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PRECURSORS OF VOLATILE COMPONENTS IN TOMATO FRUIT—I.

COMPOSITIONAL CHANGES DURING DEVELOPMENT

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Abstract—The composition of tomato fruit at nine different stages of maturity was studied. The dry matter content decreased gradually, whereas the alcohol-soluble material and non-protein nitrogen increased with ripening. Total nitrogen decreased up to the large green stage and then increased during ripening. Starch accumulated until nearly the large green stage and then diminished rapidly. Of the two components of starch, amylose decreased much faster than amylopectin, and the ratios of amylopectin and amylose thus increased during ripening. Among the amino acids, glutamic acid was present in an exceptionally high concentration, and its concentration doubled during ripening. A concomitant decrease in the concentration of several other amino acids was noted. The possibility of amino acids serving as precursors for the synthesis of volatile aroma components in tomato is discussed.

INTRODUCTION

As TOMATO fruit develops, changes in physiological and biochemical characteristics occur. Dalal *et al.*[1] observed that the biosynthesis of some non-volatile compounds in tomato increased with advanced growth stages of the fruit. They reported a progressive increase in quantity of volatile reducing substances, reducing sugars, water-soluble pectins, and organic acids as the fruit advanced in maturity. Rosa[2] reported that the starch content decreased steadily during ripening. A similar observation was made by Andreotti and Ceci.[3] The reducing sugars which were shown to increase progressively in quantity with advanced growth of the fruit may arise, at least partly, from hydrolysis of starch. As to the changes in concentration of the two components of starch, namely, amylose and amylopectin, no information is available.

Rowan *et al.*[4] reported an increase in the protein nitrogen to total nitrogen ratio during the climacteric rise, and then a steady decrease in the later stages. Freeman and Woodbridge[5] determined the amino acid content in tomato fruit and observed that glutamic and aspartic acids increased markedly, while alanine, arginine, leucine, valine, etc. decreased with ripening. Davies[6] recently reported that glutamic acid increased approximately 10-fold and aspartic acid more than doubled from the mature green to the red stage of ripeness. The presence of glutamic acid in the highest concentration in ripe tomatoes was also noted by Hamdy and Gould[7] in their studies on eight varieties of fruit.

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In the course of studying the precursors of the aroma components in tomato fruit, it was deemed of value to look further into the changes in composition during the development of the fruit. The present paper presents some experimental data using two varieties of tomatoes, namely, Fireball and V. R. Moscow. Fireball is an early and V. R. Moscow is a mid-season variety.

RESULTS

The dry matter content of tomato fruit decreased during development up to the red stage and then increased slightly (Fig. 1), ranging from 5·8 to 7·2 per cent for Fireball, and 5·8 to

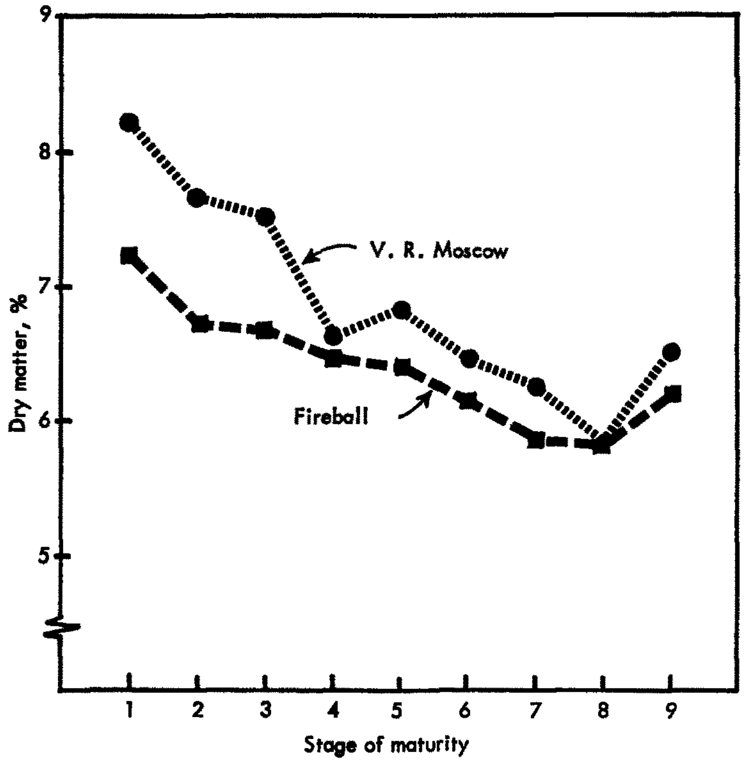

FIG. 1. DRY MATTER CONTENT OF DEVELOPING TOMATOES AT DIFFERENT STAGES OF MATURITY.
For Fireball and V. R. Moscow LSD 0·05 is 0·41 and 0·34 respectively; and LSD 0·01, 0·59 and 0·49.

8·2 per cent for V. R. Moscow, respectively. In general, the Fireball variety appeared to contain less dry matter than the V. R. Moscow variety. As shown in Fig. 2, a progressive increase in the concentration of the alcohol-soluble material was observed in both varieties. Except for the first stage of the Fireball variety, over 50 per cent of the dry matter of green tomatoes was soluble in 80% ethyl alcohol. Ripe tomatoes contained much more alcohol-soluble material; the increase was about 60 per cent when compared with tomatoes of earlier growth stages. As with the results of the dry matter study, the V.R. Moscow variety contained more alcohol-soluble material than the Fireball variety, regardless of maturation stages.

The total nitrogen was high at the earlier growth stages for both varieties of tomatoes

(Fig. 3). A gradual decrease followed until the large green or the 5th stage, when the concentration reached its minimum. Thereafter, the concentration increased steadily up to the red or the 8th stage, followed by a decline. A gradual decrease in the concentration of protein was observed during the development of the fruit (Fig. 3). For V. R. Moscow only, a slight increase was observed during ripening. Calculated as the difference between the values o total nitrogen and protein nitrogen, the non-protein nitrogen was found to increase with advancing maturity of the fruit. These trends can be seen more clearly when both protein nitrogen and non-protein nitrogen were expressed in terms of percent of total nitrogen, as shown in Fig. 4.

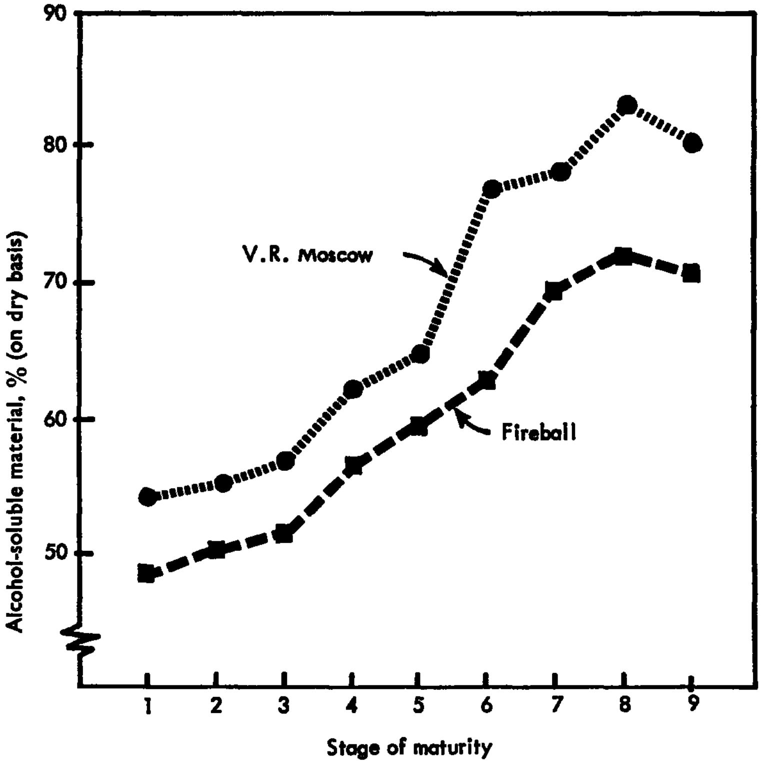

FIG. 2. ALCOHOL-SOLUBLE MATERIALS OF DEVELOPING TOMATOES AT DIFFERENT STAGES OF MATURITY. For Fireball and V. R. Moscow LSD 0·05 is 9·2 and 18·3 respectively; and LSD 0·01, 13·1 and 26·2.

The results obtained from the analysis of amino acids are shown in Table 1. The concentration of most of the amino acids changed markedly during maturation of the fruit. Alanine, arginine, cysteine, isoleucine, leucine, methionine, phenylalanine, tyrosine, and valine all decreased as the fruit advanced from the green stage to the breaker or the 6th stage. Glutamic acid was present in the highest concentration, followed by aspartic acid, for all stages of maturity. The concentration of either of these amino acids was many times higher than that of any of the others. These two amino acids increased markedly in concentration during ripening, in contrast to many other amino acids. Glutamic acid increased more than 100 per cent at the 7th stage compared to the previous stage. Total concentration of the amino acids increased, peaked at the pink stage, after which it decreased gradually. The concentration of NH_3, which would come primarily from the hydrolysis of glutamine and asparagine, appeared to follow this pattern also.

Tomatoes accumulated starch gradually as they grew until before the large green stage, when the concentration started to diminish rapidly. The concentration ranged from 1·22 to

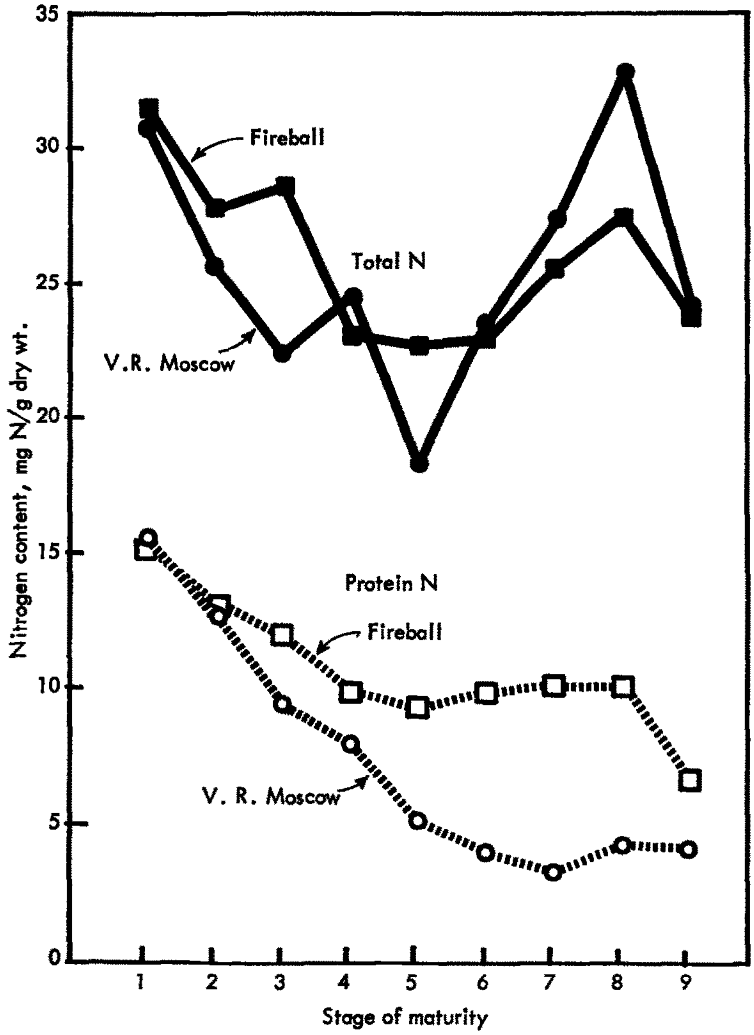

FIG. 3. NITROGENOUS COMPOUNDS OF DEVELOPING TOMATOES AT DIFFERENT STAGES OF MATURITY.
For Fireball and V. R. Moscow the LSD 0·05 for total N is 3·4 and 3·9 respectively; LSD 0·05 for Protein N, 2·2 and 2·5; LSD 0·01 for total N, 4·9 and 5·5; and LSD 0·01 for protein N, 3·1 and 3·6.

0·07 per cent for the Fireball variety, and 0·7 to 0·07 per cent for the V. R. Moscow variety. Tables 2 and 3 show the changes in total starch and amylose content for the two varieties of fruit. In general, the Fireball variety appeared to accumulate more starch than the V. R. Moscow variety.

The amylose content of fruit at different stages of maturity showed a trend similar to that of the total starch. The decrease as fruit ripened was, however, more marked. For example, the amylose content of Fireball at the breaker or the 6th stage decreased suddenly to less than

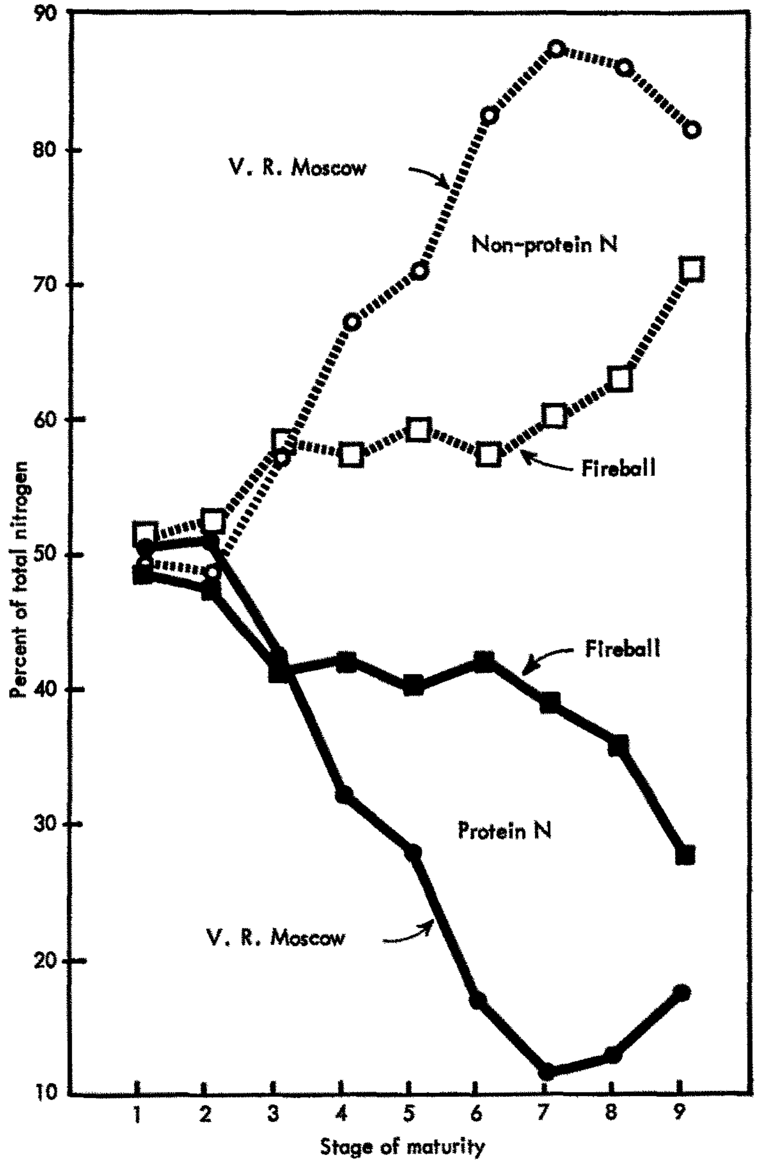

FIG. 4. PROTEIN NITROGEN AND NON-PROTEIN NITROGEN AS PERCENT OF TOTAL NITROGEN. For Fireball and V. R. Moscow the LSD 0·05 is 10·4 and 13·2 respectively; and LSD 0·01, 14·9 and 18·9.

one-tenth that at the previous stage (Table 2). The decrease was most marked between the 4th and the 5th stages in the V. R. Moscow variety (Table 3). The amylopectin content, which was assumed to be the difference between total starch and amylose, also decreased progressively as the fruit ripened. However, the rate of decrease was much smaller than that for amylose.

The ratios of amylopectin and amylose, therefore, changed markedly as the fruit grew to maturity. The ratios were about 2:3 in the earlier stages of growth, but they became increasingly greater as the fruit ripened (Tables 2 and 3).

TABLE 1. AMINO ACID COMPOSITION OF DEVELOPING TOMATOES (V. R. MOSCOW VARIETY)

Amino acid	Stage of maturity 4	6	7	8	9
	Concentration (μmoles/100 g fresh tomato)				
Alanine	87·0	60·5	32·5	37·0	35·0
Arginine	22·5	9·0	27·5	22·5	10·0
Aspartic acid	204·5	202·5	252·5	375·0	259·5
Cysteine	29·0	16·0	12·5	Trace	Trace
Glutamic acid*	830·0	740·0	1500·0	1630·0	1680·0
Glycine	47·0	37·0	32·5	30·5	30·0
Histidine	19·5	20·5	22·5	21·0	20·0
Isoleucine	40·0	20·0	20·0	25·0	11·5
Leucine	34·5	18·5	17·5	22·5	14·5
Lysine	40·0	28·0	38·5	28·5	22·5
Methionine	13·5	6·5	7·5	11·0	6·0
Phenylalanine	51·0	23·0	40·0	43·5	23·5
Serine	54·5	95·5	79·0	54·0	36·5
Threonine	53·5	32·5	69·0	54·5	36·0
Tyrosine	19·5	6·0	10·0	21·0	3·0
Valine	71·0	22·5	22·5	16·0	10·0
NH_3	935·0	1020·0	1075·0	550·0	525·0
Total	2552·0	2358·0	3259·0	2941·5	2723·0

* Proline was not separated from glutamic acid, and is included in glutamic acid values.

TABLE 2. STARCH AND AMYLOSE CONTENT OF DEVELOPING TOMATOES (FIREBALL VARIETY)

Stage of maturity	Starch (mg/100 g fresh tomato)	Amylose (mg/100 g fresh tomato)	Ratio amylopectin/ amylose
1	480	126	2·81
2	652	200	2·26
3	1110	428	1·59
4	1220	490	1·49
5	610	244	1·50
6	140	18	6·78
7	136	12	10·33
8	180	14	11·86
9	70	2	34·00

For starch and amylose LSD at 0·05 is 120·3 and 18·3 respectively; and LSD at 0·01, 172·9 and 26·3.

TABLE 3. STARCH AND AMYLOSE CONTENT OF DEVELOPING TOMATOES (V. R. MOSCOW VARIETY)

Stage of maturity	Starch (mg/100 g fresh tomato)	Amylose (mg/100 g fresh tomato)	Ratio amylopectin/amylose
1	464	140	2·31
2	700	204	2·43
3	600	226	1·65
4	400	140	1·86
5	130	20	5·50
6	96	6	15·00
7	74	4	17·50
8	68	2	33·00
9	74	4	17·50

For starch and amylose LSD at 0·05 is 81·0 and 20·7 respectively; and LSD at 0·01, 116·4 and 29·7.

DISCUSSION

The increase in alcohol-soluble material with the progressive development of the fruit is consistent with the findings of Dalal *et al.*[1] that reducing sugars, volatile reducing substances, etc. increased with advanced maturity of the fruit. Similar results have been reported by Woodmansee *et al.*[8] for Brookston tomatoes, and by Singleton and Gortner[9] for pineapples. The total nitrogen was high in the earlier growth stages of the fruit, but then gradually decreased until about the large green stage. A steady rise was then observed during ripening (Fig. 3), possibly indicating an enhanced protein synthesis during this period. This latter observation confirms the earlier report made by Rosa,[2] but our results showed that the concentration dropped when the fruit passed the red stage (Fig. 3). Woodmansee *et al.*[8] found a small increase in protein during ripening. A progressive decrease in protein nitrogen and a concomitant increase in non-protein nitrogen are evident from the results shown in Figs. 3 and 4 and Table 1. It appears that at least part of the amino acids may come directly from hydrolysis of protein present in the fruit. According to Gortner and Singleton,[10] the protease activity in the developing pineapple fruit was high until the final period of ripening.

The extremely high concentration of glutamic acid in the tomato fruit is noteworthy. This is consistent with the results reported by other workers.[5–7] Because glutamic acid was not successfully separated from proline, the concentration of glutamic acid shown in Table 1 included that of proline. It may be assumed, however, that the amount of proline was not large. It is also noteworthy that the concentration of glutamic acid doubled as the fruit grew from the 6th to the 7th stage. This may indicate that during this period, a greatly enhanced metabolic activity occurs *in vivo*, resulting in the production of glutamic acid. Freeman and Woodbridge[5] showed, in addition, the presence of a high concentration of γ-aminobutyric acid. No attempt was made to investigate this compound in the present study.

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The increase in the concentration of glutamic acid appears to be related to the decrease in concentration of many other amino acids such as alanine, cysteine, leucine, valine, etc. Similar results have been reported by Freeman and Woodbridge.[5] Dent *et al.*[11] and Steward *et al.*[12] showed that under conditions of protein synthesis in the potato many amino acids decreased markedly, whereas glutamic and aspartic acids remained high in concentration. They suggested the latter phenomenon to be due to protein synthesis. The present study also showed that only trace of cysteine could be detected in the extracts from the ripe fruit (Table 1). A marked decrease in the concentration of NH_3 was also observed during ripening. The absence of cysteine in the extracts from ripe tomatoes may indicate a rapid incorporation of this amino acid into some important components, such as a specific enzyme protein or some sulfur-containing aroma components. The decrease in NH_3 may reflect deamination of amides, such as glutamine and asparagine, during the ripening period. This might partly account for the large increase in the concentration of both aspartic and glutamic acids.

Green tomatoes gradually accumulated starch until before the large green stage, when a rapid decrease began (Tables 2 and 3). The loss of starch during ripening has been reported for bananas,[13] apples,[14] as well as for tomatoes.[2] The present study further demonstrated that the rate of decrease in amylose during ripening was much more marked than that of amylopectin. Expressed in terms of percent of total starch, the amylose diminished very rapidly as the fruit began to change its color. As a result, the ratios of amylopectin and amylose became greater during ripening. Interesting in this context are the findings that the ratio of amylose and amylopectin in wrinkled-pea starch increased as the pea matured,[15] and that no significant variation was found in the ratio in potato-tuber starch at different growth stages.[16]

According to Dalal *et al.*,[1] the free reducing sugar content of tomatoes increased uniformly. The present study has shown that the starch content increased gradually with advanced maturation of the fruit, and that it reached the maximum concentration when the fruit was nearly large green. This would indicate that the increase and decrease in free reducing sugar and starch content were not parallel. In other words, the sugars may not necessarily be produced at the expense of the starch already present in the fruit. This consideration seems to be in agreement with the work reported by Barnell[13] and Hulme.[14]

Tomato fruit develops its characteristic flavor and aroma only upon ripening. Results of the present study have confirmed the earlier findings by other workers that marked changes take place in the nitrogenous compounds, especially the amino acids during ripening. Whereas utilization of a variety of stored amino acids for protein synthesis could account for the marked decrease in some amino acids during ripening of tomatoes,[5, 11, 12] it is also possible that these amino acids may serve as the precursors for the synthesis of aroma components in tomatoes. In support of this hypothesis are the similar patterns of changes noted for the volatile components[1] and the amino acids. In addition, Pinto and Chichester[17] have recently shown that in the roasting of cocoa beans, destruction of amino acids and reducing sugars occurs, followed by the production of volatile carbonyl compounds. They postulated that the majority of the latter compounds produced during the course of the roasting process are the result of the oxidative deamination of free amino acids. Similar changes may possibly occur in the

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tomato fruit through enzymatic reactions. This possibility must, however, be tested in further research.

EXPERIMENTAL

Materials

Two varieties of field-grown tomatoes, Fireball and V. R. Moscow, were used. The sample tomatoes were sorted and classified into nine stages of maturity according to the method of Dalal *et al.*[1] Several tomatoes (4–15, depending on sizes) of the same stage were selected and cut into halves. Only one-half from each fruit was used. The halves were then cut into small pieces, from which samples were randomly taken. Each experiment was carried out at least twice, and for each experiment duplicate samples were used, and the values thus obtained were averaged.

Dry Matter

About 20 g of fresh tomatoes of various stages of maturity were weighed and heated in a forced-draught oven at 80° for about 48 hr. (This dry matter was ground in a mortar and the powdered sample was used for further analyses.)

Alcohol-soluble Material

Determination of the alcohol-soluble material was based upon the method of McCready *et al.*[15] Exactly 0·200 g of powdered dry matter was used for extraction with 80% ethanol. The alcohol-soluble material was obtained from the difference in weight between the original sample and the dried residue which remained after the ethanol extraction.

Total Nitrogen

The method described by Snell and Snell[18] was employed, using 20 mg of the dried sample. The nitrogen content of each sample was determined from a calibration curve constructed from absorbance readings of $NH_4H_2PO_4$ solutions of known concentration which were carried through the digestion procedure.

Protein Nitrogen

Protein nitrogen was considered as that fraction of total nitrogen which remained after repeated extraction of the dry samples with hot 80% ethanol. The residue from the alcohol extraction was then subjected to acid digestion and the nitrogen content determined by the same method as described in total nitrogen determination. The difference between total nitrogen and protein nitrogen was considered as non-protein nitrogen.

Amino Acids

Tomatoes representing five growth stages were used for determination of amino acids. Fifty grammes of fresh tomato samples were extracted with 150 ml hot 80% ethanol, in two portions, by blending them together in a Waring blendor for a few minutes. The homogenate was centrifuged, and the supernatant was condensed in a Rotavapor. The residue in the flask was then washed into a 25-ml volumetric flask and made up to volume with distilled water. This extract was re-centrifuged briefly and the supernatant was used as the sample for amino acid determination. The sample was hydrolyzed with 6 *N* HCl in a sealed glass ampule under nitrogen. This procedure was necessary in order to carry out the analysis in an automatic amino acid analyzer. Alcohol-soluble oligopeptides are, therefore, included in the amino acids, possibly giving higher values than those reported by other workers. Only V. R. Moscow variety was used in the determination.

Starch and Amylose Content

Extraction of starch and the determination of amylose were performed using the methods of McCready *et al.*[15] Total starch was determined by the procedures of Dubois *et al.*[19] The amylopectin content was obtained by the subtraction of amylose from the total starch.

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