under these conditions should increase early growth and inoculation with mycorrhizal fungi may also prove beneficial. Inoculation with mycorrhizal fungi may be the only means available to grow healthy, vigorous seedlings in raised seedbeds where fumigation is intended to eradicate *P. parasitica.* Application of low rates of P in conjunction with inoculation may increase the growth response to mycorrhizal infection.

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Amino Acid Composition and Flavor of Fresh Market Tomatoes as Influenced by Fruit Ripeness When Harvested[1]

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Abstract. **Four amino acids (glutamic, γ-aminobutyric, glutamine, and aspartic) make up about 80% of the total free amino acids in fruits of tomato (*Lycopersicon esculentum* Mill., cv. Cal Ace). Fruits harvested at the table-ripe stage contained more alanine and less glutamic acid than those picked green or at the breaker (incipient red color) stage and ripened at 20°C to table-ripe. The higher glutamic acid concentrations in fruit picked at the breaker or earlier stages were paralled to higher scores for "off-flavor," as described by a taste panel, relative to fruits picked table-ripe. However, when monopotassium glutamate (60, 120, or 180 mg/100g) was added to diced table-ripe fruits, the panelists were not able to detect flavor differences due to increased glutamic acid concn. Differences in amino acid composition associated with fruit ripeness when picked do not appear to be directly related to flavor differences.**

Reviews (1, 5, 11) of information on amino acids content in tomato fruits show limited quantitative data and considerable variation among reports. Some of this variation was atrributed to cultivars, truss position, and fertilization practices. Freeman and Woodbridge (4) found that glutamic and aspartic acids increased markedly, while alanine, arginine, leucine, and valine decreased with ripening. Davies (2) reported that glutamic acid concn increased approx 10-fold and aspartic acid more than doubled from the mature-green to the red stage of ripeness. He also found that cultivars differend significantly in glutamic acid but not in aspartic acid. Yu et al. (14) reported that glutamic acid concn doubled during ripening. Yamanaka et al. (13) observed that γ-aminobutyric acid decreased about 33% during ripening, and glutamic and aspartic acids increased

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approx 3.5- and 2.5-fold, respectively. Leucine, isoleucine and valine decreased slightly, and a small amount of proline and methionine was detected in over-ripe fruits.

Studies of organoleptic characteristics of pure amino acids in powder form or in aqueous solutions (7, 8, 9, 10, 12) show that various amino acids can be described as being sweet, sour, salty, or bitter. Solms (9) reported that L-glutamic acid has a unique taste-potentiating property. He concluded that the contribution of amino acids to flavor of foods often exceeds the taste properties of the pure compounds. Monosodium glutamate is widely used as a flavor potentiator. Kirimura et al. (7) determined threshold values of amino acids using solid and aqueous samples and found them to be between 0.3 and 30 mg/l. They concluded that while some amino acids contribute to the inherent tastes of foods, the buffer action of amino acids can also affect taste.

The possible significance of amino acids in tomato flavor has not been given much attention. DeBruyn et al. (3) investigated the impact of adding glutamic acid, aspartic acid, glutamine, and asparagine to tomato juice on taste and found that a 3- or 4-fold increase in their content was unfavorable.

In our studies on the effect of fruit ripeness when picked on tomato flavor (6), we found that picking fruits before fully ripe had a negative effect on their flavor. One striking observation was the detection of increased "off-flavors" in fruits harvested as greens or breakers and ripened off the plant relative to those picked table-ripe. Although the magnitude of this "off-flavor" was coincident with the concn of some volatile compounds, nonvolatile components (sugars and acids) appeared to influence the impact on these volatiles (6). The objectives of this study were to determine if differences exist in free amino acids composition among tomato fruits picked at several stages of maturity and ripeness and whether possible differences are related to the "off-flavor" defect.

Materials and Methods

Fruits. 'Cal Ace' tomato fruits were obtained during the period, Aug 18 to Sept 22, 1975, from plants grown in the field at Davis, California, using standard cultural practices. Field ambient temp during this period fluctuated between a mean minimum of 14.7°C and a mean maximum of 33.3°. Fruits were harvested at the dark-pink (DP), light-pink (LP), breaker (B), or full-sized green stages of maturity and ripeness (6), then held at 20° in glass 20-liter jars under a humidified air flow (about 100% relative humidity) until the table-ripe (TR) stage. No effort was made to exclude light during ripening. Green fruits were sorted after 5 days and those showing any red coloration were separated as typically-mature-green (TMG) when harvested. The remaining green fruits were sorted after an additional 5 days and those showing any red color were selected as partially-mature-green (PMG) while those that were still green were classified as immature-green (IMG) when harvested. When the fruits reached the table-ripe stage, a sample of 10 fruits was frozen whole in Scotch-pak bags and kept at –40° until analyzed. Four replicates of each maturity and ripeness stage were used in this experiment.

In the 1977 experiment, 'Cal Ace' fruits were also obtained from plants grown in the field at Davis, California. At each sampling date, at least 60 fruits were selected at the breaker stage (incipient red color) and divided into 3 treatments of 20 fruits each as follows: A) Fruits harvested and immediately frozen in Scotch-pak bags. B) Fruits harvested and placed at 20°C in 20-liter glass jars without excluding light, under a humidified air stream (15-20 liters/hr) until they reached the table-ripe stage, then were frozen. C) Fruits tagged and left to ripen on the plant until the table-ripe stage, then harvested and frozen. This procedure was repeated 3 times during the period between Sept 2 and Oct 18. All frozen samples were kept at –40° until analyzed. Field temp during the duration of this experiment fluctuated between a mean minimum of 12° and a maximum of 30°.

Amino acid analysis. Fruits were allowed to thaw overnight at 10°C, then blended. The extracted juice was centrifuged at 1,000 rpm for 10 min and the supernatant was separated. To 5 ml of this serum, 20 ml of 6% sulfosalicylic acid were added, then the homogenate was centrifuged at 9,500 rpm for 10 min to precipitate proteins. A 2-ml aliquot of the supernatant was frozen until analyzed. Free amino acid analysis was carried out using a Beckman Model No. 121M amino acid analyzer.

Sensory evaluation. Procedures used in 1975 to score flavor characteristics including "off-flavor" were described in a previous report (6). In 1976 table-ripe fruits with or without added monopotassium glutamate at 60 mg/100 g of diced fruits were compared in 2 triangle tests by a trained panel of 15 judges. In 1977, fruits picked at the table-ripe stage were diced and monopotassium glutamate was added at 60, 120, or 180 mg/100 g of diced fruits. Changes in acidity were monitored. These samples were presented randomly in 3 replications to a trained panel of 18 judges. The panelists were asked to detect differences with a triangle test and to describe the direction and magnitude of the differences.

Titratable acidity was determined by titrating tomato juice to pH 8.1 with 0.1N NaOH and pH was measured with a Corning digital 109 pH meter.

Results and Discussion

Amino acid composition. Glutamic acid, γ-aminobutyric acid, glutamine, and aspartic acid represent about 80% of the total free amino acids in tomato fruits ripened on or off the plant (Table 1). Fruits harvested at the light-pink or dark-pink stages and ripened off the plant to table-ripe had a lower content of the 4 predominant free amino acids than fruits harvested table-ripe, but the differences were significant for only glutamic acid and glutamine. The other free amino acids in tomato fruits are listed in Table 1, in a descending order, according to their concn in fruits harvested at the table-ripe stage. Fruits ripened on the vine contained more alanine than those picked at earlier stages of ripeness and ripened off the plant. They were also higher in asparagine content than fruits harvested PMG, LP, or DP and ripened at 20°C. Differences in total free amino acids between fruits harvested as table-ripe and those harvested green or breaker were small. However, the total free amino acids in fruits harvested at the light-pink stage and ripened at 20° was about 58% relative to that of fruits harvested at the table-ripe stage. Freeman and Woodbridge (4) reported that fruits ripened off the vine contained more total amino acids and larger quantities of both aspartic and glutamic acids than fruits ripened on the vine. Our data for fruits picked green or breaker (Table 1) are in agreement with their findings on glutamic acid, but not for aspartic acid or total free amino acids. This may be due in part to differences in cultivars used, method of analysis since they used paper chromatography (a semiquantitative method), and lack of statistical analyses in their report.

In the 1977 study, 5 amino acids were significantly different among fruits analyzed at the breaker stage and those analyzed at the table-ripe stage whether ripened on or off the plant (Fig. 1). Fruits ripened off the plant contained more glutamic and aspartic acids and less threonine, serine, and alanine than those ripened on the plant, but these differences were statistically, significant only for threonine and alanine. Table-ripe fruits whether ripened on or off the plant, had about a 3- and a 2-fold increase in glutamic and aspartic acids relative to breaker fruits. Fruits ripened off the plant lost more threonine and serine during ripening than those ripened on the plant. Although fruits ripened on or off the plant were subjected to different temperatures, they required about the same duration

Table 1. Free amino acids composition of 'Cal Ace' tomatoes harvested at various stages of maturity and ripeness[z], then ripened at 20°C *vs* those harvested at the table-ripe stage. Means of 4 replicates.

Amino acids	Free amino acid composition (mg/100 ml juice)[y]						Fruits picked TR
	Picking stage for fruits ripened off the plant to TR						
	IMG	PMG	TMG	B	LP	DP	
Glutamic	165.8ab	187.9a	172.0ab	159.8abc	92.9cd	89.7d	112.8cd
γ-Aminobutyric	66.4	63.8	58.9	51.1	31.7	57.0	71.0
Glutamine	54.8a	70.0a	55.1a	51.5ab	20.3c	25.7bc	62.4a
Aspartic	38.9	35.5	45.4	40.6	25.6	25.4	43.4
Serine	4.8	5.4	7.7	9.5	5.4	6.5	11.8
Asparagine	7.4ab	4.9b	6.9ab	6.8ab	3.6b	4.0b	10.1a
Alanine	2.7b	2.4b	3.4b	3.4b	3.4b	4.3b	6.9a
Threonine	5.3	4.4	5.0	5.4	3.1	4.2	6.8
Phenylalanine	5.5	4.7	5.6	6.0	3.7	3.8	6.7
Histidine	5.2	3.9	5.2	5.4	3.4	4.6	5.0
Arginine	4.0	3.6	4.1	4.2	2.8	3.0	3.8
Lysine	3.6	3.1	3.7	4.0	3.5	3.9	3.6
Leucine	2.4	2.4	2.8	2.6	2.3	2.4	3.0
Isoleucine	1.7	1.5	1.9	1.4	1.0	1.3	2.9
Tyrosine	1.4	1.2	1.3	1.2	1.1	1.3	1.7
Valine	1.2	1.8	1.2	1.3	0.9	1.1	1.6
Methionine	1.2	1.1	1.4	1.2	1.4	1.1	1.5
Tryptophan	1.3	1.2	1.2	1.1	0.8	0.8	1.3
Glycine	0.7	0.7	0.7	0.9	0.8	0.8	1.1
Total	374.1a	399.3a	383.4a	357.3ab	207.5c	240.8bc	357.9ab

[z]IMG = immature-green, PMG = partially mature-green, TMG = typical mature-green, B = breaker, LP = light-pink, DP = dark-pink, TR = table-ripe.

[y]Mean separation in rows by DMR test, 5% level.

to reach table-ripeness.

Glutamic acid concn in table-ripe fruits (ripened on or off the plant) in 1977 (Fig. 1) was higher than in those analyzed in 1975 (Table 1), but the relative differences were similar. This variation may have been due to changes in cultural practices, especially fertilization, which are known to influence amino acid composition in tomato fruits (5). Since no information is available for the effect of climactic factors on the amino acid composition of tomato fruit, we cannot rule out that such factors, especially temp, did influence the observed differences among fruits ripened on or off the plant. However, for the purpose of this study we were more concerned with the fact that differences associated with picking stage existed than with the reasons for such differences.

Variation in amino acid composition vs. flavor. Only 4 amino acids (glutamic, glatamine, asparagine, and alanine) varied with maturity and ripeness stage at picking. Three of these amino acids (alanine, asparagine, and glutamine) known to be sweet (8) were generally higher in fruits ripened on the plant than in those ripened off the plant. Although, the differences in concn of these amino acids were higher than their reported threshold values (7), their importance to sweetness of tomato fruits is questionable because of their low concn relative to the concn of reducing sugars.

Glutamic acid which is sour in taste (8) was higher in fruits harvested green or breaker and ripened off the plant than in those ripened on the plant. Differences in glutamic acid concn were parallel to differences in the magnitude of the "off flavor"

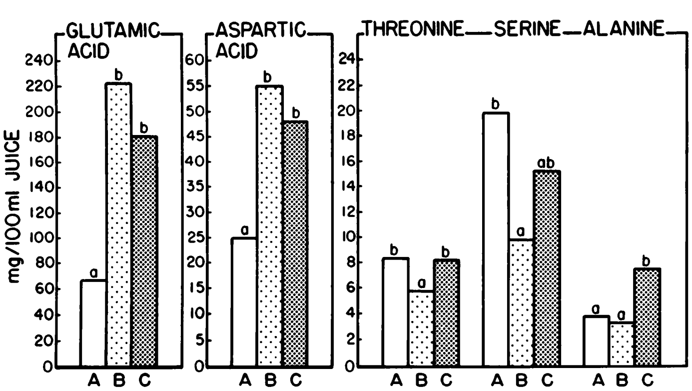

Fig. 1. Mean concn of 5 free amino acids which showed significant differences among tomato fruits: (A) analyzed at the breaker stage, (B) harvested breaker and ripened at 20°C, (C) ripened on the plant. Means not labeled with the same letter differ significantly at the 5% level, DMR test.

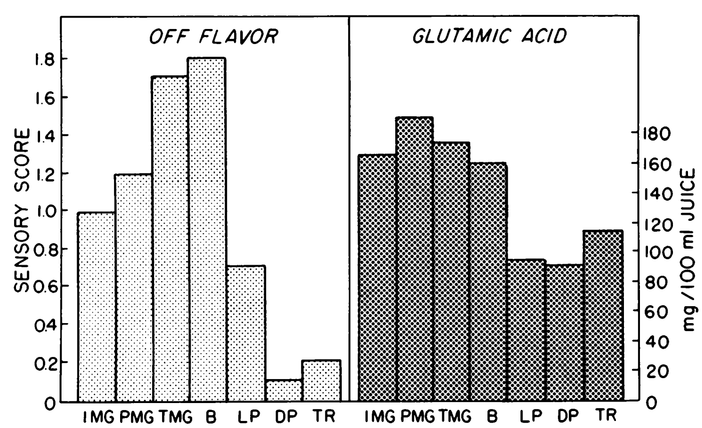

Fig. 2. Mean concn of glutamic acid and "off flavor" in 'Cal Ace' tomatoes picked at various stages of maturity and ripeness and ripened to table-ripe at 20°C *vs* those picked at the table-ripe stage. The panelists scored "off flavor" by placing a vertical line on a 10-cm horizontal line marked "weak" on one end and "strong" on the other; scores were measured as the distance from the "weak" end.

Table 2. Effect of adding monopotassium glutamate (MPG) to dices of table-ripe (TR) fruits on their acidity and flavor.

Season	Comparison	Relative change[z] in acidity		No. correct answers/no. panelists[y]
		pH units	TA (% citric)	
1976	TR *vs* TR + 60 mg MPG	–	–	11/29
1977	TR *vs* TR + 60 mg MPG	+.03	–.046*	27/50**
	TR *vs* TR + 120 mg MPG	+.06	–.054**	19/50
	TR *vs* TR + 180 mg MPG	+.10*	–.057**	22/50

[z]Due to the addition of indicated MPG amounts per 100 g fresh wt.

[y]Indicates the ability of the panelists to correctly identify the odd sample in triangle tests.

*, **Significant differences at the 5% (*) and 1% (**) level; other data not significantly different.

defect in fruits picked at the breaker or green stages and ripened off the plant relative to those ripened on the plant (Fig. 2). This "off-flavor" characteristic was detected by the panelists and was scored among other flavor characteristics in the 1975 study (6).

To test if high glutamic acid concn in fruits can influence their flavor, monopotassium glutamate (60-180 mg/100 g diced fruit) was added to table-ripe fruits which were evaluated by a sensory evaluation panel. The panelists were not able to detect any significant differences except in one triangel test in 1977 for the 60-mg addition treatment (Table 2). Panelists' comments about flavor differences were inconsistent and more related to sweetness and sourness than to "off-flavor." The addition of monopotassium glutamate resulted in an increase in pH and a decrease in titratable acidity in proportion to added concn (Table 2). This may be due to potassium effects on the buffering systems in tomato. These changes in acidity were below 0.1% which is the limit of detectability by sensory methods.

We conclude that glutamic acid does not play a direct role in the "off-flavor" defect of tomato fruits harvested at the green or breaker stage and ripened off the plant, relative to those ripened on the plant. It is also clear from this study that differences in amino acid composition are less important to tomato flavor than differences in other non-volatile components and in volatile compounds which we previously reported (6). Future studies, however, should consider possible interactions among all constituents including the major amino acids in relation to flavor quality.

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