THE PHYSIOLOGY AND NUTRITION OF TOMATO SLICES AS AFFECTED BY FRUIT MATURITY AND STORAGE TEMPERATURE

Fisiologi dan Nutrisi Irisan Tomat Akibat Pengaruh Kematangan Buah dan Suhu Simpan

Darwin H. Pangaribuan 1) and Donald Irving 2)

1) Staf Pengajar Jurusan Budidaya Pertanian Fakultas Pertanian Universitas Lampung, Lampung
2) School of Agronomy and Horticulture The University of Queensland Gatton Queensland

ABSTRAK

Penelitian ini bertujuan mengevaluasi pengaruh kematangan buah tomat dan suhu simpan terhadap ciri fisiologis dan kandungan nutrisi irisan buah tomat yaitu produksi etilen, laju respirasi, kebocoran elektrolit serta kandungan asam askorbik dan likopen. Penelitian yang menggunakan tomat cv. 'Revolution' ini dilaksanakan di Laboratorium Pascapanen Hortikultura milik 'School of Agronomy and Horticulture' The University of Queensland, Australia. Perlakuan terdiri atas 4 tingkat kematangan buah yaitu hijau-kuning ('turning'), oranye ('pink'), merah muda ('light-red') dan merah ('red') dan 3 tingkat suhu simpan yaitu suhu 0, 5 dan 10 °C. Buah tomat dikerat dengan alat 'slicing machine'. Percobaan menggunakan rancangan acak lengkap dengan 5 ulangan. Hasil penelitian menunjukkan bahwa irisan tomat dari buah hijau-kuning menunjukkan laju produksi etilen dan tingkat respirasi yang lebih tinggi, kebocoran elektrolit yang lebih rendah, kandungan asam askorbik dan likopen yang lebih rendah daripada irisan tomat dari buah berwarna merah. Irisan tomat yang disimpan pada suhu 0 °C menghasilkan laju produksi etilen dan tingkat respirasi yang lebih rendah, kebocoran elektrolit yang lebih rendah, kandungan asam askorbik yang lebih tinggi, kandungan likopen yang lebih rendah daripada irisan tomat yang disimpan pada suhu 10 °C.

Kata kunci: tomat, kematangan buah, suhu simpan, etilen, respirasi, nutrisi

INTRODUCTION

Fresh-cut products remain biologically and physiologically active, as the tissues are living and respiring (Reyes 1996). The main problems with fresh-cut products are two-fold. Firstly, fresh-cut products are highly perishable because mechanical processes such as cutting, slicing, shredding, and trimming disrupt cellular structures (Rolle & Chism 1987, Watada et al. 1996). Secondly, mechanical wounding results in increased production of ethylene and respiration rates (Watada et al. 1996). In addition, the cellular breakdown leads to undesirable enzymatic reactions, leakage of ions and other cellular components, and consequently storage life is often reduced (Burns 1995, Luna-Guzman et al. 1999). If all these changes cannot be properly controlled, they can lead to rapid senescence and deterioration of the product.

The ripening of climacteric fruits such as the tomato is stimulated by ethylene. Dramatic physiological changes occur in tomato fruit during ripening, and those events are associated with changes in ethylene production, respiratory and enzyme activity, including cell wall and membrane-associated proteins. The capability to control tomato ripening by modulating ethylene responses could extend the storage life of tomatoes.

The commercial value of ready-to-eat tomatoes is dependent on maintenance of quality during storage. Although tomatoes continue to ripen during storage (Artes et al. 1999, Mencarelli & Saltveit 1988), commercial manufacture of tomato slices is challenging because the tomato fruits must first be ripened, processed, and then stored until consumed (Gorny et al. 1998). The quality of fresh tomato slices are affected by a number of factors such as cultivar, growing conditions, fruit maturity, postharvest handling and storage temperature (Kader et al. 1977, Madhavi & Salunkhe 1998). Among these factors, fruit maturity and storage temperature are the focus of this study.

The experiment reported in this paper examines the postharvest physiology of tomato slices in relation to maturity of the fruit and storage temperature. Physiological aspects that characterise slice quality including ethylene production, respiration rate, electrolyte leakage, ascorbic acid and lycopene were measured.
MATERIALS AND METHODS

Plant materials
Fruits of tomato cv. ‘Revolution’ were harvested from a commercial field. Medium-sized fruits were chosen with a mean fresh weight of 150.0 ± 14.6 g, and equatorial and longitudinal dimensions of 68.7 ± 1.6 mm and 65.4 ± 2.4 mm, respectively. The four stages of maturity were characterised by colour (hue angle, h°) (Cantwell and Kasmire, 2002) and firmness (Newtons, N) as ‘turning’ (h° 80-100, 21 ± 0.9 N), ‘pink’ (h° 63-77, 18 ± 0.8 N), ‘light-red’ (h° 55-65, 15 ± 0.6 N), and ‘red’ (h° 46-54, 12 ± 0.5 N).

Slice preparation
Experiment was conducted in the Postharvest Laboratory the University of Queensland, Australia. A total of 5 slices (each 7 mm-thick) were cut parallel to the equatorial region fruit with a commercial slicing machine. To ensure uniformity only equatorial slices were taken, and the upper stem-end and lower blossom-end slices were discarded. The five equatorial slices from each fruit were considered as one replicate and were arranged within containers as vertical stacks. Each plastic container (high density polyethylene with length: 16.5 cm, width: 10.5 cm, depth: 6.5 cm) was capped with a lid perforated with 2 holes (10 mm-diameters). The holes were packed with clean cotton wool to assist in maintaining sterility, and to enable adequate ventilation of the atmosphere inside. Each container containing two layers of absorbent paper on the bottom to prevent juice accumulation (Gil et al. 2002). All containers were held in storage rooms at 0, 5 or 10 °C with 95% relative humidity.

Assessments and experimental design
For ethylene determination, samples were injected into a gas chromatograph (Shimadzu model GC-8A) fitted with a flame ionisation detector. Temperatures of the injector port, column and detector were 120, 90, and 120 °C, respectively. The 900 mm-long and 5 mm internal-diameter glass column was packed with activated alumina mesh size 80/100. The Shimadzu CRGA Chromatopac integrator output was calibrated using an ethylene standard gas (0.09 ± 0.02 μL L⁻¹, BOC Gases β-grade) and the balance gas was nitorgen. The carrier gas (1 kg cm⁻² pressure) was high purity nitrogen (BOC Gases). Oxygen (0.3 kg cm⁻²) was supplied as medical grade air, and hydrogen (0.45 kg cm⁻²) was high purity grade, both from BOC Gases.

Ethylene production rate on a fresh weight basis was calculated as follows:

\[
\Delta \text{C}_2\text{H}_4 \times \frac{\text{head space vol}(L)}{\text{fresh wt(g)}} \times \frac{1}{t(h)} \times \frac{273K}{273K + \text{temp}°\text{C}} \times \frac{1000}{22.4}
\]

where:

\[\Delta \text{C}_2\text{H}_4 = \text{ethylene concentration in sample background (μL L}^{-1}\text{)}\]
\[t = \text{incubation time}\]

For CO₂ analyses, headspace samples were injected into a gas chromatograph (Shimadzu model GC-8A) fitted with a thermal conductivity detector. Temperatures of the injector port, column and detector were 30, 35, and 30 °C, respectively. The 1.5 m-long and 1.8 mm-interanl-diameter glass column was packed with activated alumina mesh size 80/100. The gas chromatograph signal was recorded using a Shimadzu CRGA Chromatopac integrator calibrated with a CO₂ standard of 0.575% (v/v) in nitrogen (BOC Gases β-grade). The carrier gas (1 kg cm⁻² pressure) was high purity helium (BOC Gases).

The rate of carbon dioxide production was used to indicate the respiration rate. The respiration rate on a fresh weight basis was calculated using the following equation:

\[
\Delta \text{CO}_2 \times 10^4 \times \frac{\text{head space vol}(L)}{\text{fresh wt(g)}} \times \frac{1}{t(h)} \times \frac{273K}{273K + \text{temp}°\text{C}} \times \frac{1}{22.4}
\]

where:

\[\Delta \text{CO}_2 = \text{CO}_2 \text{ in sample } - \text{CO}_2 \text{ in background (}% \text{v/v)}\]
\[t = \text{incubation time}\]
Electrolyte leakage rate was determined from conductivity measurements according to the procedure of King & Ludford (1983), Hong et al. (2000). Four sections of pericarp discs (about 6 mm diameter) were excised from each slice and combined. The discs were weighed (about 2 g) and washed three times in distilled water and placed in beakers containing 30 ml 0.4 M mannitol solution. In an initial experiment it was found that conductivity of the bathing solution did not increase appreciably with incubation for up to 6 h. The discs were held at 30 °C for 6 hours and then 20 mL of solution was taken and initial electrical conductivity readings were taken using a conductance meter (Actinon Model 301, Conductivity Meter, Australia). The beakers were swirled for 10 s before the electrolyte measurements were taken. The discs and bathing solutions were then frozen at -20 °C for 24 hours and then thawed. Final conductivity readings were taken and electrolyte leakage (% was calculated (McCollum & McDonald 1991):

\[
\text{Electrolyte leakage} \quad \% = \frac{\text{Initial conductivity}}{\text{Final conductivity}} \times 100
\]

Ascorbic acid concentrations were determined using High Performance Liquid Chromatography (HPLC, LC-10 AD Liquid Chromatograph, Shimadzu, Japan). Approximately 20 g of slices stored at -80°C were thawed and homogenised with a homogenizer (Janke & Kunkel IKA, Ultra Tunax T25) at 13,500 rpm for 2 minutes at room temperature. Ten gram of homogenate was weighed and mixed with 25 ml of 6% (w/v) metaphosphoric acid. The solution was centrifuged (P Selecta, Centronic, Spain) for 20 min at 6,000 rpm (2500 x g). The extract was transferred into a 100 ml volumetric flask after filtration through Whatman No. 1 filter paper. The residue from the filtration was extracted with metaphosphoric acid once more and the second extract was combined with the first. The mixture was filtered through Whatman No. 1 filter paper and the filtrate was diluted to 100 ml with 6% (w/v) metaphosphoric acid. An aliquot of the acid extract was then filtered through a Millipore filter (Millex HA) prior to injection of 10 μL into the HPLC (SIL-10AXL, Shimadzu, Japan). The ascorbic acid was separated on a column of Luna 5μ C18 (Phenomenex, USA) with length 150 mm x diameter 4.6 mm, equipped with a guard column C18 5μ. The mobile phase was 0.2 N orthophosphoric acid, the flow rate was 0.8 mL/min, and the detection wavelength was 254 nm. L (+) ascorbic acid (0.01 mg/mL) (Merck) was used as the external standard for quantification. To determine recovery of the procedure, a known amount of pure ascorbic acid standard (i.e. 2 x the amount found normally) was added to disc samples and then the extraction and chromatographic procedures were applied to samples with and without standard ascorbic acid, in duplicate. The recovery was 96%, indicating complete extraction. Ascorbic acid concentration on a fresh weight basis was calculated:

\[
\text{Ascorbic acid (mg/100 g)} = \frac{\text{Area of standard \(0.01 \mu g\)} \times \frac{100 \mu L}{\text{Area of sample } 1 \mu L} \times \frac{1 \text{mg}}{100 \text{g}} \times \frac{1000 \mu g}{\text{g sample}}}{1 \mu L}
\]

Lycopene analyses were performed according to Hakim et al. (2000). Pigments were measured by homogenizing ca. 3 g of frozen pericarp tissue with a homogenizer (Janke & Kunkel IKA, Ultra Tunax, T25) at 13,500 rpm in 10 ml of acetone in a centrifuge tube at room temperature. The tubes were covered with aluminium foil to prevent light-induced lycopene oxidation. The tubes were shaken on a shaker (B. Braun, Melsungen AG, Germany) for 15 min at 150 cycles/min and then centrifuged (F Selecta, Centronic, Spain) at 6000 rpm (2500 x g) for 10 minutes at room temperature. The supernatant was decanted and adjusted to 15 ml with acetone. Lycopene concentrations were determined from the absorbance at 503 nm in an acetone extract using a spectrophotometer (Pharmacia LKB, Ultraspex III, Japan). This wavelength is best suited for tomato lycopene because the influence of carotenoids is negligible. Lycopene content was calculated using the formula using the molecular extinction coefficient of 17.2 \times 10^4 \text{ mol cm}^{-1} \text{ (Mencarelli & Saltveit 1988)} and was expressed on a fresh weight basis:
Lycopene (μg g⁻¹) =
\[
\frac{A_{503}}{17.2 \times 10^4 \text{ /mL} \times \text{cm} \times 10^3 \text{ mL}} \times \frac{10 \text{ mg}}{\text{kg tissue}} \times \frac{10 \text{ mL}}{\text{kg tissue}} \times \frac{A_{503} \times 0.0312}{g \text{ tissue}} = \frac{A_{503} \times 31.2}{g \text{ tissue}}
\]

The experiment was laid out in a completely randomised design. The factors were 4 levels of fruit maturity (‘turning’, ‘pink’, ‘light-red’, ‘red’), and 3 levels of storage temperature (0, 5 and 10 °C). Each treatment was replicated 5-fold. Experimental assessments were made on days 1, 4, 7, and 10 after slicing. In the following graphs, where maturity is the main factor, data from all temperatures are combined. Where temperature is the main factor, all maturities are combined.

RESULTS

The rates of ethylene production by slices decreased (P<0.001) with time in storage. The decrease in ethylene during the first 4 days was quite rapid especially for ‘turning’ and ‘pink’ stages (Fig. 1A). After day 4, rates remained relatively stable until day 10. Slices held at 10 °C had significantly higher (P<0.001) rates of ethylene production, but storage at 0 or 5 °C greatly reduced the rates (Fig. 1B). There was a decline in ethylene production from day 1 to day 4, then the rates of ethylene production stabilised throughout the rest of the storage period, except at 10 °C after day 7.

Respiration rates by slices also decreased (P<0.001) with time in storage. On the first day, the slices obtained from the ‘turning’ and ‘pink’ stages had the highest rates of respiration (Fig. 2A), whilst slices obtained from ‘red’ maturity fruit had the lowest rates. After 4 days, the respiration rates of slices from ‘turning’, ‘pink’, and light- red’ fruit were not different. Respiration rates of the slices at 10 °C were always higher compared with those at 0 and 5 °C (Fig. 2B). There was a gradual decline in respiration rate during storage at 0 and 5 °C with a slight increase after 4 days of storage at 10 °C.

Over the 10 days storage period, electrolyte leakage gradually increased from all slices. Slices obtained from ‘turning’ and ‘pink’ stages of maturity showed the least leakage up to day 4. After day 4, ‘pink’ slices leaked as much as more electrolytes than either ‘light-red’ or ‘red’ slices. Significant (P<0.001) but lesser increases in leakage occurred from ‘light-red’ and ‘red’ maturity stage slices (Fig. 3A). Electrolytes gradually leaked from the tomato slices over the first 7 days of storage at 0, 5 and 10 °C (Fig. 3B). During the period from 7 to 10 days of storage, electrolyte leakage was faster at 5 and 10 °C than at 0 °C.

The ascorbic acid content of tomato slices at all maturity stages decreased (P<0.001) during storage (Fig. 4A). Despite these changes, ‘red’ slices had the highest ascorbic acid contents throughout storage. Overall, the slices from ‘turning’ maturity fruit lost ascorbic acid slightly faster (5.4% per day) than slices from ‘red’ maturity fruit (3.9% per day). Temperature did not affect ascorbic acid concentration during the first day of storage. The ascorbic acid content of the slices at 0 °C was always higher than those at 5 and 10 °C (Fig. 4B). There was a steady decline in ascorbic acid content during storage at all temperatures tested. Slices stored at 0 °C lost ascorbic acid content at a rate of around 3.0% per day but those stored at 10 °C lost ascorbic acid at the rate of around 6.2% per day.

As expected, lycopene content was significantly higher (P<0.001) in ‘red’ slices than in ‘turning’ slices (Fig. 5A). Lycopene in ‘red’ slices was relatively stable during storage, whereas lycopene from ‘pink’, ‘turning’ and ‘light-red’ slices gradually increased. Lycopene content was significantly higher (P<0.001) in slices stored at 10 °C than at lower temperatures (Fig. 5B) after 4 days storage. Lycopene content in slices stored at 10 °C gradually increased whereas lycopene level in slices at 0 and 5 °C changed only slightly during storage.
Figure 1. Changes in ethylene production of slices from fruit of different stages of maturity (A) and storage temperatures (B) during storage. Vertical bars represent LSD 0.05.

Figure 2. Changes in respiration rate of slices from fruit of different stages of maturity (A) and storage temperatures (B) during storage. Vertical bars represent LSD 0.05.

Figure 3. Changes in electrolyte leakage of slices from fruit of different stages of maturity (A) and storage temperatures (B) during storage. Vertical bars indicate LSD 0.05.
Figure 4. Changes in ascorbic acid on a fresh weight basis of slices from fruit of different stages maturity (A) and storage temperatures (B) during storage. Vertical bars represent LSD 0.05.

Figure 5. Changes in lycopene content on a fresh weight basis of slices from different stages of maturity (A) and storage temperatures (B) during storage. Vertical bars represent LSD 0.05.

**DISCUSSION**

The beginning of intact tomato ripening is usually indicated by an increase in the production of CO₂ and ethylene (Grierson & Kader 1986). After slicing, slices from fruit at different stages of maturity produced different amounts of ethylene relative to each other. The ‘turning’ stage slices produced much more ethylene than ‘red’ stage slices. This may be because early maturity fruit and slices have greater potential for ethylene production, which means more ACC synthase and more ACC oxidase capacities than tomatoes at later stages of maturity (Abeles et al. 1992). These results indicate that the more advanced the maturity, the lesser the rate of ethylene production. Respiration patterns were similar to the ethylene patterns, in which the ‘turning’ tomato slices had higher respiration rates than ‘red’ tomato slices. This could also be
associated with greater capacities for ethylene production from less mature fruit and slices.

Storage at 0 and 5 °C substantially decreased the rate of ethylene production during the storage period. Madrid & Cantwell (1993) showed that storage of cantaloupe pieces at 0 - 2.5 °C completely suppressed wound-induced ethylene compared with higher storage temperatures. Artes et al. (1999) also reported that low temperature storage of tomato slices (2 °C) reduced wound-induced ethylene production. These effects are most likely due to the enzymes responsible for ethylene formation (ACC oxidase and ACC synthase) being sensitive to low temperatures (Lurie 2002).

Storage at 0 and 5 °C substantially decreased the respiration rate of sliced tomatoes during storage. Temperature is the most important environmental factor in the postharvest life of fresh produce because of its dramatic effect on rates of biological reactions including respiration (Lurie 2002). The Van't Hoff rule states that the respiration rate of fresh fruits and vegetables generally increases two to three folds for every 10 °C rise in temperature (Shewfelt 1986).

Electrolyte leakage is generally considered to be an indirect measure of plant cell membrane damage and integrity (King & Ludford 1983, Marangoni et al. 1996). The experimental results indicate that membrane permeability increased with advanced maturity (Fig. 3A). This was also shown in intact tomatoes in studies by Autio & Bramlage (1986). A rapid increase in electrolyte leakage might be due to the changes in membrane fluidity by cutting-induced oxidative stress (Hong & Gross, 1998). Therefore, the differences in electrolyte leakage at the different stages of maturity are possibly associated with senescence, where slices from advanced maturity stages (light-red and red) may be more susceptible to oxidative stress than those from early stages (turning and pink). In the present experiment, the effect of storage temperature on electrolyte leakage was not significant until day 7 of storage. Sharom et al. (1994) reported the differences appeared after day 7 of storage in whole tomatoes stored for 20 days at 5 °C, as did Murata & Tatsumi (1979) with tomatoes stored at temperatures between 2 and 12.5 °C. Kuo & Parkin (1989) reported that cucumbers did not show any changes in electrolyte leakage during storage at 2 °C for 10 days. Some researchers have used electrolyte leakage as a marker for chilling injury (Cote et al. 1993, Kader & Ludford 1983, Sharom et al. 1994).

Recently, Hong & Gross (2000) demonstrated the development of chilling injury in fresh-cut tomato slices during cold storage using tomato cvs. ‘Sunbeam’ and ‘Mountain Pride’ at the ‘light-red’ and ‘red-ripe’ stages of maturity. They used water-soaked areas as an indicator that expressed the degree of chilling injury. They also found that ethylene may inhibit development of chilling injury in tomato at ‘light-red’ and ‘red’ stages of maturity. Gil et al. (2002) also observed the development of water-soaked areas during their experiments using ripe fruits of tomato cv. ‘Durinta’. Generally, translucency of water-soaked areas was not the main disorder observed in this current study. The only exception was that the ‘red’ maturity slices often exhibited translucency. Gil et al. (2002) reported that translucency developed more frequently in fruit from advanced ripening stages. In an experiment using tomato cv. ‘Solarset’ at ‘turning’ maturity, Hakim et al. (2000) did not report any symptoms of chilling injury in fresh-cut slices. Moreover, Conesa et al. (2000) observed chilling injuries did not develop on slices of tomato cv. ‘Calibra’ when stored at 0 °C. The difference in cultivars in these studies may explain the differences in sensitivity to chilling injury.

There is a need for ready-to-eat tomato slices that have sufficient storage life while retaining nutritional value. Levels of vitamins make a significant contribution to nutritional value of fresh tomato slices. Concentrations of ascorbic acid in tomato slices are affected by fruit maturity. In this study, the slices from ‘red’ and ‘light-red’ stages of development contained more ascorbic acid than earlier stages of development (‘turning’ and ‘pink’) (Fig. 5A). Shi et al. (1999) also reported that the ascorbic acid content of greenhouse plum tomato var. ‘Heinz 9478’ increased during ripening. This trend is most likely due to the
tomatoes antioxidant function when the ripening cells absorb high amounts of oxygen as a result of an increasing rate of cell respiration (Abushita et al. 1997). In addition, antioxidant concentration increases in response to various oxidative stress (Lee & Kader 2000).

The current experiments have shown that higher temperatures promote a loss of ascorbic acid content, which is in agreement with the results of Watada et al. (1987). Increasing storage temperatures from 0 °C to 10 °C or 21 °C resulted in greater losses of ascorbic acid levels in kale, cabbage, and snap beans (Ezell & Wilcox 1959), and carotene in kale, collards, and turnip greens (Ezell & Wilcox 1962). These results suggest that higher storage temperatures can lead to a significant decline in antioxidant constituents of tomato, suggesting that free-radical accumulation is enhanced at higher storage temperatures (Toivonen 2003). This study also clearly showed that ascorbic acid content during storage at all temperatures tested gradually declined during storage (Fig. 5.9B). Ascorbic acid reduction after cutting or peeling was also reported by Chiesa et al. (2003) who reported that ascorbic acid levels in fresh-cut lettuce decreased during 10 days of storage at 4 °C with a strong decrease after 3 days of storage. Klein (1987) reported that ascorbate can be degraded by light and oxygen to which sliced tissues are exposed. Smirnoff (1996) explained that ascorbate loss in slices may be related to oxidation in injured tissues. By the end of storage, Klieber & Franklin (2000) reported that fresh-cut Chinese cabbage lost 13% of their ascorbic acid. These observations suggest that preserving the integrity of slices during storage is important during storage of tomato slices if the levels of ascorbic acid are to be held at similar levels to intact stored tomatoes.

Increase in lycopene level is directly related to the stage of ripeness. It was found that lycopene levels were higher in slices from more mature fruit (Fig. 10A). This finding is consistent with the observation that ripe ‘red’ tomatoes contain higher levels of lycopene than ‘mature-green’ tomatoes (Klein 1987). Liu & Luh (1977) also reported that ‘red’ maturity fruit produced increased carotenoid concentrations when processed into tomato paste. In the samples measured in this experiment, lycopene contents ranged from 6.5 – 8.7 µg g⁻¹, values close to those reported by Hakim et al. (2000) at ‘pink’ maturity.

CONCLUSION

Results indicate that the more advanced the maturity, the lesser the rate of ethylene production as well as the respiration rate. The ‘turning’ tomato slices had higher ethylene production and respiration rates than ‘red’ tomato slices. Storage at 0 and 5 °C substantially decreased the rate of ethylene production and respiration rate during the storage period. The results indicate that membrane permeability increased with advanced maturity. Over the 10 days storage period, electrolyte leakage gradually increased from all slices. The slices from ‘red’ and ‘light-red’ stages of development contained more ascorbic acid than earlier stages of development (‘turning’ and ‘pink’).

Higher temperatures promote a loss of ascorbic acid content and lycopene levels. Lycopene levels were higher in slices from more mature fruit.

REFERENCES


