Effect of Preharvest Carbon Dioxide Enrichment on the Postharvest Quality of Tomatoes

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Summary

The effect of preharvest application of elevated CO₂ throughout the fruit growing period on organic acid, sugar content, acid invertase activity (β -fructofuranoside fructohydrolase, EC 3.2.1.26), and color quality in tomato (*Lycopersicon esculentum* Mill. cv. Momotaro) fruit during storage at 20 °C was determined.

The CO₂-enriched tomato fruits contained significantly lower concentrations of citric, malic and oxalic acids, but had significantly higher reducing sugars and acid invertase activity at harvest and during storage. The concentration of these acids decreased with storage, whereas the activity of acid invertase and reducing sugar contents increased in the treated fruits; they were relatively constant in the control fruits. Furthermore, the elevated CO₂ resulted in a deeper red color during storage.

Introduction

Organic acids and sugars comprise the majority of the total dry matter contents of tomato (*Lycopersicon esculentum* Mill.). Citrate is usually the predominant acid in tomato fruits and depending upon variety, environment, and fruit age (Davies, 1965; Stevens, 1972) it comprises 40 to 90 % of the organic acids. The concentration of malate, the other major organic acid, ranges from 10 to 60 % of that of citrate, depending largely on the variety (Davies, 1965). These organic acids are crucial to the flavor and processing characteristics of tomatoes.

Tomatoes also contain higher levels of fructose and glucose than that of sucrose (Garvey and Hewitt, 1991; Miron and Schaffer, 1991). In the genera of *Lycopersicon*, Manning and Maw (1975) reported that the activity of soluble invertase was higher in *L. esculentum* compared to other species. There is evidence that increased invertase activity is associated with increased concentrations of hexoses and generally with decreased concentrations of sucrose (Lyne and Rees, 1971; Ricardo and Rees, 1970).

Atmospheres containing elevated levels of CO₂ are known to inhibit fruit ripening (Buescher, 1979). The application of CO₂ to delay ripening of tomatoes could be performed easily and inexpensively, but several reports have indicated that tomatoes are susceptible to CO₂ injury (Bedrosian and Schiffman, 1978; Parsons et al., 1970). Most investigations on the influence of CO₂ on tomatoes have been conducted with mature-green fruits, at reduced temperatures and in combination with reduced O₂ levels.

The beneficial effects of CO₂ enrichment during the growing period to yield, growth rate, transpiration, and photosynthesis have been reported by several authors (Calvert and Slack, 1975; Hartz et al., 1991; Kimball and Mitchell, 1979). To our knowledge, no published information is available on the effect of preharvest CO₂ enrichment on the constituents of tomato fruit during storage. The purpose of this study was to determine the effect of preharvest application of elevated CO₂ throughout the fruit growing period on the organic acids, sugar contents, acid invertase activity, and color quality in tomato fruits during storage.

Materials and Methods

Plant materials and cultural method

Tomato cv. Momotaro was grown in greenhouses at the Department of Agroindustrial Sciences, Faculty of Agriculture, Kagawa University, Japan from August 1993 to March 1994. The seeds were sown on 27 August; 14 plants were transplanted on 4m NFT (nutrient film technique) channel in two greenhouses on 28 September. The electrical conductivity and pH of the nutrient solution was maintained at $1.3 \sim 1.5 \text{ mS} \cdot \text{cm}^{-1}$ and $5.5 \sim 6.5$, respectively. The concentrations (mM) of basic elements in the solution were: $\text{NO}_3^- = 8.0$; $\text{H}_2\text{PO}_4^- = 0.7$; $\text{SO}_4^{2-} = 1.0$; $\text{K}^+ = 4.0$; $\text{Ca}^{2+} = 2.0$; $\text{Mg}^{2+} = 1.0$. CO_2 enrichment was done by supplying liquid CO_2 through a magnetic bulb controlled by a personal computer (NEC, PC-9801 UV11) connected to an infrared gas analyzer (COS, GH-250E) through A/D converter (Oga Electric, MIF-198). Application of elevated CO_2 began on 20 October, immediately after crop establishment and continued until the end of sampling on 15 February, 1994. During the growing period the CO_2 in the enriched greenhouse was 700 ppm, whereas that in the control was 250 ppm. After anthesis, each flower was treated with *p*-chlorophenoxy acetic acid (PCPA) for uniform fruit setting. The mean temperature during the CO_2 enrichment is presented in Fig. 1. Uniformly red fruits of the same age were randomly picked 90 ± 5 days after anthesis on 5, 10 and 15 February, 1994, in the test and control greenhouses. The samples were stored at 20°C and 80–90 % relative humidity, controlled by an automatic vapour regulator (Hitachi, V-50D) to prevent water loss. Ten fruits (sampled on 5 February) were used in each replication for measurement of fruit weight and color. Sugars, invertase activity, and organic acid contents were analyzed with 3 fruits in one replication at 0, 5, 10 and 15 days after storage. After 15 days of storage the fruits in both treatments deteriorated and no longer for analyzed.

Enzyme extraction

A 10 gram sample of tomato was mixed with 1 % polyclar AT (w/w) and 1 g sea sand and homogenized with 10 ml of cold 0.2 M citrate-phosphate buffer (CP, pH 5.0). The resulting

Fig. 1. Mean temperatures during the experiment (↓, start of CO_2 enrichment and ↑, first flowering date; each point represents the mean temperature over 10 days).

homogenate was filtered through four layers of cotton cloth, and the filtrate was centrifuged at $11,000 \times g$ for 10 min. The supernatant was dialyzed with 5 mM of the above buffer for 12 hr; the inner solution was designated as the soluble fraction (SF). The pellet was dissolved in 10 ml of 0.2 M CP buf containing 0.2 M of NaCl for about 24 hr and then centrifuged and dialyzed as above; the dialyzed solution was designated as cell wall bound fraction (CWBF) (Hisajima and Arai, 1978). These extractions were carried out at $0 \sim 4^\circ\text{C}$.

Enzyme assays

The standard assay medium for acid invertase consisted on 80 mM CP buf (pH 5.0), 100 mM sucrose, and an appropriate amount of enzyme solution for a total volume of 0.5 ml; the blank contained no sucrose. The assay mixture was incubated at 45°C for 15 min, cooled, and neutralized. Somogyi's copper reagent (1952) was added to the mixture which then boiled for 10 min. On cooling, the reducing sugars were quantified. Soluble protein content was determined using the method of Lowry et al. (1951) with bovine serum albumin as the standard. The enzyme activity was expressed as the amount of glucose produced per min per mg of protein.

Determination of organic acids and soluble sugars by HPLC

Sugar extractions were carried out at $0 \sim 4^\circ\text{C}$ to prevent the breakdown of sucrose (Matsui and

Kitagawa, 1988), because cultivated tomatoes have a higher invertase activity (Yelle et al., 1991). Fifteen grams of fruit tissue were mixed with 1 g sea sand and homogenized. Fifty ml of cold distilled water were added to the homogenate and the mixture centrifuged at $11,000 \times g$ for 10 min at 2°C . The mixture was filtered through polyether sulfone membrane filter ($0.45\ \mu\text{m}$ pore size) and the filtrate was analyzed for organic acids and soluble sugars by high performance liquid chromatography (HPLC). For organic acids the column (8 mm ID $\times$ 500 mm) was packed with Hitachi custom Ion-Exchange Resin # 2618. The mobile phase was 0.05 % phosphoric acid adjusted to the flow rate of $0.8\ \text{ml}\cdot\text{min}^{-1}$. The pressure was $13\ \text{kg}\cdot\text{cm}^{-2}$ and the temperature was 60°C . The ultraviolet detector was set at 210 nm. For soluble sugars, an aliquot was passed through a stainless steel column (10.7 mm ID $\times$ 300 mm) packed with silica gel (Gel pack C 610); water, the mobile phase, was pumped through the column at a flow rate of $1.0\ \text{ml}\cdot\text{min}^{-1}$. The pressure was adjusted to $9\sim 10\ \text{kg}\cdot\text{cm}^{-2}$ and the temperature to 60°C . A RI monitor (Hitachi L-3300) was used to record the peak heights and retention times of the compounds in the samples and the authentic reagents used as standards.

Analysis of color

The fruit color was monitored by measuring the L, a, and b values where L(lightness); a, red (lycopene and other carotenoids), and b, green (chlorophyll) (Baldwin et al., 1991). A digital color difference meter (Nippon Denshoku ND-101 DP) with standard plate (L = 96.9, a = -0.1 and b = 0.5) was used for color analysis.

Statistics

A completely randomized design with three replications was adopted. Data for the different parameters were analyzed with the help of two softwares CORMAT and STATISTICS. The level of significance was calculated from the F value of ANOVA.

Results and Discussion

Effect on shelf-life

No difference was observed in the shelf-life between preharvest CO_2 -enriched and control fruits.

In both treatments, the fruits lasted up to 15 days, after which they decayed rapidly.

Fruit weight

At harvest, tomatoes grown in CO_2 enriched greenhouse weighed 328 g compared to 303 g in the control (Fig. 2). Calvert and Slack (1975) and Kimball and Mitchell (1979) reported similar findings. During the storage period, no significant difference existed in weight loss between the treatments, but the control fruits lost 5 %, whereas the treated fruits lost 3 % after 15 days in storage.

Organic acid content

The preharvest CO_2 enrichment significantly decreased organic acids contents in the fruits during storage (Fig. 3). Citric and malic acid contents reached a maximum at harvest and then declined during storage; citric acid concentration was consistently higher than malic acid throughout the storage period. A small amount of oxalic acid was present and followed a pattern similar to that of malic acid. Organic acids usually decline during the ripening process as they are respiratory substrates. Winsor et al. (1962), Knee and Finger (1992), Stuart and Efiuvwevwe (1988), and Davies (1966) observed maximum acid contents at pink stage, but the level subsequently declined with ripening. Stuart and Efiuvwevwe (1988) who stored red tomato fruit at various temperatures found that citric, malic and oxalic acid con-

Fig. 2. Effect of preharvest application of elevated CO_2 on the fruit weight of tomato during storage. Each point represents the mean of 3 replications (each replicate composites of 10 fruits). Vertical bars indicate SE.

centrations continued to decrease during storage, reaching the lowest concentrations after 21 days in storage at 19 °C. These results are similar to our findings. Because of its concentration, citric acid contributes the most to the sourness of tomatoes (Stevens, 1972).

Sugar content

The CO₂-enriched tomatoes had significantly higher amounts of fructose and glucose than control fruits had at harvest and subsequently during storage (Fig. 4); no difference between treatments in sucrose contents was found. Fructose level was higher than that of glucose (Fig. 4); these two sugars, which accounted for more than 95 % of the total sugar, were in concentration 50 times higher than that of sucrose after 15 days in storage. Elizabeth et al. (1991) reported similar results. Miron and Schaffer (1991) and Dinar and Stevens (1981) reported that tomato fruits did not accumulate high amounts of sucrose, whereas Oscar and Donald (1988) reported that CO₂-enriched fruit during development accumulated more sugar than did the control fruits; these findings are in agreement with ours. Several workers found more reducing sugars in ripe fruit than in fruit

picked at green, mature-green or breaker stage (Betancourt et al., 1977 and Kader et al., 1978). In CO₂-enriched fruits, reducing sugar content increased during storage, whereas it was relatively constant in controls. This increase is not attributable to the water loss only, because control fruits lost weight more rapidly than did the treated ones.

Acid invertase activity

Figure 5 shows the effect of preharvest CO₂ enrichment on acid invertase activity, which catalyzes the hydrolysis of sucrose to glucose and fructose. Higher activity of soluble acid invertase was observed in the preharvest CO₂-treated fruits compared to the control throughout the storage period. Increase in invertase activity was observed from the time of harvest until the last sampling. The acid invertase in the soluble fraction showed a higher activity than that of cell wall-bound fraction throughout the experiment. This pattern is similar to that of kiwifruit (Matsui and Kitagawa, 1988) and tomato (Miron and Schaffer, 1991). Manning and Maw (1975) reported that in the cultivated tomato, acid invertase activity reached its maximum in overripe fruits; similar trends were observed here. The activity of acid invertase had a significant positive correlation with glucose and fructose (Table 1). In the present study, at

Fig. 3. Changes in organic acid contents in tomato fruit during storage as influenced by preharvest application of elevated CO₂. —●—: citric acid (CO₂ enriched), —○—: citric acid (control), —■—: malic acid (CO₂ enriched), —□—: malic acid (control), —▲—: oxalic acid (CO₂ enriched), —△—: oxalic acid (control). Each point represents the mean of 3 replications (each replicate composites of 3 fruits). Vertical bars indicate SE.

Fig. 4. Changes in sugar contents in tomato fruit during storage as influenced by preharvest application of elevated CO₂. —●—: fructose (CO₂ enriched), —○—: fructose (control), —■—: glucose (CO₂ enriched), —□—: glucose (control), —▲—: sucrose (CO₂ enriched), —△—: sucrose (control). Each point represents the mean of 3 replications (each replicate composites of 3 fruits). Vertical bars indicate SE.

harvest the CO₂-enrichment resulted in higher sugar contents as well as higher acid invertase activity. This is attributed to the faster translocation of photosynthate into the fruits, whereas the increased reducing sugar contents during storage in treated tomatoes could have resulted from the invertase activity.

Color

'L' and 'b' values did not differ significantly between the preharvest CO₂-treated and the control

fruits. A significant difference ($p < 0.05$) in the 'a' values existed between treatments. Fruits from the preharvest CO₂ enriched plot were redder than those in the control plot during storage (Fig. 6). We speculate that the higher sugar contents in CO₂-enriched fruits may be indirectly responsible for the red color development, comparable to what Takagi et al. (1989, 1994) found with carotenoids (a value) in developing satsuma mandarin.

We conclude that the preharvest application of elevated CO₂ concentration increases sugar contents and enhances color in tomato fruits during

Fig. 5. Acid invertase activity in tomato fruit during storage as influenced by preharvest application of elevated CO₂. —●—: soluble fraction (CO₂ enriched), —○—: soluble fraction (control), —■—: cell wall-bound fraction (CO₂ enriched), —□—: cell wall-bound fraction (control). Each point represents the mean of 3 replications (each replicate composites of 3 fruits). Vertical bars indicate SE.

Fig. 6. Hunter 'L' (lightness), 'a' (lycopene and other carotenoids) and 'b' values (chlorophyll) in tomato fruit during storage as influenced by preharvest application of elevated CO₂. —●—: 'L' (CO₂ enriched), —○—: 'L' (control), —■—: 'a' (CO₂ enriched), —□—: 'a' (control), —▲—: 'b' (CO₂ enriched), —△—: 'b' (control). Each point represents the mean of 3 replications (each replicate composites of 10 fruits). Vertical bars indicate SE.

Table 1. Correlation coefficients (r) between sugar contents and acid invertase activities (soluble fraction and cell wall-bound fraction) in tomato fruit during storage ($n=8$).

Characters	Glucose	Sucrose	Invertase activity (soluble fraction)	Invertase activity (cell wall-bound fraction)
Fructose	0.957**	-0.048 ns	0.958**	0.988**
Glucose		0.173 ns	0.859**	0.954**
Sucrose			-0.319 ns	-0.076 ns
Invertase activity (soluble fraction)				0.946**

** Significant at 1% level, ns=non significant.

the storage period, compared to fruits exposed to ambient CO₂ concentration.

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トマトの収穫後の品質に及ぼす炭酸ガス施用の影響

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摘 要

収穫前に炭酸ガスを施用し、収穫後 20°C で貯蔵したトマトにおける有機酸、糖含量、酸性インペルターゼと果色の影響について検討した。炭酸ガス施用トマト果実は、貯蔵中の対照区果実と比較してクエン酸、リンゴ酸、シュウ酸含量が有意に低かった。これらの酸含量は、貯蔵中も減少した。収穫前の炭酸ガス施用

トマトは、収穫時、あるいは貯蔵中も還元糖含量と酸性インペルターゼ活性が有意に高かった。酸性インペルターゼ活性は、貯蔵期間中に増大し、還元糖含量も炭酸ガス施用で増加したが対照区ではほぼ一定であった。さらに、炭酸ガス施用トマトは、処理果実でより強い赤色となった。