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CHANGES IN COLOUR, POLYGALACTURONASE MONOSACCHARIDES AND ORGANIC ACIDS DURING STORAGE OF TOMATOES

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; starch; glucose and fructose; malic and citric acid; ripening; polygalacturonase biosynthesis.

Abstract—The appearance of polygalacturonase and red pigmentation in mature-green tomato fruit was prevented by storing the fruit in 5% O_2, 5% CO_2 and 90% N_2. However, the breakdown of starch to give monosaccharides and the change in concentration of organic acids which is normally associated with ripening still took place. On removal of the fruit to ambient conditions, polygalacturonase was synthesized and the fruit changed colour but monosaccharide and organic acid concentrations did not change.

INTRODUCTION

The major changes in composition which occur during ripening of tomatoes include loss of chlorophyll, pigment synthesis, fruit softening and an alteration in the metabolism of organic acids and monosaccharides. These variations are associated with synthesis or alteration in the activity of a number of enzymes. The synthesis of two isoenzymes of polygalacturonase, thought to be involved in fruit softening, occurs at about the same time as lycopene production [1, 2] as do the quantitative changes in two isoenzymes of pectin methyl esterase [1]. There is an increase in invertase activity [1] due to enzyme synthesis [3], although there is little or no sucrose present [4]. Other characteristic variations also occur such as a decrease in the concentration of malic and citric acids and an increase in monosaccharides due to starch breakdown [5]. These, and a number of other events, are all considered as part of the ripening process and tend to occur at about the same time as the onset of ethylene production and the respiratory climacteric. However, regulation and co-ordination of these events is not understood.

Recent work has shown that ripening may not be quite as closely co-ordinated as previously thought. In particular, the storage of fruits under different gas atmospheres can restrict lycopene synthesis while not preventing organic acid alteration and sugar accumulation [6, 7]. There was no qualitative difference in several respiratory isoenzymes but a quantitative loss was detected [8]. No attempt to measure polygalacturonase has been made previously. However, the fruit seemed to remain firm during the experiments suggesting that different aspects of ripening could be temporally separated by storage in controlled atmospheres. The work reported here shows that under these conditions, although there are changes in starch, and in some of the substrates of glycolysis and the citric acid cycle, the fruit do not alter their colour or synthesize polygalacturonase until removed from gas storage.

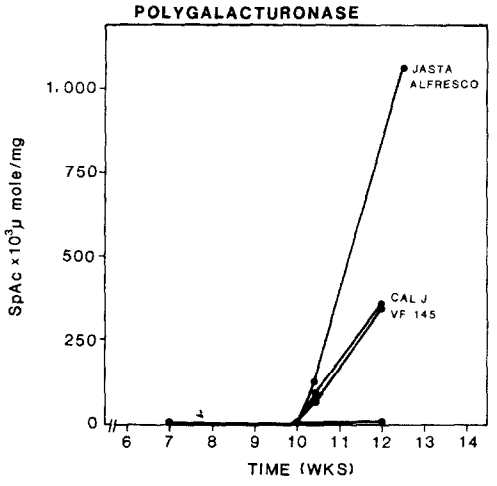
RESULTS

Synthesis of polygalacturonase and colour

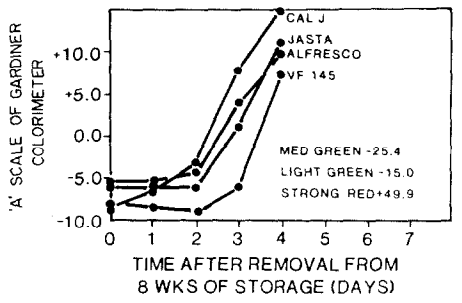
The results in Fig. 1(a) show that fruit removed after 10 weeks of gas storage had only a trace of polygalacturonase present but rapidly formed enzyme after standing at ambient temperature and gas composition for 2 days. The period of standing from 2 days to 2 weeks resulted in a linear increase in polygalacturonase until the fruit were fully ripe. Figure 1(b) shows that red colour development was not detectable initially but became apparent over the next 48 hr. Fruit could thus be removed at 7, 10 and 12 weeks of storage and allowed to stand at ambient temperature and gas atmosphere whereupon polygalacturonase and red colour were rapidly developed.

Changes in concentration of starch and monosaccharides

Table 1 and Fig. 2 show that starch was degraded during an 8 week period of gas storage and during this time monosaccharides accumulated, except in the cases of glucose concentration in Jasta and VF 145. Starch has completely disappeared by 12 weeks of storage and, although in previous experiments it has



(a)



(b)

Fig. 1. (a) Change in concentration of polygalacturonase during storage for 12 weeks. The increase in enzyme during ripening at ambient temperature and gas composition is shown for fruit removed after 10 weeks of storage. Fruit removed at 7 and 12 weeks of storage behaved similarly. (b) Increase in red colour of fruits taken from store after 8 weeks. Similar results were obtained from fruit after storage for 4, 10 and 12 weeks.

Table 1. Concentration of starch in tomato fruit during storage (cv. Cal. J.)

Time of storage (weeks)	Starch (% dry wt)
0	5.5
5	1.5
8	0.5
12	0.0

Only results from one cultivar are shown, the others all showed similar changes.

been possible to exactly equate quantities of glucose and fructose appearing with loss of starch [7], the loss reported here did not match the accumulation of monosaccharides. Attempts to detect other oligosaccharides were unsuccessful and it is possible that some starch was not amenable to the usual treatments for its solubilization [7]. The fruits were maintained in the dark and monosaccharides were therefore assumed to have been derived from storage carbohydrate. When fruits were removed from storage and allowed to change colour after treatment there was no further increase in monosaccharides, indicating that all starch had been degraded.

Changes in organic acids

The concentration of malic acid fell very rapidly during the first 4 weeks of gas storage (Fig. 3) but did not decline much further during the next 8 weeks. There was little further change when fruits were taken from storage although VF 145 had a larger amount of malic acid after colour development than before. However, the concentration was not large, compared to the original malic acid levels.

Although previous results [7] indicated that citric acid concentration has reached a peak after 5 weeks of storage, this was only found in one case (VF 145) whilst in the other three cultivars there was either little general difference (Cal J) or a general decline (Jasta, Alfresco) in citric acid during storage. There was no accumulation of citric acid during colour development and polygalacturonase synthesis after fruit were removed from storage (Fig. 3). Previous results [6] did not show significant increases in citric acid during fruit ripening on the plant of cultivars Cal J, VF 145 and Jasta.

DISCUSSION

The observed changes in monosaccharides, citrate and malate during storage may all be linked to control of starch degradation. It is well known that starch is broken down to give either glucose or phosphorylated glucose. As yet no mechanism of starch degradation has been investigated in detail in the tomato fruit. However, both glucose and fructose occur in equal quantities in the fruit during normal ripening [6] and there are two possible ways in which this can occur. Firstly, phosphorylated glucose from starch would be converted to fructose by phosphohexose isomerase and secondly, glucose from starch with existing fructose is converted to sucrose and this is inverted to glucose and fructose as found in potato tubers [9]. The rapid increase in invertase activity supports the theory of a sucrose pathway but there is no accumulation of sucrose in the tissues. If the increase in invertase removes the small amount of sucrose from the tissue then the monosaccharides must come from starch in detached stored fruit. Increased concentrations of hexose can lead to a greater metabolic flux through the glycolytic pathway and perhaps increase the amount of acetyl CoA available to condense with oxaloacetate. If, during ripening, there is a change in control of citrate metabolism in the TCA cycle, then malate will decrease and citrate accumulate.

Storage of mature-green fruit in an atmosphere of 5% O_2, 5% CO_2, 90% N_2 clearly delays polygalacturonase synthesis, which is important in fruit softening, and also pigment development. However, degradation of starch and changes in monosaccharides and organic acids still occur during controlled atmosphere storage. This is in marked contrast to the situation during normal ripening, where all these changes tend to occur at about the same time.

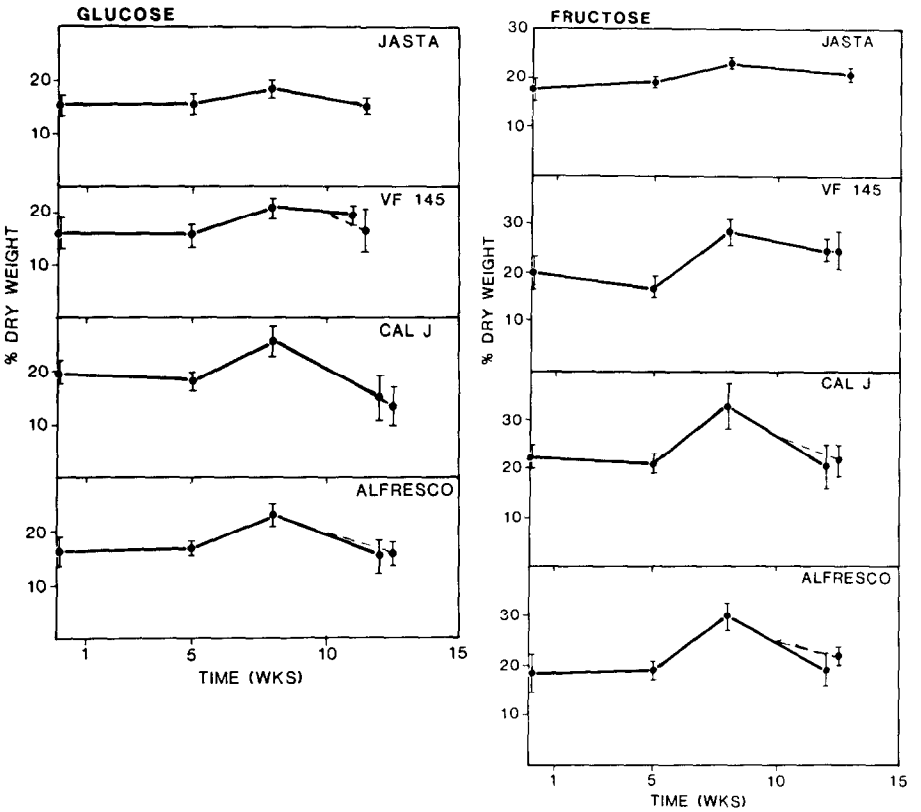


Fig. 2. Change in concentration of glucose (left) and fructose (right) as a percentage of the dry wt of fruits during storage for up to 12 weeks and also during subsequent ripening. O——O Concentration during storage. O---O Concentration after removal from 10 weeks of storage.

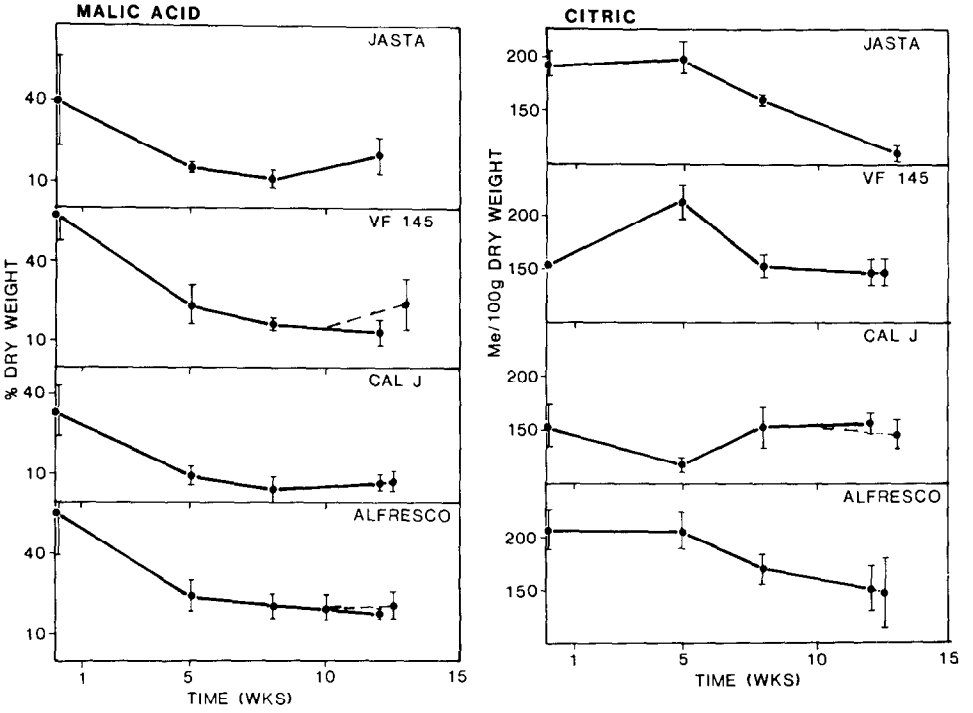


Fig. 3. Change in concentration of malic (left) and citric (right) acid as a percentage of the dry wt of fruits during storage for up to 12 weeks, and also during subsequent ripening. O——O Concentration during storage. O----O Concentration of monosaccharide after removal from 10 weeks of storage.

During gas storage respiration declines at concentrations of oxygen which would allow full activity of the cytochrome oxidase enzymes. It has been suggested that the cyanide-resistant alternate pathway of electron transport is operative in fruit and more dependent on oxygen than the cytochrome electron transport path [10]. The reduced rate of respiration may supply less ATP to the fruit biosynthetic systems and it is possible that synthesis of polygalacturonase is retarded more than regulation of enzymes controlling starch breakdown. If polygalacturonase synthesis requires transcription and translation then lack of sufficient phosphorylated molecules may slow down or prevent translation until metabolism returns to a normal level. Thus when fruit are removed to a normal atmosphere either transcription may proceed rapidly, followed by translation, or performed mRNA may be rapidly translated.

The rapidity of polygalacturonase appearance may indicate that preformed mRNA is translated. It has been shown previously that treatment of tomato fruit can separate the respiratory rise of CO_2 production from red colour development [11] but no attempt was made to measure polygalacturonase production. In the case reported here both colour and polygalacturonase synthesis are separated from hydrolytic breakdown of storage polysaccharide.

EXPERIMENTAL

Fruit culture. Fruits were obtained from plants grown out of doors and cultivated as described previously [12]. Fungicide was applied regularly to prevent development of disease. Fruits were picked at the mature-green stage (green skins but fully formed seeds) and any found to have immature seeds when sampled for analysis were discarded.

Gas composition and adjustment during storage. Fruits were stored in a sealed chamber at 12°, 93% r.h. 5%O_2, 5%CO_2, 90%N_2. Gas analysis was by two methods; O_2 analysis by GC equipped with dual TC. Columns contained molecular sieve 5A and N_2 at 30 ml/min was carrier gas; oven temp. was 60°. CO_2 was analysed by an IR gas analyser, scale 0–10% ± 0.2%.

Preparation of extracts. At the time of each sampling six fruits were removed from the chamber and added to boiling EtOH–H_2O (4:1). The insoluble solid was extracted in a Soxhlet apparatus for 12 hr with EtOH–H_2O (4:1) and the colourless solids dried for starch analysis. Other fruits were removed at the same time, cut open and locular tissue and seeds removed. The pericarp was sliced, mixed thoroughly, H_2O added (1:2.5 v/v) and this mixture was homogenized and centrifuged at 4000 *g*. The pellet was resuspended in M NaCl (pH 6 with NaOH) for 3 hr, filtered and made 75% satd with $(NH_4)_2SO_4$. The protein ppt was collected by centrifuging at 10000 *g*, resuspended in M NaCl and dialysed against M NaCl overnight [2].

Organic acids. Citric acid was analysed after conversion to oxaloacetate and acetate by citrate lyase (EC 4.1.3.6). Oxaloacetate was converted to malate by malate dehydrogenase (MDH, EC 1.1.1.39) and pyruvate (decarboxylation product of oxaloacetate) to lactate by lactate dehydrogenase (EC 1.1.1.27). The NADH oxidized in the former reactions was measured at 340 nm [13]. Malate was measured by reduction of NAD by MDH and oxaloacetate removed by glutamate–oxaloacetate transaminase (EC 2.6.1.1) to allow MDH to continue catalysing the reaction [14].

Sugars. Aliquots of EtOH extracts were evaporated to dryness TMSi derivatives were prepared and 1-μl samples injected into a GC equipped with dual FID. Columns containing 3% silicone gum rubber on Supasorb 80–100 mesh had N_2 as carrier gas at 30 ml/min. Injector and detector temps were 250° and the column temp. programmed from 150° with a subsequent 8°/min rise to 250° [6]. The RR_t of xylose to other monosaccharides was as previously given [6].

Starch. Starch was extracted from the dried residue by either boiling with H_2O or by shaking with DMSO. The supernatant was acidified to 6 M with $HClO_4$ and hydrolysed with amyloglucosidase at pH 4.6. Glucose was determined by reaction with hexokinase (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49). The reduction of NADP was measured at 340 nm and used to calculate the amount of starch [15].

Polygalacturonase. Enzyme extract was added to 0.5% polygalacturonic acid (1:4 v/v) containing 0.15 M NaCl and 0.05 M NaOAc at pH 3.8. Reducing groups of sugars were eliminated as previously described [2].

Colour development was measured by means of a Gardiner tristimulus colorimeter. The (a) scale gave a reading of slight red colour with a range from −15.0 (light green) to +49.9 (dark red).

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