and KC 146 varieties which were high in isoamylol also were higher in pentanol.

cis-3-Hexenol-1 (hexenol) (Figure 3) was present as the major volatile component for all varieties of fresh juice with the exception of the September 12 harvest of Rutgers which had more isoamyl alcohol present.

A comparison of the relative amounts of the volatiles from the different harvests showed some consistent relationships. Libby's C 52, ES 24, Heinz 1350, and Heinz 1370 varieties had more hexenol in the first than the third harvest. Glamour, Fireball, and Heinz 1409 had more hexenol in the third than the first harvest. Glamour also showed this trend with isoamylol, Fireball with pentanol, but Heinz 1409 with all three compounds. Rutgers, KC 146, and ES 24 had less hexenol in the second as compared to the third harvests. This was not the case with the other two compounds for Rutgers and KC 146. With ES 24, isoamylol only compared with hexenol in the three harvests.

Comparison of fresh and processed juice (Figure 4) shows that without exception, processing reduced the amount of these three compounds. There also were differences with varieties. Isoamyl alcohol decreased 22, 18, 6, and 5%, respectively, for ES 24, Rutgers, KC 146, and Heinz 1370 varieties. Losses of pentanol and hexenol were generally higher, being 63, 45, 35, and 5%, respectively, for ES 24, Rutgers, Heinz 1370, and KC 146. The same sequence was found with *cis*-3-hexenol-1 losses of 73, 57, 51, and 41%, respectively. ES 24 variety lost the highest and KC 146 the least percentage of the three volatiles.

DISCUSSION

Volatiles Isolation and Analysis Methods. The flash steam distillation of the tomato juice volatiles in vacuo, employing the juice as the steam source, had the advantage of no outside contamination from steam injection and a minimal amount of aqueous distillate. The steam flashing of the juice also permitted maximum evaporation of steam distillables. The low vacuum system, 10 to 11 inches of Hg, did not require an ice water condenser, and volatilized materials were quantitatively isolated since the system was essentially closed.

During extraction, the addition of ether to the aqueous distillate before the admixture of the $(\text{NH}_4)_2\text{SO}_4$ appeared to have some advantage over adding the salt first. The prior addition of the ether ensured that, during further work, only negligible quantities of a compound with a

higher boiling point than ether would be lost. If the salt were added first, the volatiles would have been forced from solution to the surface of the liquid where evaporation would have been rapid, or to the surfaces of the container. On the surfaces of the container or in the water medium itself, the extractive ability of immiscible solvent globules

would have determined the efficiency of the process. However, with ether partially soluble in the cooled aqueous distillate, the ether-soluble molecules would have been diluted when salted out by the addition of the ammonium salt.

During the ether solvent removal, the apparatus em-

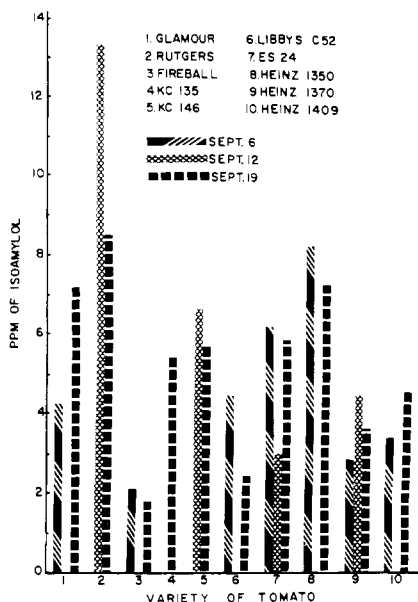


Figure 1. Isoamylool in fresh tomato juice—varietal and harvest differences

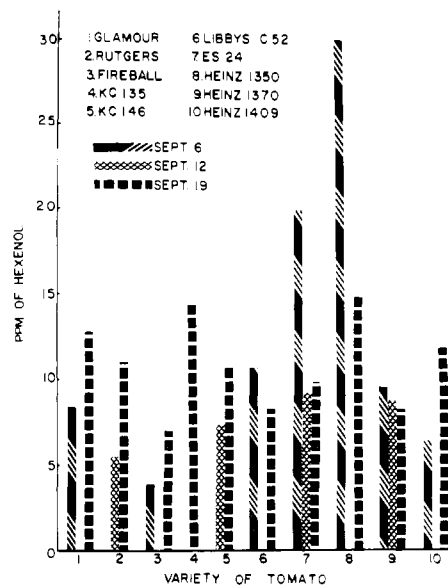


Figure 3. *cis*-3-Hexenol-1 in fresh tomato juice—varietal and harvest differences

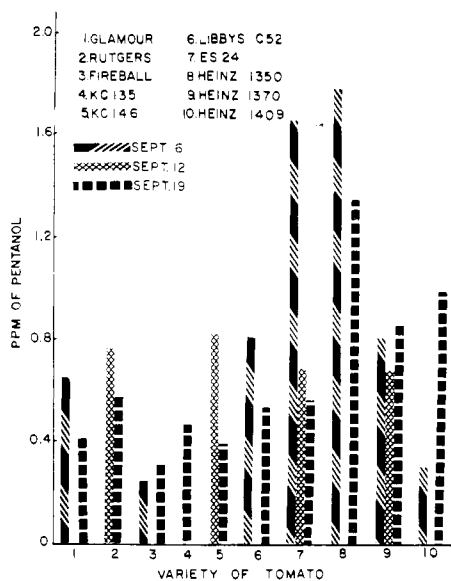


Figure 2. *n*-Pentanol in fresh tomato juice—varietal and harvest differences

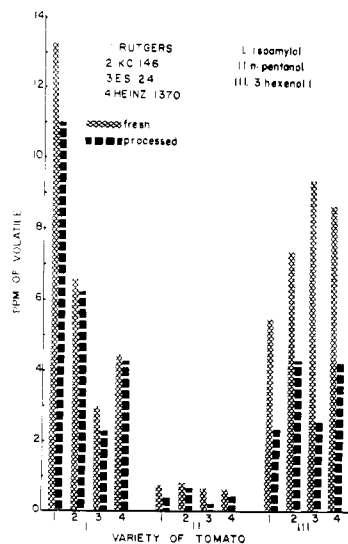


Figure 4. Some volatiles of fresh vs. processed tomato juice

ployed only an air condenser for refluxing. As opposed to a packed column, it was possible to use a small liquid volume which was unimportant at the outset with the volume near 100 ml., but was critical when the volume was reduced to about 1 ml. The lack of inert packing in the column reduced the likelihood of contamination. Again, with a packed column, a high temperature in the distilling pot would have increased the possibility of chemical action.

Varietal and Harvest Differences. Fireball, an early variety which was generally low in solids, was also low in volatiles. Rutgers and KC 146, which were related genetically (Interim Report of the Committee on Varietal Pedigree 1958-1960, 1961), showed similar relationships between harvests in the three volatiles measured in fresh juice. Both varieties had less hexenol in the first as compared with the second harvest, and both varieties tended to have a smaller amount of hexenol present compared with the amount of isoamylol detected. While these were the only varietal relationships known, nevertheless a possible connection of genetically related varieties to some volatiles seemed likely.

The data reported here show that fruits harvested one week apart differed considerably in the amount of volatiles present. Tomatoes harvested at different times would be subject to variation in weather and soil conditions.

Fertility, moisture, heat, and sunlight would affect the composition. Since less than 30 p.p.m. of the volatile was present, a small variation might have had a large effect on the volatiles.

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